UNIVERSITY OF ALBERTA

EFFECTS OF SYNCRUDE PROCESSED WASTE WATER ON GROWTH AND REPRODUCTION OF FATHEAD MINNOWS

BY

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Abstract

A waste product of SynCrude Canada Ltd.'s oilsands mine is mature fine tailings (MFT), a toxic aqueous suspension of particles, organic acids, bitumen and metals. One disposal method involves constructing lakes lined with MFT and capped with clean water. Prototype ponds support fathead minnows (*Pimephales promelas*; FHM), but the long-term viability of these populations is unknown. This study attempted to determine if exposure to MFT and tailings pond water (TPW), a related waste product, affected growth and reproduction of FHM. Laboratory larval growth bioassays (7 and 56 days) yielded no significant differences, but larval fish spawned in MFT water and TPW grew faster than control larvae. In a life cycle bioassay, male FHM raised in TPW showed delayed development of secondary sexual characteristics and were less likely to spawn. In the prototype ponds, no patterns in body size and condition were evident, but spawning was delayed in one MFT site.

I would like to dedicate this work to my grandfather,

Dr. John Rhodes Patton (1908-1998),

President of Canadian Petrofina Ltd. (1968-1973).

You were right granddad, life is one big circle.

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Chapter 1

General Introduction

The Athabasca oilsand deposit, located in Northern Alberta, is currently mined by SynCrude Canada Ltd. and Suncor Inc. Both companies use a process referred to as the Clark Hot Water Extraction (CHWE) method to extract bitumen, a crude petroleum product, from the oilsand. CHWE involves the addition of hot water and caustic soda to oilsand. The main by-products of this process are unrefined bitumen and an aqueous slurry with a pH between 8-9 composed of naphtha, residual bitumen, silt, sand and clay particles referred to as tailings (FTFC, 1995). Tailings are pumped into settling basins where they partition into three main components; large, primarily inert particles such as sand and clay, a water layer called Tailings Pond Water (TPW) and a mixture of water, ultra-fine particles, residual bitumen, heavy metals, and insoluble organic carbons referred to as fine tailings (FTFC, 1995). Over time, fine tailings consolidate by the expression of water; once fine tailings are 55% solids by weight, they are referred to as Mature Fine Tailings (MFT; FTFC, 1995). Millions of cubic meters of MFT are currently awaiting safe containment and, as plant production increases, their disposal is becoming a pressing issue (FTFC, 1995). One disposal method proposed by SynCrude involves incorporating MFT into wet landscapes.

The wet landscape option involves constructing large lake basins in geotechnically stable parts of the mine scheduled for reclamation, filling the basins with MFT, then capping the MFT with clean water from a natural source

(FTFC, 1995). The clean water cap would serve as a barrier between terrestrial organisms and the MFT and possibly as habitat for aquatic organisms. In 1989 and 1993, SynCrude constructed a number of prototype ponds to test the viability of water capping.

Undiluted MFT are acutely toxic to fishes (Verbeek, 1994; FTFC, 1995). Toxicity Identification Evaluation (TIE) reveals that the acute toxicity of both TPW and interstitial MFT water can be eliminated by removal of fractions which contain carboxylic acids, the majority of which have been identified as naturally occurring naphthenic acids (see Verbeek, 1994). Fathead minnows (*Pimephales promelas*) and brook sticklebacks (*Culaea inconstans*) have survived in many of the prototype ponds since their construction. Studies have been undertaken to determine if the presence of MFT in these ponds adversely affect fish health and metabolic processes, but none of them involved the ponds' original fish population or used similar species (see FTFC, 1995; Verbeek, 1994), despite the fact that the fathead minnow/ brook stickleback assemblage is one of the most common fish assemblages in northern Alberta (Robinson & Tonn, 1989), and that the fathead minnow is commonly used for toxicity bioassays (Environment Canada, 1992).

In toxicological studies, a distinction is often made between bioassays which test for acute and chronic effects. Acute effects are immediate and generally lethal whereas chronic effects are often defined as those that are not lethal to the individual and may only express themselves after long term exposure (Adams, 1990; Suter et al., 1987). Thus, a test for chronic effects is

officially defined as one in which an organism is exposed for at least 10% of its lifespan (Suter et al., 1987). In attempts to streamline and standardize testing, life cycle stages which appear to be less sensitive are often dropped from testing procedures and, as a result, chronic bioassays become shorter and involve fewer aspects of an organism's life history (Suter et al, 1987). One example is the fathead minnow 7 day larval growth and survival test which has been used to test acute and chronic toxicity since the 1960's. This test is currently one of the core tests used by Environment Canada and the US Environmental Protection Agency to evaluate the impact of materials discharged into aquatic environments (Environment Canada, 1992; Pickering and Lazorchak, 1995).

The fathead minnow 7 day growth and survival test has been shown to be consistent and relatively robust (Pickering and Lazorchak, 1995). Its strengths lie in that it a) uses growth as its endpoint and b) concentrates on early life stages. Growth has been referred to as the "ultimate indicator of health and condition" as growth is affected by "all of the biotic and abiotic variables acting on the organism, and reflects secondary effects of chronic stress." (Goede and Barton, 1990). Larval fish have greater sensitivity to environmental contaminants than juveniles and adults (McKim, 1977; Woltering 1984). Growth of larval fish is particularly sensitive to contaminant exposure (see McKim, 1985) as larval fish undergo significant growth and development relative to older fish (Diana, 1995); hence, metabolic stress or reduction in feeding due to contaminant exposure is evident relatively quickly.

Although the fathead minnow 7 day growth and survival test has its

strengths, researchers also accept that no one species, life stage or endpoint is consistently sensitive to all contaminants (Genoni, 1997). For example, in their comparisons of sensitivities of chronic test endpoints, Mayer et al. (1986) found reproduction to be more sensitive than growth. Suter et al. (1987) used the results of many partial and total life cycle tests carried out for suites of chemicals/chemical mixtures and determined fecundity of fish to be more sensitive than survival and growth of early life stages. Their review also outlined a number of studies which found significant effects at different life cycle stages and concluded that, when determining chronic effects, tests which incorporated only early life stages were not good substitutes for life cycle tests. In Woltering's (1984) review, larval fish survival, not growth, was determined to be the most sensitive endpoint. Contrary to the results of Pickering and Lazorchak (1995), Woltering also documented a high degree of variability in growth tests using the same chemicals and life stage endpoints which may be a result of slight variation in testing procedures and/or biological variability.

Another difficulty in determining potential chronic effects is the trade off between controlled laboratory studies and less controlled, but possibly more biologically relevant, field tests. Single species lab bioassays are considered by many to be highly repeatable and cheaper than whole community or mesocosm field tests (Cairns et al., 1996). Kimball and Levin (1985), however, review a number of examples where laboratory analyses underestimate the impact of a toxicant in a field situation. Laboratory bioassays for fluorine underestimated fluorine toxicity for algae and invertebrates in field conditions, possibly due to

differences in bioavailability. Fish, however, proved to be more sensitive to fluorine in the field than in the laboratory, possibly due to additional factors such as less than optimal water conditions in the field (see Cairns et al., 1996). Sub-optimal water conditions occur when temperature, pH, turbidity and dissolved oxygen change, and this variability is thought to influence the toxicity of many contaminants (Sprague, 1985). For example, Cairns and Scheier (1958) determined that bluegill sunfish (*Lepomis macrochirus*) were more sensitive to naphthenic acids at lower levels of dissolved oxygen than at consistently high levels.

The aim of this study was to assess if fathead minnows were affected by the presence of MFT and/or TPW. Patrick et al. (1968) determined that a naphthenic acid level of 5.6 ppm resulted in 50% mortality of bluegill sunfish over 96 hours, and growth was significantly reduced in rainbow trout (*Oncorhynchus mykiss*) fed naphthenic hydrocarbons (1% of total diet) (Luquet et al., 1984). As fathead minnows survive in reclaimed waterbodies in which naphthenic acid levels range from 1 to 9 ppm, it is possible that they are being chronically affected by the presence of the naphthenic acids in MFT. The endpoints selected for my study were those associated with growth and reproduction and allowed inclusion of different life stages and endpoints. In attempts to thoroughly examine the potential impact of SynCrude's waste waters on fathead minnows, growth and reproduction endpoints were examined in field populations, controlled field mesocosms and short term and life cycle bioassays.

Chapter 2 focuses specifically on growth of the early life stages of fathead

minnows, and includes results of 7 and 56 day laboratory growth bioassays, as well as a field experiment examining larval growth of fish in mesocosms. The waters tested included those from ponds constructed in 1989 and 1993, and that ranged in relative volume of MFT and TPW (i.e. high or low). The main focus of Chapter 3 is reproduction, and examines the results of a laboratory partial life cycle test (fish exposed post-hatch) that involved the exposure of larval fish <24 hours old to various SynCrude waters. These fish were then allowed to grow to adults (7 months), and spawn. Endpoints included condition of adults, number of days until first spawn, egg number and hatchability, and offspring growth and survival. Both of these chapters present controlled experiments in which observed effects could be contributed directly to the test waters. In Chapter 4, I examine growth and reproduction of field populations of fathead minnows living both in SynCrude ponds and natural sites, and compare findings to the laboratory and field bioassays.

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Chapter 2

Fathead minnow larval growth bioassays: laboratory and field bioassays using SynCrude Canada Ltd. processed waste water.

Introduction

The main waste material produced by companies currently mining the oilsand is an aqueous slurry referred to as tailings. To date, companies have been stockpiling their tailings, but as production increases and new mines are developed, tailings disposal becomes a pressing issue. One company, SynCrude Canada Ltd., is attempting to incorporate tailings into their reclamation program. SynCrude proposes to build lakes basins in their old mine pit and partially fill them with Mature Fine Tailings (MFT), an aqueous suspension of water, residual bitumen, heavy metals, and insoluble organic carbons, then cover the MFT with a layer of natural water (FTFC, 1995). As the rheological properties of MFT would prevent mixing, the overlying water would serve to isolate the MFT from the rest of the environment (FTFC, 1995). Whereas MFT and its associated water is acutely toxic, there is evidence that, over time, toxicity is reduced as contaminants are biodegraded, biotransformed, and volatilized (FTFC, 1995). It is hoped that aquatic biota will eventually colonize these lakes.

In 1989 and 1993, prototype ponds were constructed to test the viability of water-capped lakes. While some ponds were built as outlined above, other sites were filled with MFT, but capped with Tailings Pond Water instead of clean water. Tailings Pond Water (TPW) is a saline solution with a chemical profile similar to the water expressed during the consolidation of MFT (Verbeek, 1994).

Toxicity Identification Evaluation (TIE) tests identified naphthenic acids as being responsible for the acute toxicity exhibited by TPW and MFT expressed water, but other contaminants may induce chronic responses in biota (Verbeek, 1994). Determining the concentration at which the many elements contained in MFT cause chronic effects and establishing their synergistic/antagonistic interactions with other elements would be extremely costly and time consuming. Whole Effluent Toxicity Technique allows the evaluation of discharge waters in which observed toxic effects are not linked to specific chemical components (AEP, 1996). Survival, growth and reproduction of fish are some of the endpoints most often used by regulating agencies in toxicity testing and have been determined by Woltering (1984) to be the three endpoints most sensitive to chemical effects. The experiments in this chapter focus on growth and survival.

Goede and Barton (1990) state that "growth (of fish) is one of the ultimate indicators of health and condition as it integrates all of the biotic and abiotic variables acting on the organism and reflects secondary effects of chronic stress." Somatic growth in fish is an integrative process and differences in growth among individuals may reflect ambient temperature, food abundance, population density or a variety of other biotic and abiotic factors (Diana, 1995). Only surplus energy not needed for activity, osmoregulation and metabolic processes is allocated to growth (Diana, 1995); therefore, chemicals which directly or indirectly influence any of these processes also impact growth. In growth-based bioassays, fish are reared in regulated settings under conditions

conducive to growth, with the only difference experienced among experimental groups being the presence, absence or concentration of a test chemical/solution.

The fathead minnow (*Pimephales promelas*) has been used in toxicological tests for many years (McKim, 1985; Environment Canada, 1992). Its natural distribution extends from Quebec to Northern Alberta and south into Mexico, making it an ecologically relevant species for many areas in North America (Nelson and Paetz, 1992). It is a short-lived species, attains sexual maturity at 6 to 8 months of age under laboratory conditions, and will reproduce in captivity. Standard protocols have been published for a bioassay that monitors the survival and growth of fathead minnow larvae to 7 days and this bioassay is included among the tests required by Environment Canada (1992) for the evaluation of chemicals and effluents. The strength of this particular test lies in its use of newly hatched larval fish, a life stage that has greater sensitivity to environmental contaminants than juveniles and adults (McKim, 1977; Woltering 1984). This sensitivity possibly results from the fact that most fish undergo a degree of post-hatch development (Diana, 1995). Fathead minnow larvae, for example, hatch with a yolk sac that supplies nourishment, incomplete mouths, no body pigmentation, deflated swimbladders and underdeveloped fins (Buynak and Mohr Jr., 1979). Studies done with effluent produced by petroleum refineries in Ontario have found the 7 day fathead minnow bioassay to be a useful test in establishing sublethal toxicity of effluent, yielding positive effects when assays with other organisms displayed none (Sherry et al, 1997; Chapman et al., 1994).

Current test protocol requires the exposure of fish for only 7 days, but some researchers have expressed concern at the brevity of the test. A true chronic test has been defined as one which spans a significant portion of the life cycle of an organism (~ 10% of the life span) (Environment Canada, 1992). The 7 day test falls short of that even in a laboratory culture where fish spawn at age 6 to 8 months (180-240 days). McKim (1985) recommends exposure of fathead minnows for at least 28 days while Petrocelli (1985) suggests that all fish be exposed for up to 6 months. Whereas some studies demonstrate that the 7 day test is a good predictor of sublethal toxicity, others have determined that terminating a test at 7 days underestimates toxicity compared to tests that extend to 30 days (Environment Canada, 1992).

Difficulties can also arise when the results of laboratory bioassays are applied to field conditions. Biotic and abiotic conditions can alter the sensitivity of an organism to a toxicant and/or the presence of a contaminant can alter an organism's tolerance to natural abiotic conditions (Sprague, 1985). The tolerance of larval fathead minnows to acute levels of copper changed as abiotic factors (pH and dissolved organic carbon) were altered (Welsh et al., 1993). When oxygen levels were cycled to low levels for 8 hours then increased to 95% saturation, the toxicity of naphthenic acids to bluegill sunfish (*Lepomis macrochirus*) increased by a factor of 2.8 at the lowest oxygen levels (Cairns and Scheier, 1958). Naphthenic acids also were twice as toxic to pond snails (*Physa heterostropha*) at 20° C than at 30° C (see Sprague, 1985). The influence of abiotic conditions on the toxicity of naphthenic acids is not well understood (AEP,

1996), but, as indicated above, toxicity does appear to vary as conditions change. Hence, a field growth bioassay may yield different results from an assay carried out in the laboratory.

I attempted to address some of the concerns about the fathead minnow 7 day growth and survival test by carrying out a number of tests that varied in length and setting. I first performed two standard laboratory growth bioassays with waters collected from various sites constructed by SynCrude Canada Ltd. To ensure a thorough evaluation of the SynCrude waters, the protocols for the standard 7 day bioassay were also adapted for a longer bioassay in which fish were sampled at 7, 28 and 56 days. Finally, I included a mesocosm component which allowed me to a) include exposure of embryos as part of the test by allowing fish to spawn in the test sites before collecting eggs and larvae and b) determine if field conditions affect the test outcome.

These growth bioassays provided an industry accepted starting point for the broader analysis of the potential chronic toxicity of SynCrude tailings waste water. Determining impact of contaminants on larval fish in a field population is difficult as natural processes may act synergistically, or dominate over effects resulting from chronic exposure to a chemical (Sprague, 1985). As tests described here are part of a larger study that includes field monitoring, they will provide valuable information on a life stage difficult to monitor closely in the wild.

The two main factors that determine the relative toxicity of the water in the SynCrude constructed ponds are the age of the tailings, which has a negative relationship with toxicity, and the type of water used to cap the tailings

(TPW or natural water) (FTFC, 1995). Water was collected from sites built in 1989 and 1993 with either a) a natural water layer overlying Mature Fine Tailings (MFT) or b) a layer of Tailings Pond Water (TPW) overlying MFT.

Methods

Procedures for egg and larval handling and care, abiotic parameters (temperature, photoperiod, etc.), maintenance of *Artemia* (brine shrimp) culture for feeding the larvae, and effluent collection and transport were followed as outlined by Environment Canada's (1992) biological test method for fathead minnows.

Growth Chamber

The environmental chamber used for all bioassays was located at the University of Alberta. The room was maintained at a constant temperature of 25° C on a 16 hour light/ 8 hour dark cycle. Lights were fluorescent and illumination was >500 lux.

Water

Control water was dechlorinated water from the University of Alberta. The salt control used in the 56 day bioassays was a solution of dechlorinated water, 70.2 ppm NaCl, 147.6 ppm Na_2SO_4 and 420 ppm $NaHCO_3^-$. The other treatment waters were collected from a number of different constructed ponds on the SynCrude Canada Ltd. Mildred Lake mine site, located ~40 km north of Fort MacMurray. To ensure SynCrude waters had the potential for chronic toxicity (did not immediately kill the fish), numerous sites were tested using the 7 day bioassay. Sites then tested in the 56 day bioassay were selected based on a) age of tailings material in a pond and b) whether the capping material was TPW or natural water. Age of tailings and capping material of each site, and the

bioassay in which they were used, are outlined in Appendix A and summarized in Table 2.1.

Water from the SynCrude sites was collected monthly by submerging 20 liter polyethylene jugs 0.25 m below the surface. Jugs were transported to the University of Alberta where they were stored at 4^0 C until use. Water aged in jugs for more than 1 month was not used. Twenty-four hours before use, jugs of water were removed from the refrigerator and placed in the growth chamber in order to warm to chamber temperatures. Before use, jugs were shaken vigorously by hand so particles that had settled out would be resuspended.

Fish

Fathead minnow eggs were obtained from Aquatic Research Organisms, in Hampton, New Hampshire. Eggs were shipped on nest tiles on the day of laying. Two to three tiles were sent ensuring that fish used were not full siblings (350 to 700 eggs depending on which bioassay was run). Tiles were placed in aquaria with 12 liters of dechlorinated water and aerated vigorously. Eggs were checked daily for fungus or dead (opaque) eggs which were picked off and discarded when located. Eggs generally hatched 3 days after arrival. Once hatching began, aeration was terminated. All eggs from each shipment hatched on the same day; therefore, nest tiles were maintained in a common aquaria to ensure random selection of larvae from all clutches.

7 day growth and survival bioassay

Within 24 hours of hatch, active larvae were randomly selected and introduced into growth vessels. Ten larvae were introduced into each vessel.

Vessels were 800 ml polyethylene beakers containing 500 ml of test water. Five vessels per treatment were distributed randomly in the environmental chamber. For screening purposes, waters from several sites were included in the first test. Sites with potentially higher toxicity (based on age and tailings capping material) were retested. Temperature, dissolved oxygen, pH and conductivity were measured and monitored daily. Each vessel received a 250 µl slurry of concentrated newly hatched *Artemia* twice daily.

Beakers were cleaned and checked for surviving larvae daily. Larvae were counted by placing containers on a light table. Cleaning consisted of decanting approximately 300 ml of water into a waste beaker and then using a 10 ml disposable plastic pipette to remove debris from the bottom of the beaker. The pipette was also used to decrease the volume of solution in the vessels to ~100 ml. Test solution was then slowly and carefully poured down the side of the vessel until the original volume of 500 ml was restored.

On day 7, fish were not fed in the morning to ensure that their guts would be empty during weighing. That afternoon, fish were killed using MS-222 and preserved in 70% ethanol. Within 2 weeks of preservation, fish were removed from the ethanol, rinsed with distilled water, placed into preweighed labeled aluminum weight boats and dried in an oven at 100° C for 2 to 8 hours as outlined in the Environment Canada guidelines (1992). Ten empty boats, which served as blanks, were also weighed and dried. All weights were done on a balance which weighed consistently to 10 µg.

56 day growth bioassays

Sixty-five larvae, > 24 hour old, were introduced into 18 liter aquaria placed randomly around the environmental chamber. Each aquarium contained 12 liters of treatment water, and four aquaria were established for each of the six treatments. A salt control was included in these bioassays as salinity of tailings associated waters is relatively high.

Temperature, pH, and conductivity were checked weekly in all treatments. Dissolved oxygen was not monitored as, unlike the 7 day growth and survival bioassay vessels, aquaria were aerated continually by airstones. Larvae were fed newly hatched *Artemia* twice daily for the duration of the bioassay (Appendix B). Tanks were cleaned on a weekly basis. This involved filling the beaker assigned to each tank with the corresponding treatment water, then removing the fish using a turkey baster (0 to 28 days) or net (29 to 56 days) and placing them in the beaker. Tanks were emptied, scrubbed, rinsed with dechlorinated water and refilled with new treatment water. The volume of water and food was standardized per fish and adjusted on a weekly basis as outlined in Appendix B.

At least 10 fish per tank were removed before morning feeding at 7, 28 and 56 days and these fish were killed with MS-222, then preserved in 70% ethanol. Dry weights were attained as outlined above except that 1) each fish was dried and weighed individually, and 2) fish were dried for 24 hours at 100° C.

Larval Growth Mesocosm Study

Three sites were selected for this portion of the study, Deep Wetland (DWL), Demonstration Pond (DP) and SC10 (Table 2.1). In May 1997, four enclosures (2 m x 2 m x 1 m) of 250 μm mesh were suspended from a structure constructed with 57, 50 cm^2 interlocking floating blocks. These blocks were connected in such a way to form a 6 m x 6 m (36 m^2) square which was then divided into four equal 2 m x 2 m (4 m^2) sections creating a structure sturdy enough to walk on. A 3 m dock was also attached to the DWL and DP arrays in order to access water > 1 m in depth, thereby ensuring that the nets did not touch the bottom. A 1 m dock was used in SC10 as the lack of littoral zone in that site meant that accessing deeper water was not difficult. Data loggers were suspended from each dock to ensure temperatures in each site were similar. The data logger in DP malfunctioned between August 1 to 13 but temperatures taken once a week were comparable to those of the logger before its malfunction (Appendix C).

Sixteen adult fathead minnows (twelve females and four males) were introduced into each of the twelve nets as a source for eggs laid and fertilized in each site. These fish were trapped in Kearl Lake, located ~ 40 km northeast of SynCrude's Mildred Lake site (Appendix A), using Gee minnow traps in early June, close to the beginning of the spawning as determined by the use of breeding boards placed in the site for monitoring purposes (Chapter 4). At the time the adults used in this study were removed from Kearl Lake, males had established nesting territories under some boards, but females had not yet laid

eggs on the boards. Only males displaying secondary sexual characteristics (dark head, tubercles and spongy dorsal pad) and gravid females (distended abdomen, visible ovipositor) were selected for the study. Fish were brought to the SynCrude site and kept in the laboratory for 2 days to ensure habituation to food (*Artemia*) and to cull fish that may have been injured during transportation. Sex ratio (three females : one male) of minnows in the nets was selected based on Environment Canada guidelines (1992).

As fathead minnows often die after spawning (Price et al., 1991), dead fish were collected daily and replaced with another of the same sex, thereby keeping adult density constant. Array bottoms were "swept" every 2 days with dip nets to obtain carcasses that had sunk. To prevent food from being a limiting factor for successful spawning, adult fish were fed 50 ml of live *Artemia* slurry, 0.6 g of freeze dried tubifex and a handful of Hagen fish flakes daily. Food volumes were determined using the Environment Canada guidelines (1992), then multiplied by 50 to increase the chances of the fish encountering the food in the nets.

Breeding substrates, in the form of 33 cm x 10 cm tarpaulin-covered boards, were secured in the corners of the twelve nets. Four boards were used in each net, one in each corner. Boards were checked for eggs each morning. When nests were found, nest location was recorded and the number of eggs/nest was estimated by laying a transparent grid (1 cm^2) on top of the nest and recording how "full" each square was (i.e. half full, three quarters full). Estimates on how many eggs were in a quarter, half, three quarters or full

square were obtained by counting individual eggs in squares of each group. Counting the number of eggs in certain squares to obtain estimates was done intensively at nests in all sites at the beginning of spawning, then periodically throughout the summer to ensure that researchers did not change their assessment over the course of time. When isolated eggs were present in a square, they were counted and added to the nest assessment. Egg masses of similar size and age were removed from each site on July 8 and 13 by cutting the tarpaulin away from its board. The egg masses were brought into the lab. Masses, still attached to the tarpaulin, were placed in a 800 ml beaker containing their natal water. The tarpaulin was secured to the wall of the beaker and an airstone was placed into the beaker. Adult fish were permitted to spawn until July 13, at which point they were trapped out of the nets. The nets were then raised, allowed to dry and reintroduced into the arrays.

The collected egg masses were the source of larval fish used for the subsequent mesocosm growth bioassay; thus, eggs were monitored carefully and checked daily for dead (opaque) or fungused eggs. On July 11, 150 newly-hatched fish from each site were introduced into aquaria (16 liter). The larvae were randomly selected from two to three nests. This was repeated on July 12 and 15 resulting in three aquaria for each site, each containing 150 fish. Each tank was fed 200 µl of *Artemia* twice daily and tanks were cleaned as described previously. On July 26, 20 fish of each birth group were introduced into each net in the field arrays (sixty fish/net; four nets per array). The remaining fish were killed with MS-222 and individually dried and weighed to attain initial growth

rates. Fish were processed as outlined in the 56 day growth bioassay. As fish from all three birthdates were introduced into each net, fry weights were pooled for analysis of initial weight.

Fish in the arrays were fed 50 ml of *Artemia* and 1 g of Hagen fry food once daily. Again, food volumes were well over those suggested by the Environment Canada guidelines (1992) in order that fish would have an increased chance at encountering food in the nets. Fish were allowed to grow in the nets for 21 days (August 16), at which point thirty fish were sampled from each net (120 fish per site). These fish were killed, individually dried and weighed as outlined previously (56 day growth bioassay). The remaining fish were collected on August 18, killed and preserved in ethanol in order to determine density.

Statistics

Statistical analysis used are outlined in Zar (1984). All statistics were run on SPSS (versions 6.0 and 7.5). Data were tested for normality using the Kolmogorov-Smirnov test (Zar, 1984). Growth and survival of the fish used in the standard 7 day bioassays were both analyzed using one-way analysis of variance (ANOVA). Growth to 7 days in the 56 day bioassay was also analyzed with one-way ANOVA. Fish sacrificed at 21 and 56 days were weighed individually; therefore, data for individual fish were nested within their tanks for analysis. Significant tests were compared post-hoc using a two-tailed Dunnetts test that compares each treatment to the control. Survival of the 56 day

bioassay fish was recorded weekly and analyzed using a repeated measures ANOVA.

Dry weights of larval fish used in the mesocosm study were compared among sites using one-way ANOVA, on the day of introduction (initial) and 21 days later. Relative change in dry weight (larval growth) in the nets was determined as below

(final weight-initial weight)/initial weight

The results of each net were nested within site and compared using a General Linear Model (SPSS 7.0). Significant differences between groups were identified using a Tukey's test for honest significant difference. Results of all tests were considered significant if $p < 0.05$.

Results

7 day growth and survival bioassays

Differences in growth were tested by comparing mean dry weights for each treatment using a one-way ANOVA. Fish in each vessel were pooled when weighed and mean dry weight was standardized for the number of surviving fish. Results were compared within assays, but not between assays. In the first 7 day growth and survival assay (SGS1) there was no significant difference in survival (Fig. 2.1; $F_{6,21}$=0.87, p=0.536) or growth (Fig. 2.2; $F_{6,21}$=1.57, p=0.204) among treatments. In the second assay (SGS2), control vessels had significantly greater survival than TP7 and SC10 vessels (Fig. 2.1; $F_{3,16}$=5.22, p=0.010), but growth did not differ among treatments (Fig. 2.2; $F_{3,16}$=1.68, p=0.211).

56 day growth and survival bioassay

Survival

Survival was evaluated weekly based on the number of fish in each tank for 7 weeks. There was no significant difference in survival among treatments for assay 1 (LGS1) (Fig. 2.3a; $F_{5,18}$=0.94, p=0.250) or assay 2 (LGS2) (Fig 2.3b; $F_{5,18}$=0.09, p=0.390). Fish used in the second assay exhibited reduced survival in all treatments compared to assay 1 fish, likely due to problems in shipping which resulted in the eggs spending 3 days in an unaerated shipping container. Contrary to the results of LGS1, fish in salt control in LGS2 suffered severe mortality and this treatment was removed from the analysis.

Growth

In LGS1, no significant difference in dry weight was found among treatments for 7 (Fig. 2.4a; $F_{5,17}$=2.55, p=0.065), 28 (Fig. 2.4b; $F_{5,18}$=0.64, p=0.672) or 56 (Fig. 2.4c; $F_{5,18}$=0.89, p=0.469) day old fish. In LGS2, TP5 fish had significantly greater dry weights than dechlorinated-water control fish at 7 days (Fig 2.4a; $F_{5,18}$= 4.58, p=0.007) but this difference did not carry over to 28 (Fig. 2.4b) or 56 days (Fig. 2.4c). There was no significant difference among treatments in dry weights of LGS2 fish at 28 ($F_{5,16}$=0.23, p =0.95) or 56 days ($F_{4,15}$=0.40, p=0.807).

Larval growth - mesocosm experiment

There was minimal mortality during the 21 days the fry spent in the nets with mean densities of 58.25 fish/net in DWL, 54.4 fish/net in DP, and 57.6 fish/net in SC10 . Density did not differ significantly after 21 days in the field ($F_{2,12}$=0.78, p=0.489). SC10 fish were significantly heavier at the time of introduction into the nets than DP and DWL fish (Fig. 2.5; $F_{2,131}$=14.80, p<0.001). When weighed at 21 days, however, SC10 and DWL fish were significantly heavier than DP fish (Fig 2.5; $F_{2,349}$=95.56, p<0.001). DWL fish gained significantly more weight than SC10 or DP fish during the 21 days in the field ($F_{2,8}$=152.77, p<0.001).

Discussion

Based on the results of the bioassays, there is no reason to suspect that exposure to the contaminants found in aged SynCrude TPW and MFT expressed water would impede growth of fathead minnows in their early life stages. Larvae in tailing waters did not differ significantly in dry weight from larvae raised in either dechlorinated control water or the salt control water solution, except for the second long term growth test (LGS2) in which TP5 fish exhibited significantly increased growth compared to the dechlorinated water control at 7 days. TP5 fish were not significantly larger at 7 days in SGS1, SGS2 or LGS1 and the LGS2 test results may be anomalous.

One 7 day growth and survival bioassay (SGS2) did result in significantly lower survival of fish exposed to TP7 and SC10 water. This would seem to support the findings of Woltering (1984) and Mayer et al. (1986), that larval survival was a more sensitive bioassay endpoint than growth, and Sherry et al. (1997) who found fathead minnow survival to be the most sensitive endpoint in petroleum refinery effluent testing. However, there was no significant difference in survival when SC10 water was used in another assay (LGS2). Intra- and inter-laboratory bioassay comparisons have found significant variation between bioassays that may be attributed to different fish stocks, genetic variation, and subtle differences in test conditions (Sprague, 1985).

In both the long and short assays, there was a consistent yet non-significant trend towards faster larval growth to 7 days in the salt control and SynCrude waste water treatments compared to the dechlorinated water control.

The salt water solution and waste water treatments all contained various levels of NaCl, Na_2SO_4 and $NaHCO_3^-$ which may have acted alone or synergistically to stimulate growth. In bioassays of KCl and NaCl, Pickering et al. (1996) found NaCl to have a stimulatory effect on growth, with larvae exposed to sublethal concentrations growing significantly larger than controls. The salts may have also affected the *Artemia* nauplii fed to the larvae. Fathead minnow larvae feed predominantly on living nauplii (Silberhorn et al., 1993). As *Artemia* naturally occur in saline environments, the saltier environments may allow them to live longer with minnows benefiting from increased feeding time.

Although growth bioassays are useful in determining toxicity of effluent, there are limitations that impact their predictive ability. Many studies have attempted to determine the ability of laboratory bioassays, including the fathead minnow bioassay, to predict the effects of toxins on populations and ecosystems with conflicting results. Both Dickson et al., (1992) and Birge et al. (1989) determined that fathead minnow dry weight (Dickson et al., 1992) and embryo-larval survival (Birge et al., 1989) were predictive of community responses and species richness, particularly of invertebrates, in contaminated streams. Robinson et al. (1994) found that bioassays measuring growth and survival of larval fathead minnows and survival and reproduction of *Cerodaphnia* correlated with the response of the benthic macrocommunity (i.e. low species diversity) in streams receiving pulp mill discharge. They also compared physiological parameters in resident fish to the results of the bioassays, but found the

bioassays not sensitive enough to predict the level of contamination which would elicit these type of responses (i.e. reproductive dysfunction).

The difficulty in applying results obtained in the laboratory to a field situation is further illustrated by the fact that results obtained in the mesocosm experiments differed from the 7 and 56 day growth bioassay. In the mesocosm experiment, SC10 fish were significantly heavier than DP and DWL fish at introduction into the arrays (~2 weeks of age). This trend is similar to that of laboratory bioassays where larvae reared in water from sites containing relatively large volumes of MFT and/or TPW were larger than their control counterparts. However, after 21 days in the arrays, DWL fish had gained significantly more weight than either SC10 or DP fish, a result I would not have predicted based on the laboratory bioassay results.

Many abiotic factors, including temperature, salinity and turbidity, affect larval growth (Wootton, 1990; Diana, 1995). Conditions between arrays were similar in terms of temperature (Appendix C), net volume, age structure, and density of fathead minnows, but water quality parameters differed due to the influence of MFT and TPW (conductivity, salinity, turbidity, naphthenic acids levels; Appendix A). These differences in water quality, however, were also present in the laboratory. One factor that did differ between the laboratory and the field was light.

Young fish depend on both visual and chemical cues for the detection of food and predators (Hubbs, 1986). Preliminary studies have demonstrated that SynCrude waste water does not impede olfactory detection of food odour

(McCutchen, 1998) or alarm pheromones (Wisenden et al., 1998), but no studies have been done to determine if the ability of fish to use visual cues in tailings associated waste water (MFT and TPW) is affected. In the lab, photoperiod was constant, surface light levels were high (>500 lux), aquaria were shallow (34 cm) with transparent sides and fish had a relatively small volume of water in which to search for prey. In the field, however, the fish were in a larger volume of water and exposed to a variety of light conditions (dawn, dusk, overcast conditions). Suspended solids reduce light penetration as they cause light to be reflected and absorbed (Wetzel and Likens, 1991), and there is evidence that suspended solids and turbidity can negatively impact the detection of food (Johnson and Wildsh, 1982; Breitburg, 1988; Barrett, 1992; Miner and Stein, 1993) by larval fish. Alabaster and Lloyd (1980) state that suspended solid levels above 25 mg/L can affect fish yield of a waterbody. Johnston and Wildish (1982) found that 20 mg/L suspended solids reduced consumption rates in larval herring (*Clupea harengus harengus*). In their review, Newcombe and MacDonald (1991) cited studies in which suspended solids levels as low as 6 mg/L reduced growth rates in chinook salmon (*Oncorhynchus tshawytscha)* , 14 mg/L reduced feeding efficiency in coho salmon (*Oncorhynchus kisutch*), and 12 mg/L reduced growth rate and body condition in brook trout (*Salvelinus fontinalis*). Levels of suspended solids in August of 1997 were 20 mg/L in DP and 35 mg/L in SC10, both near or above the level of suspended solids which affected consumption rates in larval herring (Johnston and Wildish, 1982) and reduced growth and feeding in the above mentioned salmonids (Newcombe and MacDonald, 1991).

No measurements were available for DWL in 1997, but DWL did have a constant suspended solid level of 5 mg/L from May to September in 1996. It is possible that the laboratory allowed for increased light conditions relative to the field, and that the decrease in light resulting from higher levels of suspended solids coupled with dawn/dusk periods and a larger volume of water made prey detection more difficult for DP and SC10 fish.

There appears to be a trend toward increasing levels of suspended solids in sites containing SynCrude processed waste water. Upon grouping a number of sites based on the waste products contained in each site (no waste material, MFT capped with natural water, and TPW), there appears to be a trend towards increasing suspended solids with increased tailings waste (June 1996: 5.71 mg/L in sites with no waste material to 22.50 mg/L in sites capped with TPW; August 1996, 5.80 mg /L in sites with no waste material to 28.33 mg/L in sites capped with TPW; Appendix D). More data are needed to determine if there is a consistent trend in suspended solids, if the level of suspended solids will decrease with age and if this will have an effect on the growth and foraging ability of larval fish in sites containing SynCrude waste material.

Hence, while growth bioassays are a quick, useful starting point in determining chronic toxicity of effluent, the results obtained from these tests cannot be directly applied to the field or other aspects of an organism's life cycle (Carins et al., 1996; Suter et al., 1987). Many factors modify the biological impact of a substance or mixture (Sprague, 1985), and toxicants can behave in many different ways, some having obvious immediate impacts while others may

accumulate over a life time. In their review of naphthenic acids, the Alberta Environmental Protection's Environmental Regulatory Service (AEP, 1996) identified a number of studies that examined the impact of purified naphthenic acids or oilsand waste water on fish. All studies outlined were relatively short term and all except one occurred in the laboratory (AEP, 1986). The report goes on to identify data gaps which include work on ambient levels of naphthenic acids in the environment and chronic impacts on biota. The growth bioassays carried out in this chapter do address ambient levels of naphthenic acids in the ponds, although not in isolation, and the mesocosms allow for corroboration of the laboratory results. The results of the mesocosm experiment indicate that more controlled field studies may be needed to determine if there are water quality issues surrounding fish growth in sites containing tailings waste water.

Table 2.1: Summary of waters used in growth bioassays; Nap. acids refers to the range of naphthenic acids in the waters throughout the tests, SGS refers to a standard 7 day growth and survival assay, LGS refers to the longer term (56 days) growth and survival assay, Meso refers to the mesocosm study and nd means none detected. In situations where more than one bioassay was carried out, 1 refers to the first bioassay and 2, to the second (i.e. SGS1 = the first standard 7 day growth and survival assay).

Site	Date of pond construction	MFT volume and Water Cap	Nap. acids (ppm)	Test Used
Control (C)		none	nd	SGS1, SGS2, LGS1 and LGS2
Salt Control (S)		none	nd	LGS1 and LGS2
Deep Wetland (DWL)	1993	none	0.9-1.7	SGS1 and Meso
South Bison (SB)	early 1980's	none, but contains runoff from mine site	9.4-14.7	SGS1
Test Pit 3 (TP3)	1989	1000 m^3 MFT with 1000 m^3 natural water cap	1.2-4.8	SGS1, LGS1 and LGS2
Demonstration Pond (DP)	1993	70000 m^3 MFT with 80000 m^3 natural water cap	6.0-9.0	SGS1, LGS1, LGS2 and Meso
Test Pit 5 (TP5)	1989	1000 m^3 MFT with 1000 m^3 TPW water cap	5.0-10.0	SGS1, SGS2, LGS1 and LGS2
Test Pit 7 (TP7)	1989	2000 m^3 MFT; water cap comprised of expressed and melt water	12.0-25.0	SGS1 and SGS2
Tailings Pond Water Pond (TPW)	1993	no MFT, pond filled with 50,000 m^3 of TPW	27.0-60.0	SGS1 and LGS1
Storage Cell 10 (SC10)	1993	29,000 m^3 MFT with 6,300 m^3 TPW water cap	44.0-51.0	SGS2, LGS2 and Meso

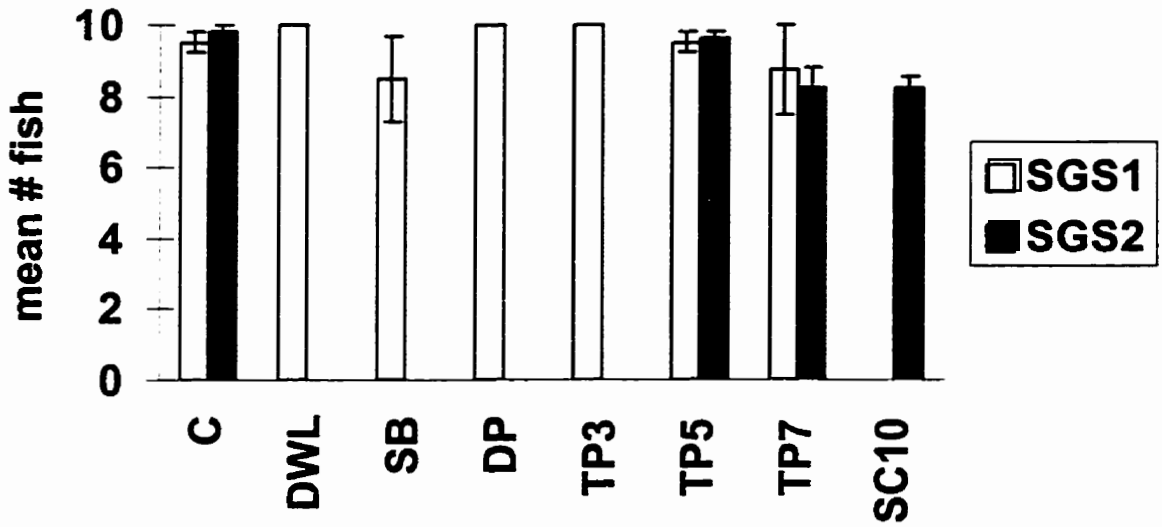


Figure 2.1 Survival (mean + standard error) of larval fathead minnows to 7 days in different SynCrude waters. Ten larval fish were introduced into each vessel initially. Sites ranged from those containing no tailings (C, DWL, and SB), those containing MFT capped with natural water (DP and TP3), to those containing MFT capped with TPW (TP5 and SC10). TP7 was MFT capped with expressed MFT water and melt water. In SGS1, n=4 for all sites and n=5 for all sites used in SGS2.

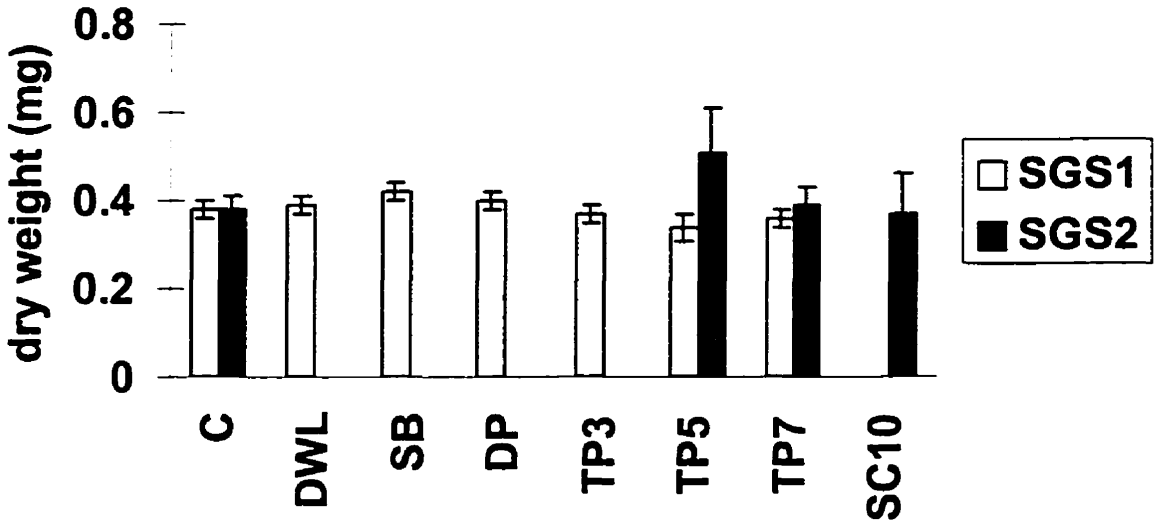
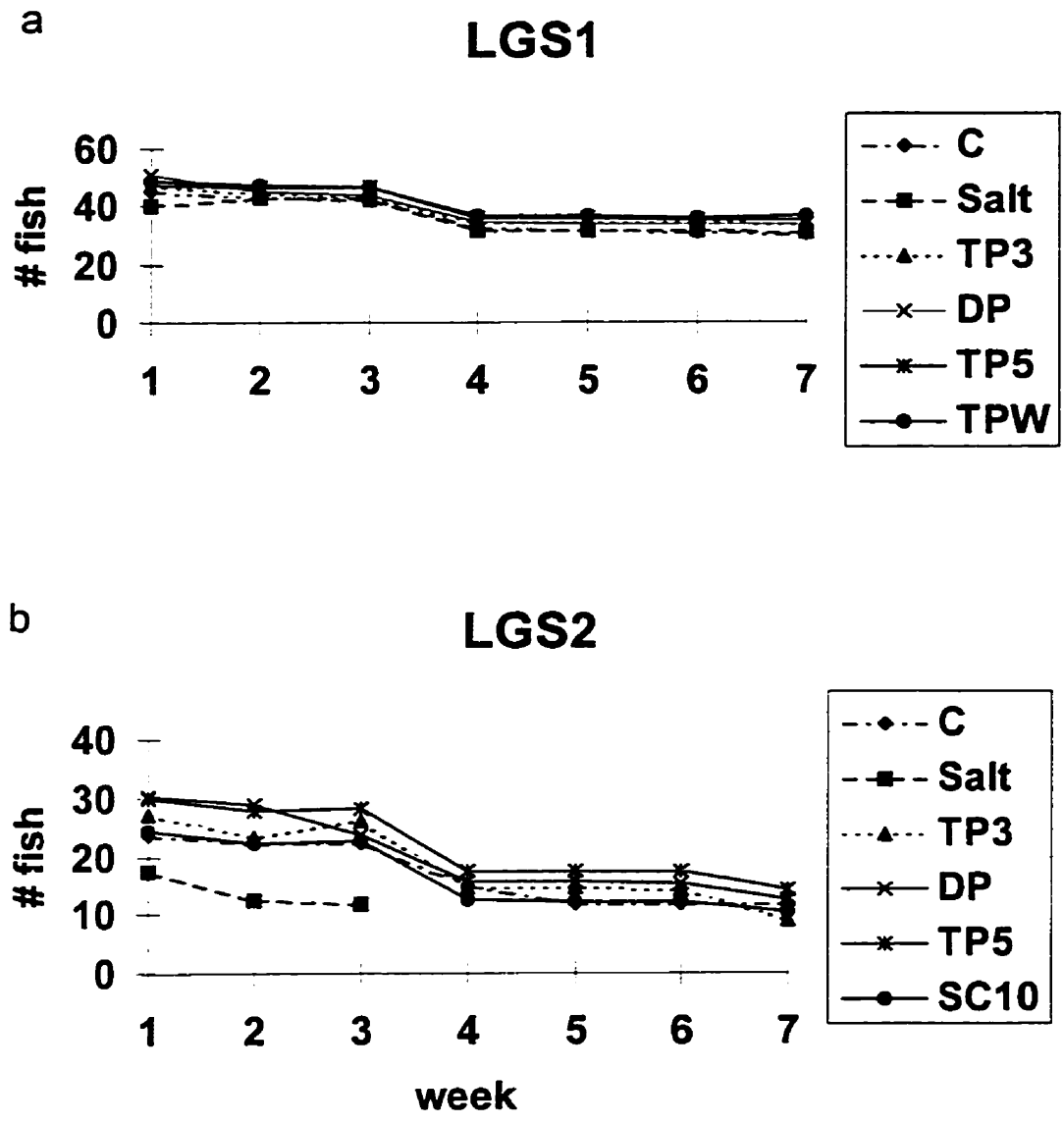


Figure 2.2 Dry weight (mean + standard error) of larval fish at 7 days in different SynCrude waters. Weights of all fish in one vessel were pooled, then standardized to the number of fish in the vessel at 7 days. Sites ranged from those containing no tailings (C, DWL, and SB), those containing MFT capped with natural water (DP and TP3), to those containing MFT capped with TPW (TP5 and SC10). TP7 was MFT capped with expressed MFT water and melt water.



Figures 2.3: Survival of fish used in the LGS1(a) and LGS2 (b) 56 day growth bioassay. 10 fish / tank were removed for growth assessment on week 4 resulting in the associated decrease in numbers. The waters used included those with no tailings associated waste (C and Salt), those from sites containing MFT capped with natural water (TP3 and DP), and those from sites containing MFT capped with TPW (TP5 and SC10). The site referred to as TPW in LGS1 contained tailings pond water only. n=4 for all sites in both LGS1 and LGS2.

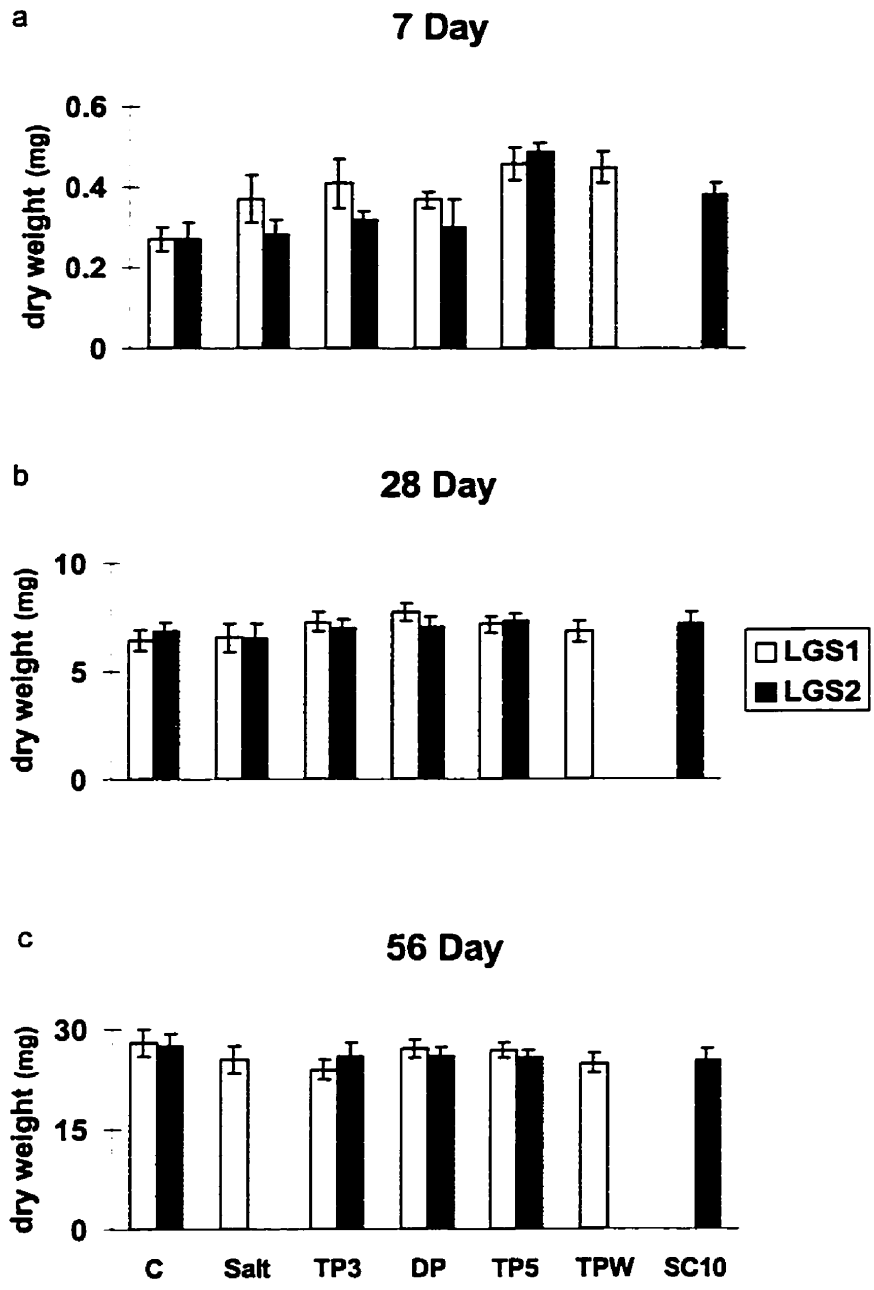


Figure 2.4 Dry weight (mean + standard error) of fish sampled at 7 (a), 28 (b) and 56 (c) days. 7 day fish were weighed in batches of ten fish, while 28 and 56 day fish were weighed individually and then nested within their tank for analysis. The waters used included those with no tailings associated waste (C and Salt), those from sites containing MFT capped with natural water (TP3 and DP, and those from sites containing MFT capped with TPW (TP5 and SC10). The site referred to as TPW in LGS1 contained tailings pond water only. n=4 for all sites in both LGS1 and LGS2.

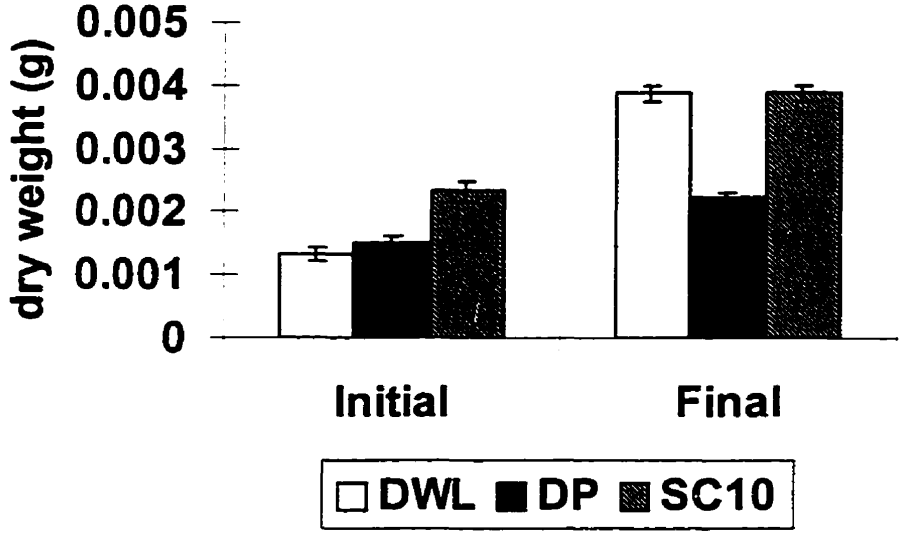


Figure 2.5 Dry weights (mean + standard error) of larval fish in each net at introduction into the outdoor mesocosms (initial) and after 21 days in the mesocosms (final). Final dry weights of individual fish were nested in their nets. DWL (n=4) contained only natural water while DP (n=4) contained MFT capped with natural water and SC10 (n=4) contained MFT capped with TPW.

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Chapter 3

Partial life cycle study examining the effects of SynCrude processed waste water on growth and reproduction of fathead minnows

Introduction

SynCrude Canada Ltd. mines the Athabasca oil-patch 40 km north of Fort McMurray, Alberta. Their main waste component is an aqueous slurry which partitions into three phases: inert solids; an aqueous suspension of water, residual bitumen, heavy metals, and insoluble organic carbons referred to as Fine Tailings; and a saline solution which remains after the solids and fine tailings settle, referred to as Tailings Pond Water (TPW). Fine Tailings consolidate slowly. Once they have attained 55% solids by weight, they are referred to as Mature Fine Tailings (MFT; FTFC, 1995). SynCrude is currently stockpiling the aforementioned waste products but, as production increases, permanent waste disposal solutions are crucial. SynCrude has proposed a plan in which MFT are incorporated into a lake being constructed in the old mine pit. This lake, referred to as Base Mine Lake, would be lined with MFT and capped with a 20 m layer of natural water. As the rheological properties of MFT would minimize mixing of the MFT and water phases, it is possible that aquatic organisms could be introduced into or colonize the lake and, as a detrital layer built up to further separate the MFT from the rest of the environment, a viable aquatic community could be established (FTFC, 1995). SynCrude refers to the process as "water capping".

In 1989 and 1993, SynCrude constructed a series of prototype ponds that were lined with MFT and capped with either natural or TPW water. These sites were an attempt to determine if water capping would work and to monitor the toxicity of water overlying MFT under natural conditions. Fresh TPW and the water expressed from fresh MFT have similar chemical profiles, are acutely toxic to fish and have been shown to decrease in toxicity over time (Verbeek, 1994; FTFC, 1995). Toxicity Identification Evaluation (TIE) has identified carboxylic acids, particularly naphthenic acids as being responsible for the acute toxicity of both waters (Verbeek, 1994). Based on growth bioassays carried out in this project (Chapter 2), the water phase of the constructed ponds does not appear to have an appreciable effect on growth or survival of fathead minnows.

Environmental standards and discharge limits are often based on the results of short-term bioassays. The strength of these tests is that they are quick, economical and allow for numerous single species tests, involving different species, to be carried out (Adams, 1990; Birge et al., 1985). Fish bioassays focus primarily on early life stages (embryonic to larval); hence, applying the results of these short, controlled bioassays to a field situation or to other life stages is difficult. Life cycle tests are thought by many to be the most sensitive as they allow the monitoring of organisms at all life stages, from embryonic development of young to gamete production in adult fish (Donaldson, 1990).

Reproduction is particularly sensitive to stress and can be influenced by environmental factors such as temperature, photoperiod, turbidity, salinity,

dissolved oxygen and substrate availability (Donaldson, 1990). In their comparison of studies encompassing twenty-eight chemicals and seven fish species, Mayer et al. (1986) determined that reproductive endpoints were sensitive to chemical stress, particularly when compared to growth. Suter et al. (1987) examined studies that encompassed ninety-three chemicals and eighteen species, and determined fecundity to be more sensitive to chemical stress than all early life-stage endpoints. Lanno and Dixon (1994), determined adult maturation and gamete production to be more sensitive to waterborne thiocyanate than larval growth and survival. Similarly, Buckler et al. (1981), saw potential negative impacts of kepone on reproduction at concentrations one tenth those which affected growth. Donaldson (1990) argues for the inclusion of reproductive endpoints in chemical assessment, stating that although reduction in survival and growth can indirectly lead to impaired reproduction, there is no guarantee that adequate survival and growth ensures that fish will reproduce.

Life cycle tests can be difficult to carry out as many fish spawn poorly in captivity, test length can vary depending on the life cycle of the fish (years in some cases), and they are costly. Fathead minnows (*Pimephales promelas*) are particularly suited to life cycle tests as they are relatively short-lived, maturing within 6 to 8 months in a laboratory setting, will spawn in captivity (Environment Canada, 1992), and are widespread, making them an ecologically relevant species in many areas in North America (Nelson and Paetz, 1992). Fathead minnow reproduction involves relatively complex behaviour including parental care. Males establish and defend territories under structures such as

submerged logs or, in a laboratory situation, clay or PVC spawning tiles. When a female spawns with a male, she attaches her egg to the underside of the spawning structure at which point the male fertilizes the egg. The male remains with the nest, cleaning and aerating the eggs until they hatch (~4 to 5 days at 25° C). The impact of a contaminant on fathead minnow reproduction could occur directly at the physiological level, impacting processes such as sexual maturation, or gamete production and viability. Alternately, contaminants could affect reproductive success indirectly through alterations in behaviour.

I performed a partial life cycle study with fathead minnows to examine the impact of various SynCrude waters on growth and reproduction of adult fish and growth and survival of their offspring. The test is referred to as a partial life cycle test because fish are introduced into the treatments post-hatch, instead of at the egg stage, and sacrificed after spawning. This study is intended to provide a bioassay possibly more sensitive to stress than a short-term growth bioassay, allow for the study of reproduction in a controlled environment, and support field observations presented in Chapter 4. The endpoints selected are outlined in Donaldson (1990) and include body condition, timing of reproduction, number of clutches produced and eggs/clutch, percent hatch of offspring, and survival and growth of offspring. Body condition was measured to determine the "state of well-being" of the adult fish before spawning (Wootton, 1990). All other endpoints have been affected by contaminants in other life-cycle studies (see Donaldson, 1990), and can result in reduced reproductive output.

The two main factors that determine the relative toxicity of the water in the SynCrude constructed ponds are the age of the tailings, which has a negative relationship with toxicity, and the type of water used to cap the tailings (TPW or natural water) (Verbeek, 1994; FTFC, 1995). Water was collected from sites of different ages (8 years and 4 years) with either a) natural water overlying Mature Fine Tailings (MFT) or b) Tailings Pond Water (TPW) overlying MFT.

Methods

This study was carried out in two locations. The bioassay was initiated in October, 1996, at the University of Alberta, Edmonton. Fish were housed in the aquaria facilities at the University of Alberta until May 8, 1997, at which point fish were transported to the SynCrude Canada Ltd., Mildred Lake site, located ~ 40 km north of Fort MacMurray. The rest of the bioassay was carried out at the Mildred Lake site.

Water

Dechlorinated water from the University of Alberta was used as the control water. Water from sites of two different ages (4 or 8 years) and two different capping waters (natural water or SynCrude processed water) were selected for bioassay treatments. As MFT associated water and TPW is relatively saline, a salt control, reflecting the levels of NaCl, Na_2SO_4 and $NaHCO_3^-$ found in DP, was included. In total, six treatments were established; 1) a dechlorinated control, 2) a salt control, 3) a site aged 8 years capped with clean water, 4) a site ages 8 years capped with TPW water, 5) a site aged 4 years capped with clean water, and 6) a site aged 4 years which only contained TPW water. Treatments are described in Table 3.1.

Water from SynCrude Canada Ltd. Mildred Lake site was shipped to the University of Alberta monthly in 20 liter polypropylene containers and stored at 4° C until used. In accordance with Environment Canada guidelines (1992), conductivity and pH were measured weekly. During the second part of the bioassay, dechlorinated water from the University of Alberta was shipped to

SynCrude Canada Ltd. bi-weekly, as needed, throughout the experiment. Once at Mildred Lake Plant site, one 20 liter container of dechlorinated water from each shipment was sampled for pH and conductivity whereas the SynCrude Canada Ltd. waters were monitored weekly at the site, during collection. Water samples of sites used in this study were obtained and characterized by SynCrude Canada Ltd. employees (Appendix A).

Fish

Fish used in this study were purchased as eggs from Aquatic Research Organisms, Hampton, New Hampshire. Eggs were shipped to the University of Alberta and hatched in dechlorinated water following the methods outlined in Chapter 2. Four clutches of eggs were sent, and hatched larvae combined, ensuring that not all fish used were full siblings. When 24 hours of age, fish were introduced into 40 liter treatment tanks. Two tanks per treatment, each containing 55 fish, were initially established.

Fish were maintained at 25° C and a photoperiod of 16 hour light : 8 hour dark. They were fed standardized quantities of live, newly hatched *Artemia* and Tetra fish flakes *ad libitum*. Feeding was standardized from tank to tank by administering *Artemia* with a Gilson pipette. Fish flakes were not weighed, but ~ 2.5 cc were fed daily. Tanks were cleaned by siphoning out 50% of the water twice a week. At age 7 months, 60 fish from each treatment were randomly selected from both tanks for each treatment, and moved to SynCrude Canada Ltd. Mildred Lake Site, north of Fort MacMurray. There the group was again divided in half and introduced into 60 liter tanks resulting in a density of

one fish/2 liters water. Photoperiod, feeding and cleaning regimes were maintained as previously described (Chapter 2). Temperature in the environmental room was monitored with minimum/maximum thermometers. Thermometers were read every morning at 07:30.

At 8 months (1 month after fish were moved to the Mildred Lake Site), reproduction trials began. Six 18 liter aquaria were established, randomly assigned one of the rearing treatments, and filled with 12 liter of treatment water. Only males displaying secondary sexual characteristics (dark banding, spongy dorsal pad and tubercles) and gravid females (distended abdomen, obvious ovipositor) were used. A male and female from each treatment were weighed, measured (total length) and introduced into the tank containing their rearing water. No adult fish was used more than once. A new set of six tanks, one tank per treatment, were set up at approximately 1 week intervals for 7 weeks. Adult pairs were fed 1 ml of live *Artemia* slurry twice daily. Tanks contained a couple of aquatic plants to provide structure and a spawning tile under which males established and defended territories.

Tiles were checked every morning for egg clutches. When found, the tile with eggs was replaced. Eggs were counted and placed, still attached to the tile, into aquaria with natal treatment water and vigorously aerated. Clutches were checked daily and dead or fungused eggs were removed. Eggs generally began to hatch by 4 to 5 days and once less than ten eggs remained, larvae were subsampled for growth and survival analysis. Growth tests were carried out following the protocol published by Environment Canada (1992), and outlined in

the methods section of Chapter 2, with the exception that larvae spawned in a treatment water were maintained and allowed to grow only in their natal water. Fifty larvae per clutch were introduced into 800 ml beakers, resulting in five beakers/treatment each containing ten fish. Larvae were allowed to grow for 7 days, after which point they were killed, dried and weighed. The remaining larvae of the clutch were killed and preserved in 10% buffered formalin until they could be counted to determine percent hatch.

Due to space, tank and time constraints, each adult pair was allowed to provide three clutches of eggs. Pairs that had not spawned after 10 days were exposed to a second male from the same treatment for a few hours between 1:00 and 5:00 in the afternoon. The second male was fin clipped (caudal) and placed into the tank containing the spawning pair. The original male often became aggressive after the introduction and often defended a territory under the spawning tile. This procedure was carried out with a total of eight pairs; one control, one DP, one TP5 and five TPW pairs. Eggs were found 2 to 3 days after exposure to the second male in 63% of the cases. Three of the TPW pairs did not spawn even after exposure to a second male. If a pair did not provide one clutch of eggs within 1 month of introduction, they were removed.

Statistics

Statistical analysis used are described in Zar (1984). All data were analyzed on SPSS (version 6.0 and 7.5). Data were first tested for normality using the Kolmogorov-Smirnov test. Results of all tests were considered significant if $p < 0.05$.

Adults

Body condition ($K=W/L^3$) of the adult fish used in this study was determined from weight and lengths attained at the time of introduction into spawning tanks, and analyzed with a one-way ANOVA. Post-hoc differences were identified with a Tukey's test for honest significant difference.

Delays in spawning (number of days it took each pair to produce its first clutch) were also analyzed with a one-way ANOVA and post-hoc differences examined with a Tukey's test for honest significant differences. Only pairs that actually spawned were included in the analysis of number of days to first clutch.

As previously stated, each fish pair was allowed to provide a maximum of three clutches, and data documenting the number of clutches/pair (nesting success) was not normal. Therefore, the data were analyzed with the nonparametric log-likelihood ratio (G-test). Nesting success data were scored with pairs providing three clutches being assigned "1" and pairs providing less than three clutches assigned "0".

Clutch size and hatch rate

Clutch size (number of eggs) was analyzed using a repeated measures ANOVA, as three clutches were included per pair, and differences identified using a Tukey's test for honest significant difference.

The percentage of eggs which hatched per clutch was determined for a subsample of clutches by counting the preserved larvae and then comparing the number of larvae to the initial number of eggs. In some instances, the number of larvae counted was more than the number of eggs initially documented. As few

clutches were smaller than 150 eggs, clutches with up to 125 % hatch were included in the analysis. Data were normalized with an arcsine transformation, and results analyzed with a one-way ANOVA. Post-hoc differences were examined using a Tukey's test of honest significant difference.

The number of days it took eggs to hatch after being laid (incubation period) was also assessed for each clutch of eggs. Temperature can affect the incubation period of eggs (Diana, 1995); hence, incubation rate was standardized for temperature by multiplying mean temperature by the number of days it took each clutch to hatch (Bengtsson, 1980), then comparing incubation period of eggs in each treatment by nesting each fish pair within treatment and clutch within pair. Temperatures were within the limits of fathead tolerance for most of the summer, but there was a length of time where temperatures were very high (>29^{o} C) and fungus became a problem. All unhatched egg clutches and larvae that were in the laboratory at that point are excluded from analysis.

Larvae

Larvae were killed and weighed as described in Chapter 2. Temperature can impact larval survival and growth (Diana, 1995). Therefore, tests for interactions between temperature and treatment for survival and growth were also carried out. When non-significant interaction terms were obtained, results of individual beakers were nested within water, pair and clutch, and analyzed using temperature as a co-variate on the GLM feature of SPSS 7.0. Post-hoc differences were examined using a Tukey's test of honest significant difference.

Results

Adults

Body condition of adult females used in this bioassay did not significantly differ among treatments (Table 3.2; $F_{5,42}$=0.55, p=0.741). There was a significant difference in males, with condition of TPW males being significantly lower than Control, Salt and TP3 males (Table 3.2; $F_{5,42}$=4.90, p<0.001). Lengths, weights and body condition of adults are presented in Table 3.2.

Tukey's test identified TPW fish as taking significantly longer to produce their first clutch of eggs compared to all other sites (Fig. 3.1; $F_{5,37}$=2.59, p=0.042). All of the pairs used in this study laid three clutches of eggs with the exception of one salt pair which laid 2 clutches and three TPW pairs who were not successful in providing any clutches. When analyzed statistically, TPW pairs produced significantly fewer (~50% fewer) clutches compared to pairs in all other treatments (Fig. 3.2; $G_{5,1}$=12.15, p=0.033).

Clutch size and hatch rate

There was no difference among treatments in the total number of eggs per clutch (Fig. 3.3; $F_{5,111}$=1.00, p=0.436). There was also no statistically significant treatment effect in the percentage of eggs which hatched from each clutch (fig 3.4; $F_{5,41}$=0.81, p=0.552).

As temperature increased, incubation period decreased, but this difference was not significant across treatments (Fig. 3.5; $F_{5,111}$=0.82, p=0.543).

Larvae

There was a significant interaction between temperature and treatment for larval survival to 7 days ($F_{5,1}$= 4.40, $p<0.001$). In most cases, there was a slight negative relationship between survival and temperature with the exception of TPW fish which seemed to have a slightly positive relationship (Fig. 3.6).

There was no significant interaction between temperature and treatment for growth of larval fish to 7 days ($F_{5,1}$=1.93, p=0.088); hence, a nested analysis of covariance (ANCOVA) was run. The results of that test yielded significant differences between treatments, and Tukey's test for honest significant difference identified control fish as weighing significantly less than TP3, DP, TP5 and TPW fish (Fig. 3.7; $F_{5,423}$=75.36, $p<0.001$). Responses among the remaining treatments were graded, as salt treatment fish weighed significantly less than TP5 and TPW, and DP and TP3 fish weighed significantly less than TPW fish.

Discussion

In toxicological testing, the importance of the duration of exposure, and the use of multiple endpoints and generations are recognized by many scientists (Lander et al., 1985; Cairns et al., 1996). This bioassay involved the exposure of fish from hatch to sexual maturity, thereby covering a significant portion of the life of the fish, and included assessment of offspring. This study determined that SynCrude waste water does affect fathead minnow reproduction and growth of resulting larvae. The impact on adult body condition and reproduction was negative, with TPW males having a significantly lower body condition and TPW fish taking longer to initiate spawning and producing about half as many clutches as other treatments. Effects were also observed in the offspring as growth was stimulated in offspring whose parents had been reared in SynCrude processed waste water.

The failure of pairs in TPW to reproduce as did pairs in other treatments, could be linked to adult male body condition. Body condition was significantly lower in TPW males compared to Control, Salt and TP3 males. Weights and lengths of adult fish used in this bioassay were similar to or greater than those published in other studies (Table 3.2; Lanno and Dixon, 1994, Suedel et al., 1997). Abiotic factors (i.e. density, food, temperature) with the exception of those associated with MFT or TPW (i.e. conductivity, pH) were also constant among tanks; hence, the lower body condition displayed by the TPW males may indicates a physiological, and/or behavioral effect of the water.

Body condition (K) is used to define the "state of well-being" of a fish (Wootton, 1990). Fish with higher K values weigh more for their body length. Goede and Barton (1990) cite studies in which a decrease in body condition was observed in fish exposed to high rearing densities and adverse environmental conditions. TPW males were of slightly smaller length than other males, but were noticeably lighter, and as a result had a significantly lower body condition (Table 3.2). Differences in body condition can result from a number of factors including gonadal development (Wootton, 1990). Male gonad weights of natural fish populations in the Fort MacMurray area ranged from 0.01 to 0.32 g (Chapter 4). It is possible that the gonads of TPW males were under-developed, resulting in lighter fish. There is evidence that exposure to contaminants can impede testicular development. For example, the testis of Atlantic cod (*Gadus morhua*) exposed to the water soluble fraction of crude oil exhibited histopathological changes. These changes included delayed gametogenesis and the premature development of multinucleated cells in the seminiferous tubules which could impair semen concentration (Khan and Kiceniuk, 1984). Multinucleated cells are thought to be involved in the removal of debris and normally occur after spermiation (Khan and Kiceniuk, 1984). Payne et al. (1978) observed smaller testes in cunner (*Tautogolabrus adspersus*), after a 6 month exposure to oil, but did not observe histopathological changes. Pesticide exposure impaired testicular recrudescence in the freshwater murrel (*Channa punctatus*), delaying the development of spermatids (Saxena and Mani, 1987), and exposure to crude petroleum inhibited testicular development in landlocked salmon (*Salmo salar*)

(Truscott et al., 1983). Finally, there is evidence that the body to gonad relationship for male white suckers (*Catostomus commersoni*) exposed to bleached kraft mill effluent may be altered (Gagnon et al., 1995). There are also studies document a reduction/disruption of female gonads and reproductive output (see Donaldson, 1990). While possible effects of SynCrude waste water cannot be eliminated without further study, all females in my experiment appeared gravid and all appeared to be in breeding condition (i.e. obvious ovipositor). Unfortunately, adult fish were not killed immediately after spawning for the third time; hence, accurate GSI data are not available for male or female fish used in this experiment.

TPW fish also took longer to begin spawning than pairs in all other treatment tanks. This could be related to the lower body condition of the males as it is possible that TPW water may have delayed male maturation. There is evidence of delayed maturation in stressed fish. Bengtsson (1980) did not measure specific behaviours or histopathological endpoints in his assessment of the impacts of PCB on the European minnow (*Phoxinus phoxinus*), but observed a delay in the initiation of spawning in fish exposed to "medium" and "high" levels of PCB (Chlophen A50). At lower exposure levels, Lanno and Dixon (1994) observed a significant spawning delay in fathead minnows exposed to waterborne thiocyanate, and spawning was inhibited after fathead minnows underwent prolonged exposure to 0.68 mg/liter carbaryl (Carlson, 1972). There is also evidence of delayed spawning in field populations of fathead minnows exposed to SynCrude waste water, as discussed in Chapter 4.

Finally, TPW fish pairs produced fewer clutches than fish in the other treatments. Although delayed maturation of males could certainly have affected that result, there could also be a behavioural component as well. Fathead males must establish and defend territories in order to successfully attract a mate. In his review of behavioural stress, Beitinger (1990) cites a number of examples where aggressive and territorial behaviours were depressed in fish during exposure to toxicants. Those include a decrease in maintenance of territories in young Atlantic salmon exposed to fenitrothion, and decreased aggression in bluegill sunfish (*Lepomis macrochirus*), with the exception of the dominant fish, during exposure to a mixture of cadmium and zinc.

Behavioural depression can be indicative of hypoactivity syndrome where narcosis or narcosis-like responses are elicited from fish exposed to chemicals which depress nervous system activity (Drummond and Russom, 1990). A dramatic response to outside stimuli (hyperactivity) can also disrupt normal interactions between individuals, and is thought to indicate disruption of a metabolic activity or function (Drummond and Russom, 1990). Exposure to carboxylic acids resulted in a hyperactive response in juvenile fathead minnows (Drummond and Russom, 1990). Naphthenic acids are primarily mixtures of mono- and poly-cycloalkane carboxylic acids (AEP, 1996). Although the carboxylic acids used in Drummond and Russom's (1990) study did not include naphthenic acids, it is possible that exposure to similar compounds could elicit similar responses (Drummond and Russom, 1990).

Disruption in behaviours associated with territoriality and aggression can occur at very low levels of a toxicant (see Beltinger 1990; Pyron and Beitinger, 1989), whereas disruptions in spawning behaviour generally occur at close to lethal levels of a toxicant (Lanno and Dixon, 1994; Beltinger 1990). In this study, there was a 50% reduction in spawning of TPW fish, but mortality of adults and larval fish was not affected (current results and Chapter 2). Additionally, males in the TPW pairs who did not spawn a) did not respond to exposure to a second male, unlike males in other treatments who became very aggressive and b) were not often observed under the spawning substrate (pers. obs.). These observations could suggest a depression in territorial behaviours in males.

There was no treatment effect in the number of eggs laid per clutch but high within-treatment variation may have contributed to the lack of statistically significant differences. For example, Suedel et al. (1997) observed a three-fold difference in clutch size among fathead minnows exposed to PCB's, but this finding was not statistically significant due to high within-treatment variability. Fathead minnows have been documented to lay 9 to 1,136 eggs per clutch (Gale and Buynak, 1982). The range of clutch sizes in this study was 15 to 467 eggs. Analysis of incubation period, after transforming the data by multiplying the mean temperature by the number of days to hatch as described in Bengtsson (1980), indicated that this was not significantly affected by the SynCrude waste waters used in this study.

Survival of larvae did not seem to be affected by the SynCrude waters although TPW fish responded differently to temperature than all other treatments

(Fig. 3.6). The TPW fish seem to have increased survival with increase in temperature whereas all other treatments had a negative relationship. There was a significant impact of water type on growth, with TPW fish growing larger than all other treatments with the exception of TP5. All fish exposed to SynCrude water (TP3, DP, TP5 and TPW) grew bigger than Control and Salt treatments. The possibility of prehatch selection, or that treatment waters killed the weaker fry, is not an issue as the percent of larvae which successfully hatched did not differ among treatments. Hence, differences in growth were not due to the selection of robust (larger) fry at hatch.

Growth stimulation, as a result of chemical exposure, has been reported in a number of studies. Beitinger and McCauley (1990) refer to a number of examples in their review. The authors refer to this phenomenon as homoresis which they define as "an enhancement or stimulation of a physiological process by exposure to a low concentration of a toxicant" (Beitinger and McCauley, 1990). European minnows exposed to PCB's had significantly higher growth rates at the highest PCB exposure level whereas rates at medium and low exposure levels were higher than the controls, but not significantly so (Bengtsson, 1980). Pickering et al. (1996) found sublethal concentrations of NaCl stimulated growth of fathead minnows. Larval fathead minnows that developed embryonically in uncontaminated water grew larger than control fish at 13 μg/liter Mirex. During the Mirex life cycle test, the eggs of fish reared in 7 μg/liter had increased hatching success and the larval fish experienced stimulated growth (Buckler et al., 1981). Increased sensitivity to the effect of a

toxicant has been exhibited in fry that developed embryologically in a test solution or whose parents had been exposed (Lander et al., 1984; Lanno and Dixon, 1994), and may account for the non-significant growth trends reported in Chapter 2 where larvae were maintained in control water before exposure to the SynCrude waste water. In those bioassays, the trend towards increased growth rates in fish exposed to SynCrude waters was evident, but not significant.

Based on the results of this study, reproduction of fathead minnows in sites containing SynCrude waste water aged 8 years as well as sites capped by MFT for 4 years, should not be negatively affected by water quality. There are, however, factors which still require investigation. These include determining the mechanisms behind the reduction in reproduction of TPW fish and the increased growth exhibited by the larvae spawned in all SynCrude waste waters. The fact that two important endpoints (growth and reproduction) have been affected to varying degrees by SynCrude waters, suggests the possibility that tailings water may have endocrine effects (Pickering, 1993). Pollutants which affect endocrine systems, often impact levels of catecholamine and corticosteroid hormones (Donaldson et al., 1984). Increases in the concentration of these two classes of hormones, referred to as the primary stress response, then affect numerous physiological and biochemical changes (Donaldson et al., 1984). Disruptions in normal endocrine function have been associated with reductions in many endpoints including disease resistance, reproduction success, and larval recruitment (Thomas, 1990).

The release and regulation of circulating sex steroids are controlled by the hypothalamus-pituitary-interrenal axis (Donaldson et al., 1984; Donaldson, 1990). Both estrogens and androgens are necessary for gonadal development and sexual maturation, as well as general growth and development (Weatherley and Gill, 1987; Thomas, 1990; Pickering, 1993). Increased levels of sex steroids can have an anabolic impact on growth (Weatherley and Gill, 1987). In his review, Thomas (1990) discusses numerous studies where contaminants result in increased/decreased levels of sex steroids circulating in the blood. In fathead minnow, the emergence of tubercles are known to be androgen dependent (see Smith, 1978). All males had some evidence of tubercles when selected for the bioassay, implying that androgen levels were somewhat elevated in selected males, but the tubercles had regressed in the male minnows that required exposure to an additional male to stimulate spawning. After exposure, however, tubercles re-emerged within 36 hours, and spawning was initiated in all but TPW males (pers. obs.).

Prostaglandin stimulates female sexual behaviour in many teleost fish, and its detection induced fathead minnow males to commence courtship behaviours (Cole and Smith, 1987). Female fathead minnows in TPW did not appear to be negatively affected by their treatment water; however, additional data including behavioural observation are required to be certain. Male detection of prostaglandin could also have been impaired, which may account for the failure of about half of the TPW males to reproduce.

Although the life cycle test is the most thorough laboratory analysis, it is still unable to predict what happens in a field situation. Changes in temperature, dissolved oxygen, food availability, and parasites have been shown to moderate or exaggerate the effects of toxicants on aquatic biota (Sprauge, 1985). A fish's ability to swim (Beitinger and McCauley, 1990) or detect pheromones and food odour (Hara, 1992) could also be impacted by contaminants, but these effects may be less apparent in a laboratory situation where fish are in small tanks which may increase encounter rates with food and interaction with conspecifics. Field validation of laboratory results are ideal. Some growth and reproductive endpoints were monitored in populations on and off the SynCrude site, and will be discussed in the next chapter.

Table 3.1 : Waters used in partial life cycle experiment. SynCrude sites containing material of 2 different ages and 2 capping waters were selected for study. A salt control was included in an attempt to control for the salinity of the water associated with tailings.

Water	Source	Age of Waste Material in Pond
Control (C)	dechlorinated; University of Alberta	none present
Salt Control (Salt)	mixture of dechlorinated water and 70.2 ppm NaCl, 147.6 ppm Na_2SO_4, and 420 ppm $NaHCO_3^-$	none present
Test Pit 3 (TP3)	pit containing MFT capped with natural water	8 years
Demonstration Pond (DP)	pond containing MFT capped with natural water	4 years
Test Pit 5 (TP5)	pit containing MFT capped with TPW	8 years
Tailings Pond Water (TPW)	pond containing TPW	4 years

Table 3.2 Mean length, weight and body condition of adult males and female fish used in this study. Treatments included control waters (C and Salt), water from sites containing MFT capped with natural water (TP3 and DP), water from sites containing MFT and capped with TPW (TP5) and one site which only contains TPW. Standard error are included in parenthesis. Sample size (n) for males and females in each treatment is 8.

Site	Males			Females		
	Length (mm)	Weight (g)	Body Condition	Length (mm)	Weight (g)	Body Condition
C	64.88 (1.82)	3.61 (0.21)	1.32 (0.04)	54.5 (0.85)	1.68 (0.09)	1.04 (0.04)
Salt	63.25 (1.29)	3.18 (0.21)	1.25 (0.03)	52.62 (1.39)	1.70 (0.07)	1.20 (0.11)
TP3	64.63 (1.84)	3.44 (0.29)	1.25 (0.04)	53.13 (0.97)	1.66 (0.08)	1.11 (0.06)
DP	64.63 (1.00)	3.15 (0.22)	1.16 (0.05)	51.75 (1.50)	1.49 (0.17)	1.06 (0.10)
TP5	64.38 (1.84)	3.16 (0.21)	1.19 (0.06)	52.75 (0.70)	1.56 (0.08)	1.06 (0.03)
TPW	62.75 (1.50)	2.56 (0.22)	1.02 (0.05)	56.13 (1.52)	1.98 (0.14)	1.14 (0.11)

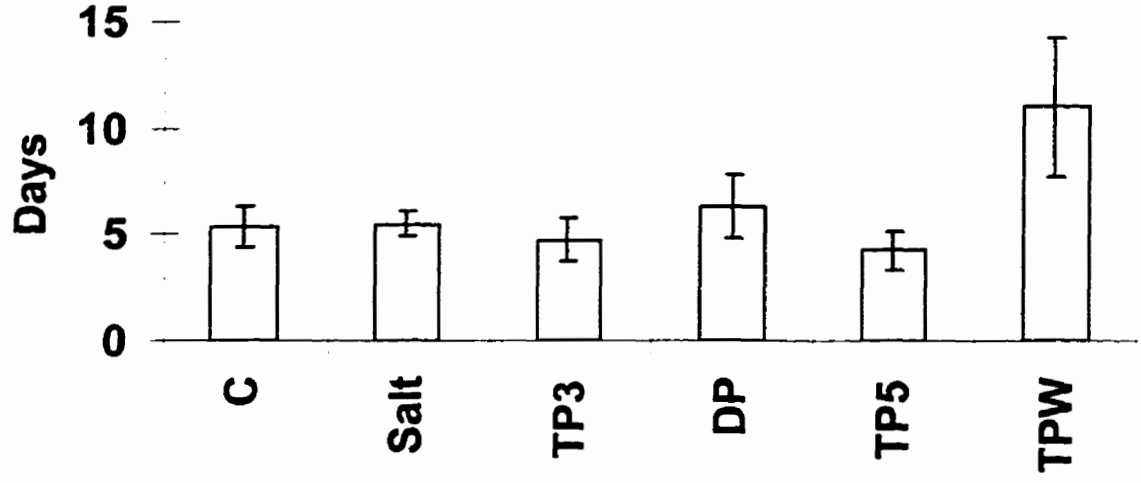

Figure 3.1 The number of days (mean + standard error), after the introduction of a pair into a tank, until the first clutch of eggs was laid. Treatments included control waters (C and Salt), water from sites containing MFT capped with natural water (TP3 and DP), water from sites containing MFT and capped with TPW (TP5) and one site which only contained TPW. Four TPW pairs that did not lay any eggs were excluded from the analysis. Pairs were allowed 1 month before being removed. n=8 for all sites except TPW where n=4.

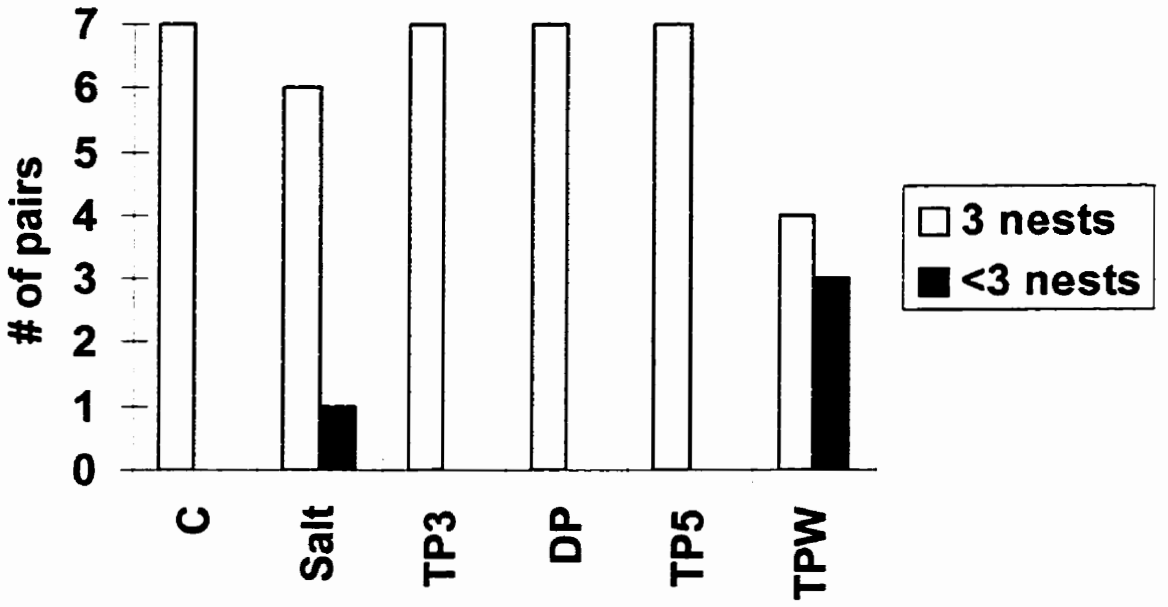

Figure 3.2 The number of pairs that produced 3 clutches (maximum permitted in the study) compared to pairs which produced fewer than 3 clutches. Treatments included control waters (C and Salt), water from sites containing MFT capped with natural water (TP3 and DP), water from sites containing MFT and capped with TPW (TP5) and one site which only contains TPW. n=7 for all treatments.

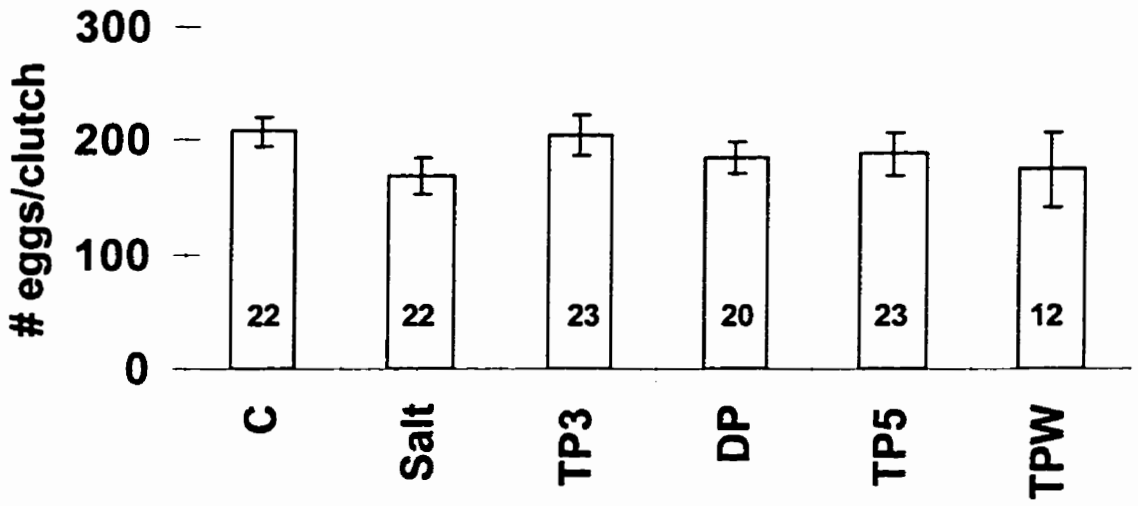

Figure 3.3 Number of eggs laid per clutch (mean + standard error). Treatments included control waters (C and Salt), water from sites containing MFT capped with natural water (TP3 and DP), water from sites containing MFT and capped with TPW (TP5) and one site which only contains TPW. Values in bars represent the number of clutches (n).

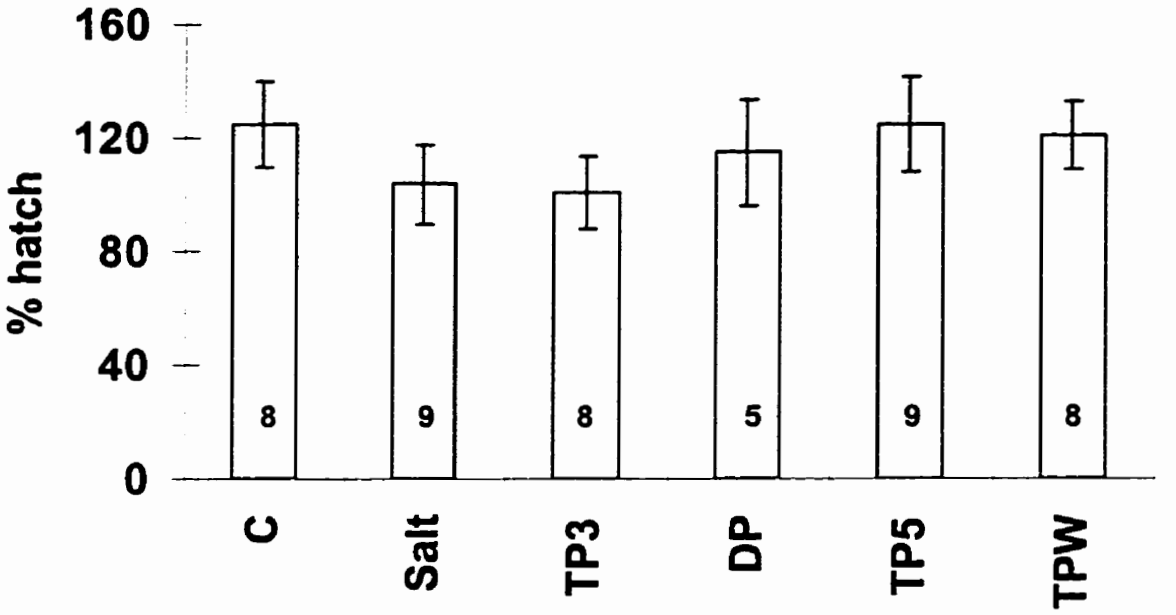

Figure 3.4 Percentage of eggs (mean + standard error) which successfully hatched. Treatments included control waters (C and Salt), water from sites containing MFT capped with natural water (TP3 and DP), water from sites containing MFT and capped with TPW (TP5) and one site which contains only TPW. Values in bars represent the number of clutches analyzed in each treatment (n).

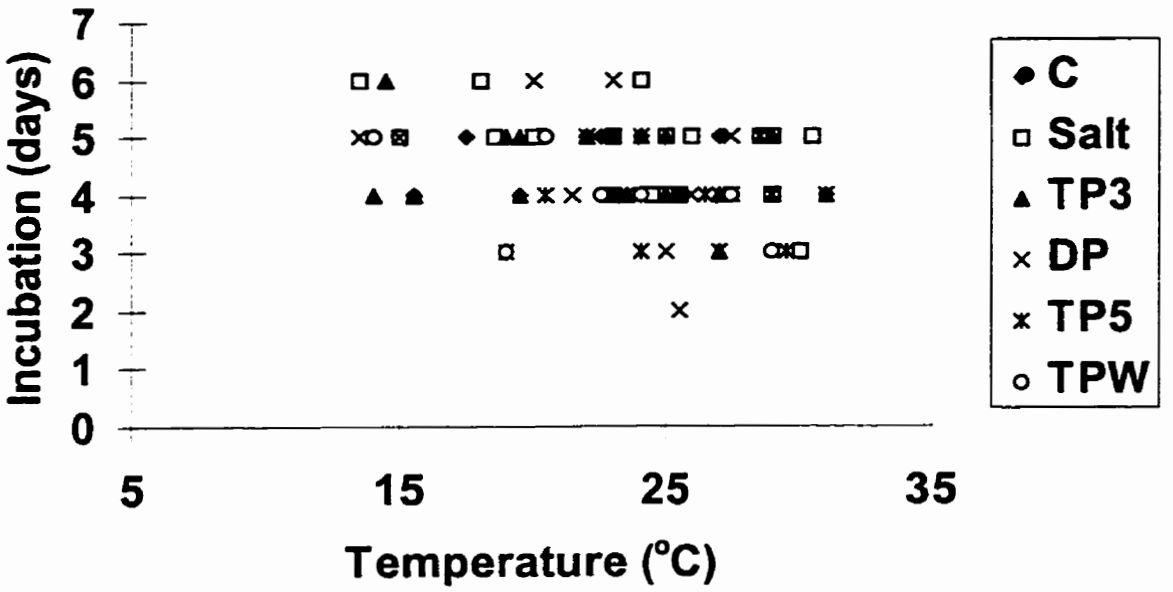

Figure 3.5 Relationship between length of incubation period of egg clutches (# of days from being laid to hatching) and temperature. Treatments included control waters (C and Salt), water from sites containing MFT capped with natural water (TP3 and DP), water from sites containing MFT and capped with TPW (TP5) and one site which only contains TPW.

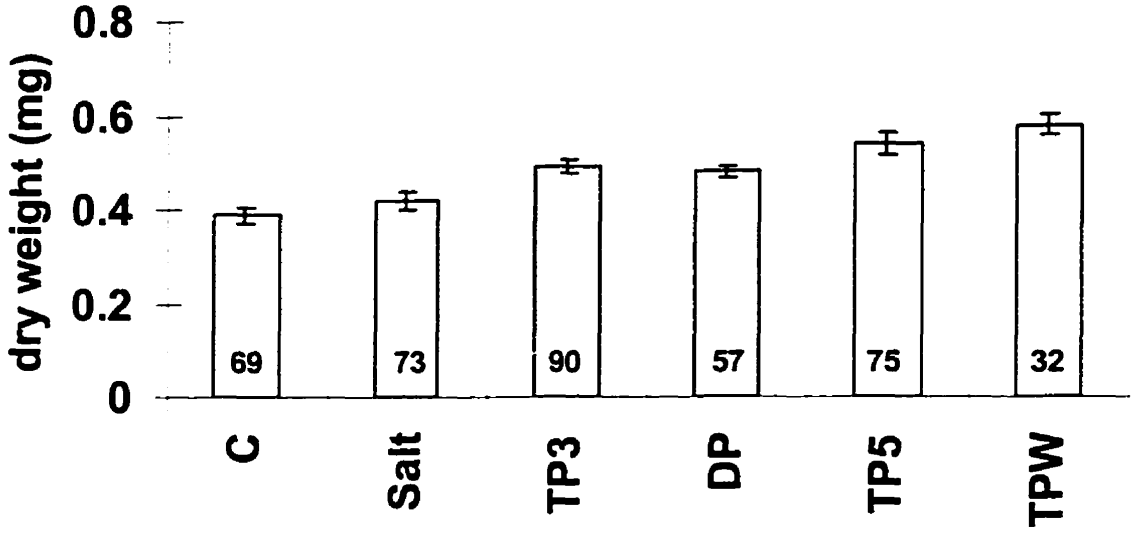

Figure 3.7 Dry weights (mean + standard error) of offspring to 7 days. Weight of all fish in each vessel were pooled and then divided by the number of fish in the vessel. Treatments included control waters (C and Salt), water from sites containing MFT capped with natural water (TP3 and DP), water from sites containing MFT and capped with TPW (TP5) and one site which only contains TPW. The number in the bars represent the total number of vessels for each treatment.

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Chapter 4

Growth and reproduction of fathead minnows in ponds containing mature fine tailings (MFT).

Introduction

The Athabasca oilsand deposit, located in Northern Alberta, is currently mined by SynCrude Canada Ltd. and Suncor Inc. Both companies use a process referred to as the Clark Hot Water Extraction (CHWE) method to extract bitumen, a crude petroleum product, from the oilsand. The aqueous by-products of CHWE partition into three main components; large, primarily inert particles such as sand and clay, a saline water layer containing water soluble organic and inorganic compounds called Tailings Pond Water (TPW) and a mixture of water, ultra-fine particles, residual bitumen, heavy metals, and insoluble organic carbons referred to as fine tailings (FTFC, 1995). Over time, fine tailings consolidate by the expression of water; once fine tailings are 55% solids by weight, they are referred to as Mature Fine Tailings (MFT; FTFC, 1995). Millions of cubic meters of MFT are currently awaiting safe containment and, as plant production increases, their disposal is becoming a pressing issue (FTFC, 1995). One disposal method proposed by SynCrude involves incorporating MFT into wet landscapes.

The wet landscape option involves constructing large lake basins in geotechnically stable parts of the mine scheduled for reclamation, filling the basins with MFT then capping the MFT with clean water from a natural source (FTFC, 1995). The clean water cap would act as a barrier between terrestrial fauna and the MFT, and possibly serve as habitat for aquatic organisms. In

1989 and 1993, SynCrude constructed a number of prototype ponds to test the viability of water capping and, while pumping in the clean water cap, introduced fathead minnows (*Pimephales promelas*) into their sites.

Undiluted fine tailings are acutely toxic to fish (Verbeek, 1994; FTFC, 1995). Toxicity Identification Evaluation (TIE) reveal that the acute toxicity of both TPW and interstitial MFT water can be eliminated by removal of fractions which contain carboxylic acids, the majority of which have been identified as naturally occurring naphthenic acids (FTFC, 1995). As tailings age, their acute toxicity decreases, likely due to microbial degradation of the naphthenic acids (FTFC, 1995). Fathead minnows have survived in SynCrude water-capped sites since 1989, but while laboratory bioassays and fish health tests have been carried out, there had been no studies of the potential chronic effects of exposure to MFT to fathead minnows living in the ponds at the time this study was initiated.

Laboratory bioassays are good tools for determining potential chronic effects as they are highly controlled and repeatable and allow the researcher to eliminate confounding factors. The results of laboratory bioassays, however, cannot be directly extrapolated to a field situation. Many factors influence the toxicity of contaminants on aquatic organisms. Biotic factors which have been shown to increase the sensitivity of organisms to contaminants include parasites, nutrition and disease. Sprague (1985) outlines a number of papers in which fish experiencing high levels of parasitism, poor nutrition and disease were impacted by lower contaminant levels than their healthy counterparts. Goldfish

(*Carassius auratus)* heavily parasitzed by a skin fluke were affected by lower levels of NaCl (Aldeman and Smith, 1976). Dixon and Hilton (1981) determined that rainbow trout (*Oncorhynchus mykiss*) fed diets higher in carbohydrates became less tolerant to copper. As the level of dietary carbohydrate increased, the liver contained higher levels of glycogen relative to protein and became less effective in detoxifying copper.

Abiotic factors which can impact toxicity include sorption of contaminants to particulates, dissolved oxygen, temperature and salinity (Sprague, 1985). For example, fathead minnows tolerated higher levels of Hydrothol-191, a substance used to control macrophyte and algae growth, at 25° C than at 15° C (Keller et al., 1988). During a study in which oxygen levels were cycled to low levels (2 ppm) for 8 hours then increased to 95% saturation, the toxicity of naphthenic acids to bluegill sunfish (*Lepomis macrochirus*) increased by a factor of 2.8 at the lowest oxygen levels (Cairns and Scheier, 1958). Naphthenic acids were twice as toxic to snails at 20° C than at 30° C (see Sprague, 1985). The influences of abiotic conditions on naphthenic acids are not well understood (AEP, 1996). It is known that they are relatively non-volatile and are not likely to disperse into the atmosphere (AEP, 1996). The potential for photodegradation is not well documented (AEP, 1996) but would be reduced with increasing turbidity. There is no information as to the potential of naphthenic acids to bind to suspended matter (AEP, 1996).

The potential toxicity of a material can also reduce the tolerance of aquatic organisms to abiotic factors such as extremes in temperature or low

levels of dissolved oxygen. In their review, Beitinger and McCauley (1990) cite numerous examples in which temperature tolerance, resistance to hypoxia and resistance to salinity were impacted by the presence of contaminants. Fathead minnows exposed to the synthetic pyrenoid, cyfluthrin, a compound used in insecticides, were less able to tolerate high and low temperature extremes (Heath et al., 1994). The toxicity of copper to larval fathead minnows was found to be affected by both pH and dissolved organic carbon (Welsh et al., 1993). Pulpwood fiber was found to decrease the ability of fathead minnows to absorb oxygen from the water, thereby reducing their ability to function in high temperatures and low dissolved oxygen conditions (MacLeod and Smith, 1966).

Assessing the health and viability of natural populations experiencing chronic levels of pollutants can be difficult. Confounding factors such as exploitation (Munkittrick and Dixon, 1989), varying abiotic conditions (Sprague 1985), interactions with other species and the environment (DeAnglies et al, 1990), and indirect effects of contaminants (i.e., no effect on monitored species but reduction in forage base; Brazner and Kline, 1990) may impede detection of chronic effects. Determining which endpoints to measure is also important in monitoring field populations potentially impacted by pollutants. Munkittrick and Dixon (1989) proposed age, growth, condition, age at maturity, egg size and population size as good indicators of fish population characteristics. A short study carried out in 1995 determined that body condition, mean age within a population, and possibly, timing of the initiation of spawning differed between fathead minnows in a SynCrude water capped tailings site and a constructed site

which did not contain tailings (van den Heuvel and Dixon, 1998). Comparing individuals from a contaminated site and an uncontaminated reference site is one approach used to evaluate the impact of contaminants of populations (Donaldson, 1990). In this study, I compare size and reproduction of fish sampled from constructed sites which contain MFT, constructed sites which do not contain MFT and natural reference sites.

At the beginning of the open water season in northern Alberta, fathead minnow populations are generally comprised of large adult males and females, which will spawn during the summer, and smaller juveniles which may or may not spawn by summer's end (Price et al., 1991). Reproduction takes place from late May/early June to early August (pers. obs.). Adult fish are sexually dimorphic with the males developing a larger head, dark banding, a dorsal pad and cornified tubercles once mature. Males are thought to use a chemical cue released by a female to synchronize gonadal development and breeding behaviour with females (Cole and Smith, 1987). Breeding behaviour begins with males establishing territories underneath a structure. A gravid female attaches her eggs onto the nesting substrate, at which time they are fertilized by the male. Female fathead minnows are fractional spawners which allows them to spawn multiple times with multiple males during the breeding season (Gale and Buynak, 1982). Males remain with the eggs, protecting and cleaning them, until they hatch (~5 days at 25° C) (Environment Canada, 1992). There is no parental care of fry. Most adult fathead minnows, particularly males, are thought to die shortly after spawning (Price et al., 1991)

Negative impacts on growth and reproduction can be disastrous for any fish population (Donaldson, 1990; Wootton, 1990). The results of laboratory bioassays indicate that reproduction might be affected by the presence of MFT and TPW aged 4 years or less (Chapter 3), and that larval fish grow faster in sites containing tailings-associated waste than in sites free from these materials (Chapter 2 and Chapter 3). However, it is not known if faster growth as larvae results in larger adult fish and if faster larval growth can be maintained in field conditions where other factors (i.e. turbidity and temperature) may be involved (Chapter 2).

Populations of fathead minnows were weighed and measured to compare size, body condition and gonad to body ratio among populations. Body condition is considered to be indicative of the "wellness" of a fish and can be influenced by prey abundance, sexual maturation and environmental stress (Goede and Barton, 1990; Wootton, 1990). In the 1995 study, van den Heuvel and Dixon (1998) determined that fish in a pond containing MFT had a lower body condition than fish in a constructed pond which did not contain tailings. Gonad to body ratio (gonadosomatic index; GSI) is also commonly measured when comparing reproductive potential of populations, and reduction in GSI has been linked with contaminant exposure (see Goede and Barton, 1990; Munkittrick et al., 1991; Gagnon et al., 1995) . Finally, reproduction was monitored directly through the use of breeding boards. Monitoring reproduction of fatheads minnows is facilitated by their need for a substrate to which they attach their eggs, and by the fact they readily use artificial substrate (Jones and Paszkowski, 1997;

Vandenbos, 1996). This allows for intensive reproductive monitoring of fathead minnows and enables researchers to get relatively accurate data on endpoints normally restricted to laboratory studies (i.e., delays in spawning, length of spawning period). In this study, breeding boards were used to monitor timing of reproduction and length of spawning season.

Based on the results obtained in Chapters 2 and 3, I made the following predictions: 1) that adult fathead minnows in minnows in sites containing MFT will have a smaller body size than other populations and 2) that fathead minnows in ponds containing MFT may have a delayed spawning period.

Field Methods - Adults

Sites

All sites used in this portion of the study, with the exception of the two natural reference sites, were constructed by SynCrude Canada Ltd. and located on the Mildred Lake mine site. The two natural sites are within 50 km of the mine site. The SynCrude sites were selected based on the presence or absence of tailings in the site and the presence of fathead minnows. As all the ponds on the SynCrude site were constructed and introduced fish were from the same source (a ditch referred to a West Interceptor Ditch) two natural reference sites were selected to provide a baseline for comparison of results from the constructed sites. Kearl Lake and Thickwood Beaver Dam (Thickwood) were selected based on accessibility and abundance of fathead minnows. Yellow perch (*Perca flavescens*) were added into South Bison (SB) and Demonstration Pond (DP) in 1995, 1996 and 1997. Neither reference site contained piscivorous fish. Site descriptions are presented in Table 4.1.

Data collection

Population estimates and dissection collections were made in May and reproduction monitored from the end of May to August in 1996 and 1997. Monitoring included using mark-recapture surveys to estimate changes in population size. Annual decreases in fish number can be indicative of a number of stressors including reduced food resources, and reductions in recruitment. Fish were measured during marking and collections were made for dissection. Dissections were performed to determine sex and gonadal development of each

fish. Water analyses for all sites was carried out by SynCrude Canada Ltd. and are presented in Appendix A. Site descriptions are also included in Appendix A.

Mark- recapture

Fatheads were sampled in mid-May using randomly set Gee minnow traps. Twenty-four traps were set at each site with the exception of TP3 where only six traps were set and DP where minnow traps did not yield any fish. Fyke nets were used to sample in DP. Minnow traps (mesh size ~ 3 mm^2) were left in the water for 24 hour sets. Fyke nets (mesh size ~ 5 mm^2) were set overnight for 12 hours. Whenever possible, 100 to 200 minnows were measured (total length), and the presence of secondary sexual characteristics were noted (males: dorsal pad, tubercles and dark bands on the body; females: ovipositor). The tip of the upper (1996) or lower (1997) caudal fin was clipped and the fish were released. Recapture trapping took place 48 hours after the initial session.

Dissections

Within a week of the mark-recapture, approximately 100 fathead minnows were collected, killed with an overdose of MS-222 and frozen for dissection. Fish were weighed, measured (total length) and assessed for the presence of the secondary sexual characteristics listed above. Gonads were removed from fish and weighed to obtain GSI (Wootton, 1990).

Breeding boards

Reproduction was monitored by introducing five to ten breeding boards into each site. The number of boards in each site was determined by the size of the site with ten boards being introduced into the larger sites (i.e. DP) and five

into the smaller sites (i.e. TP3). The exception was Kearl Lake which was the largest site but , due to time constraints, only had five boards. Breeding boards were floating 5.1cm x 10.2 cm boards cut into 1 m lengths and covered with a black tarpaulin. Boards were anchored to the bottom with bricks. Boards were monitored three times per week for nests. When nests were present, the number of eggs/nest was estimated by laying a transparent grid (1 cm^2) on top of the nest and recording how "full" each square was (i.e. half full, three quarters full). Estimates on how many eggs were in a quarter, half, three quarters or full square were obtained by counting individual eggs in squares of each category (i.e. half full). Counting the number of eggs in certain squares to obtain estimates was done intensively at nests from all sites at the beginning of the summer then periodically throughout the summer to ensure that researchers did not change their assessment over the course of time. When isolated eggs were present in a square, they were counted and added to the nest assessment.

In 1996, after the fathead minnows had begun spawning, a barrier was introduced into SB by other researchers. This barrier was made of 2.54 cm^2 wire and did not likely impede the movement of fathead minnows but did impede the movement of perch. Based on trapping carried out by the other scientists, this appeared to split the pond into an area with many perch and one with few or no perch. As there was the potential for predation to impact fathead minnow reproduction (Jones and Paszkowski, 1997; Duffy, 1998), boards in SB were divided into two groups, with SB-A boards on the side without perch and SB-B boards on the side with perch. This barrier was removed in 1997.

Statistics

Statistical analysis used are based on Zar (1984). All data were analyzed on SPSS (version 6.0 and 7.5). Data were first tested for normality using the Kolmogorov-Smirnov test. Results of all tests were considered significant if $p < 0.05$ unless otherwise stated.

Mark recapture estimates were calculated using the Peterson mark-recapture method (Krebs, 1989). Poisson confidence intervals were used in all cases except for TP3 1996, and Thickwood 1996, in which case binomial confidence limits were used, as the fraction of marked animals was more than 0.10 (Krebs, 1989). The assumption of a closed population was violated at the Thickwood site due to some inflow/outflow which connected that site to at least two wetland areas.

Length and weight of adult fish was transformed using a $\log_{10}$ transformation. As natural population fluctuations could affect intersite comparisons, length and weight of fish caught in 1996 from each site were compared to 1997 values using t-tests with a Bonferroni adjusted p value of 0.017.

Lengths recorded during the mark/recapture portion of this study were added to the dissection data set, thereby increasing the sample size for length. The 1996 Thickwood dissection samples were lost; hence, they are not included in the between-year comparison for weight. TP3 experienced a total winterkill during the winter of 1996/97; hence, it is not included in 1997. Body condition of

fish was derived using the formula $K=W/L^3$ (K= body condition, W= weight, L= length; Wootton, 1990).

Populations were divided into males, females and juveniles based on gonad development (Price et al., 1991). Lengths, weights and GSI were compared among sites using one-way ANOVAs. GSI of males and females was analyzed with one-way ANOVA with the exception of 1996 males where there were insufficient data for comparison. GSI was not determined for juveniles. In 1996, predominantly juveniles were caught in DP, and therefore this site was not included in the 1996 GSI results.

Timing of spawning and length of spawning season was determined by calculating the proportion of the total number of eggs laid weekly on each breeding board, then determining in what week 25%, 50%, 75% and 100% of eggs had been laid on that board. The time it took for boards to achieve each of the above mentioned targets was analyzed using one-way ANOVA's with a Bonferroni adjusted p value of 0.0125. Differences between sites were determined using a Tukey's test for honest significant difference.

Results

Population estimates

Estimates and 95% confidence intervals are presented in Table 4.2. Confidence intervals are high due in part to relatively low recapture rates (i.e. captures ranged from 36 to 2131 whereas recaptures ranged from 1 to 90).

Between-year comparison

T-tests indicated that populations differed in lengths and weights from 1996 to 1997 and this difference could affect the outcome of intersite comparisons. In DP, fish were longer (Fig. 4.1a; df=392, t=-38.34, $p<0.001$) and heavier (Fig 4.1b; df=117, t=-16.45,$p<0.001$) in 1997 than in 1996. SB, Thickwood, and Kearl Lake fish were significantly longer (Fig. 4.1a; SB- df=322, t=7.49, $p<0.001$; Kearl Lake- df=435, t=14.38, $p<0.001$; Thickwood- df=249, t=9.39, $p<0.001$) in 1996 compared to 1997. SB and Kearl Lake fish were also heavier (Fig. 4.1b: SB- df=131, t=6.97, $p<0.001$; Kearl Lake- df=103, t=6.71, $p<0.001$) in 1996 compared to 1997. Figure 4.2 illustrates relative proportions of specific size classes for each site in both 1996 and 1997.

Inter-site comparisons

Length

In 1996, fish collected from DP were significantly shorter ($F_{4,899}=795.81$, $p<0.001$) than fish from all other sites (Fig. 4.1a). Other sites that differed significantly from each other in length were: SB< Kearl Lake, TP3 and Thickwood; Kearl Lake and TP3 < Thickwood. In 1997, Thickwood fish were

significantly longer ($F_{3,688}$=53.51, p<0.001) than fish from all other sites. Other sites that differed in length in 1997 include SB< DP and Kearl Lake.

Body Condition

The body condition of DP fish was significantly lower ($F_{2,143}$ =332.19, p<0.001) than the fish from Kearl Lake and SB in 1996 (Fig. 4.1c). In 1997, both Thickwood and SB fish had a significantly higher body condition ($F_{3,293}$=66.82, p<0.001) than fish from Kearl Lake and DP (Fig. 4.1c).

GSI, length and body condition for males, females and juveniles are given in Table 4.3. Results pertaining to DP adult males and females in 1996 should be viewed with caution due to small sample size (two and three individuals respectively).

Adult fish - males

In 1996, SB, Thickwood, TP3 and Kearl Lake males were significantly longer than DP males ($F_{4,207}$=31.89, p<0.001). Other significant differences included SB males being significantly shorter than Thickwood, TP3 and Kearl Lake males, and Kearl Lake and TP3 males being shorter than Thickwood males. SB males, however, had a significantly higher body condition ($F_{2,50}$=68.22, p<0.001) than males in DP and Kearl Lake.

In 1997, Thickwood males were significantly longer than males from other sites ($F_{3,248}$=34.42, p<0.001), but body condition of SB males was significantly higher than other sites ($F_{3,113}$=30.06, p<0.001). There was no significant difference in male GSI among sites ($F_{3,55}$=0.45, p=0.722) in 1997.

Adult fish - females

In 1996, female fish from DP were significantly shorter than SB, Kearl Lake and Thickwood females ($F_{4,248}$=34.42, p<0.001). Other significant differences in length included Thickwood females being longer than females from DP, SB and Kearl Lake. SB females had a significantly higher body condition ($F_{2,36}$=78.56, p<0.001) and GSI ($F_{2,36}$=6.37, p=0.004) than fish from Kearl Lake and DP.

In 1997, females from SB were significantly shorter ($F_{3,263}$=13.03, p<0.001) and had a significantly higher body condition ($F_{3,117}$=45.92, p<0.001) than female fish from other sites. The body condition of Thickwood females was significantly higher than that of DP and Kearl Lake females. Both Thickwood and SB females had a significantly higher GSI than those collected from DP and Kearl Lake ($F_{3,112}$=5.02, p=0.003).

Juveniles

In 1996, SB, Thickwood, TP3 and Kearl Lake males were significantly longer than DP juveniles ($F_{4,434}$=473.72, p<0.001). Other significant differences included SB juveniles being significantly shorter than Thickwood, TP3 and Kearl Lake juveniles, and Kearl Lake and TP3 juveniles being shorter than Thickwood juveniles. DP juveniles had a significantly lower body condition that SB juveniles ($F_{1,52}$=151.28, p<0.001).

In 1997, SB juveniles were significantly shorter than at all other sites ($F_{3,207}$=78.31, p<0.001), but had a significantly higher body condition than DP and Kearl Lake juveniles ($F_{3,61}$=23.90, p<0.001).

Reproduction

In 1996, reproduction commenced May 28 and continued until August 8. Only one nest was found in DP throughout 1996; hence, DP was not included in the analysis. The time it took the minnows to lay 25% (Fig. 4.3a; $F_{4,20}$=1.89, p=0.151), 50% (Fig. 4.3a; $F_{4,20}$=3.53, p=0.025), 75% (Fig. 4.3a; $F_{4,20}$=3.32, p=0.030) and 100% (Fig. 4.3a; $F_{4,20}$=3.93, p=0.016) of the total eggs on each board did not differ significantly among sites in 1996. There was, however, a non-significant trend as TP3 fish laid 50%, 75% and 100% of their eggs before other sites, but began laying June 3, a week later than SB and at the same time as Kearl Lake fish.

In 1997, spawning commenced June 4 and continued until August 6. As no fish were caught in TP3, this site was excluded in 1997. There were significant differences among sites in the timing of spawning in 1997 with DP taking significantly longer than other sites to attain the 25% point (Fig. 4.3b; $F_{3,23}$=30.39, p<0.001). Both DP and Kearl Lake took longer than Thickwood and SB to attain the 50% point (Fig 4.3b; $F_{3,23}$=29.45, p<0.001) and DP took longer than Thickwood to attain the 75% point (Fig 4.3b; $F_{3,23}$=11.29, p<0.001). DP fish were the last to begin spawning in 1997 (June 16), and the first to stop (July 9), laying 100% of their eggs significantly earlier than Kearl Lake and SB fish (Fig 4.3b; $F_{3,23}$=6.75, p=0.002).

Discussion

Population Structure

Populations in all sites differed from 1996 to 1997, implying that natural processes may be exerting a strong influence on the population dynamics of fathead minnows in the area. This may disguise any subtle effects of tailings exposure. Fish exposed to contaminants have been reported as being smaller (Munkittrick et al., 1991) or larger (Gagnon et al., 1995) at age of maturity than fish in uncontaminated sites. The total length of the fathead minnows in DP were, on average, smaller when compared to other sites in both 1996 and 1997, but did not differ significantly from Kearl Lake fish in 1997.

Contaminants present in tailings-impacted sites could have influenced fathead minnow size. DP had higher levels of naphthenic acids throughout the study. Additionally, there was an unexplained increase in salts and naphthenic acids in SB in August 1996; as of June 1996, naphthenic acids levels in particular were equal to or higher than those in DP (SB: 9.4 ppm; DP: 9 ppm; Table 4.1). These levels persisted for the duration of the study. The presence of these contaminants could account for the decreased size in both DP and SB fish compared to Thickwood fish in 1997. Although laboratory bioassays did not result in decreased growth of DP- or SB-exposed larvae, larval growth in mesocosms was negatively affected in sites containing SynCrude waste materials (Chapter 2). Contaminants can affect fish growth via numerous physiological mechanisms including appetite suppression, decreases in food

assimilation and increased metabolic demands (Luquet et al., 1984). Contaminants could also be affecting growth indirectly through reductions in prey items of the fathead minnows (Branzer and Kline, 1990).

One factor which seems to have influenced the fathead minnows in DP and SB was the way in which those ponds were colonized. Fish were introduced into DP in 1993 when the natural water cap was pumped into the pond (T. VanMeer, pers. comm.). Based on the size of screen used on the water pump, it is likely that only larvae were introduced. In 1995, these fish would be 2 years old and therefore ready to spawn (Price et al., 1991; Held and Peterka, 1974). Most adult fatheads do not live to 3 years; hence, the 1996 fathead population in DP would consist predominately of young fish hatched the year previously, which is consistent with the lack of fish captured >55 mm (Price et al., 1991). As there was evidence of very limited spawning in 1996, this same cohort grew and dominated the population, resulting in significantly larger and heavier fish caught in May 1997 compared to 1996. Juveniles and adult fish were introduced into SB in 1995. There was evidence of some spawning the year of introduction (pers. obs.), and the result may have been a more typical population comprised of newly hatched fry, spawning adults, and larger juveniles (Fig. 4.1, Price et al., 1991). As only 500 minnows were introduced into a site which had been fishless to that point, it is unlikely that fish growth was limited as a result of density or food availability. This is supported by unpublished data which documented increased growth rates of fathead minnows in SB compared to DP or Deep Wetland, a constructed site containing no MFT or TPW (Siwik,

unpublished data). Both fathead minnow and perch populations had increased in 1996, which may have increased competition for resources hence contributing to the shorter, lighter fish caught in that site in 1997.

Other factors which could have affected average size of the pre-spawning fathead minnow population include low winter oxygen levels and piscivory. Low oxygen levels resulted in a winterkill in Kearl Lake in the 1980's (Golder Associates, 1996) and TP3 experienced a winterkill in 1996-97. Fathead minnows have been found in sites with overwinter oxygen readings of <1 ppm (Held and Peterka, 1974; Klinger et al., 1982). Unlike most species, however, weight specific oxygen consumption in fathead increases with an increase in weight (Wares and Igram, 1979), which means that larger fathead minnows are even more vulnerable to the effects of low oxygen. This may explain why winter conditions eliminated the oldest individuals in one of the lakes studies by Held and Peterka (1974). Winterkills have been documented in ponds in the Fort MacMurray area, and any one of the sites in this study could have experienced some overwinter mortality which may have altered the size of fish caught the subsequent May.

Piscivores can influence the population dynamics of their prey fishes. Fathead minnows have been reported in the stomach contents of yellow perch (Lott et al., 1996), and there is evidence that the perch introduced in DP and SB in 1995 , 1996 and 1997 were eating fathead minnows (R. Gould, pers. comm.) and foraging on their egg masses (pers. ob.). Besides direct mortality, piscivory

can lead to shifts in habitat use of prey fishes which can result in an overall decrease in fish size, and delays or reduction in spawning (Tonn and Paszkowski, 1987; Fraser and Gilliam, 1992; Duffy, 1998). Mean size of fathead minnows decreased in populations experiencing predation by yellow perch, northern pike (*Esox lucius*) and walleye (*Stizostedion vitreum vitreum*), possibly due to direct mortality (Duffy, 1998) or reductions in feeding in the presence of a predator (Abrahams, 1994).

Low oxygen conditions and piscivory are only two examples of the many factors which were likely influencing the growth of the fathead minnows in the study ponds. While it is interesting that adult fish from DP fish were often smaller, based on length, than other sites, pollutants may have had little to do with these changes and differences.

Reproduction

In 1996, spawning took place in all sites from late May to early August with the exception of DP in which only one nest was found. That nest was abandoned within 2 days of discovery. An apparent total winterkill in TP3 resulted in no nests at that site in 1997.

The dominance of one year class in DP is likely responsible for the lack of spawning in 1996, but the fact that 1997 spawning was delayed in DP is worthy of note. Spawning was not monitored in a preliminary study carried out in 1995, but the authors observed that Deep Wetland, a constructed site containing only natural water, contained obviously gravid females and males with secondary sexual characteristics whereas DP fish, sampled at the same time, showed no

evidence of spawning (van den Heuvel and Dixon, 1998). This suggests that the delay observed in the current study may not be an isolated event. Fish reproduction is highly attuned to abiotic conditions such as photoperiod and water temperature, as well as other factors such as abundance of food or changes in water chemistry (Smith, 1978; Wootton, 1990). All sites were experiencing similar photoperiod during the summer, and DP was similar in temperature to sites which commenced spawning the week of June 11, 1997 (Kearl Lake : 14.2° C, TWBD : 17.6° C, SB : 20.3° C, DP : 18.4° C). DP was, however, the only site monitored in 1997 which contained tailings, a mixture of many potential contaminants.

In Chapter 3, reproduction of fish exposed to TPW water was both delayed and reduced, and numerous laboratory studies which documented a reduction or delay in spawning as a result of exposure to contaminants were discussed. Cases included a delay in spawning of fathead minnows exposed to thiocyanate (Lanno and Dixon, 1994), a delay spawning of European minnows (*Phoxinus phoxinus*) after exposure to polycyclic-chlorinated biphenyls (Bengtsson, 1978), and two examples in which exposure to petroleum products resulted in abnormal testes in cunners (*Tautogolabrus adspersus*) and Atlantic cod (*Gadus morhua*) (Payne et al, 1978; Khan and Kiceniuk, 1984). Both Munkittrick et al. (1991), and Gagnon et al. (1995) documented reduced reproductive output in stream populations of white sucker (*Catostomus commersoni*) exposed to bleached kraft mill effluent (BKME). The study by Munkittrick et al. (1991) found male fish to lack secondary sexual characteristics,

and all fish to be shorter and older at maturity with smaller gonads. Gagnon et al. (1995), concluded that increased size at age of maturity, a reduction in female GSI and highly variable reproduction were indications of reduced reproductive output of white suckers exposed to BKME. The lack of nests in DP in 1996 was likely due to the predominance of young, small fish in the site, particularly as reproductive output was higher in DP in 1997 compared to 1996. While DP fish did not have significantly lower GSI, length and body condition, these measures were often lower than other sites. Hence, the delayed and somewhat compressed reproductive season of DP fish in 1997 may be a result of MFT in the pond. It is worth noting, however, that the timing of spawning in TP3 fish showed a similar, yet non-significant trend in 1996.

Fish reproducing in DP water in the lab did not exhibit delayed spawning; however, fish in TPW water did. It is possible that the varying conditions in the field may have intensified the impact that SynCrude waste water has on reproduction in the fathead minnow. One possible reason for delayed spawning could be linked to an increase in 7-ethoxyresorufin catalytic enzyme (EROD) activity as documented in DP fish by van den Heuvel and Dixon (1998). EROD is one assay toxicologists use when measuring mixed function oxidase enzymes (MFO's). MFO's metabolize or detoxify contaminants and elevated MFO activity is often used an indicator of contaminant stress (Passino, 1984). Elevated levels of EROD is thought to indicate higher levels of contaminant biotransformation. MFO activity can also be induced by natural processes, including reproduction. Levels of MFO's can differ depending on the reproductive status of the fish

which, is not surprising as it is thought that one function of MFO's is to metabolize certain steroid hormones (Passino, 1984).

van den Heuvel and Dixon (1998) reported: a) that EROD activity was significantly higher in DP fish compared to Deep Wetland fish in spring 1995 and b) males had a five fold increase in EROD activity compared to females in both sites. It is possible that increased MFO activity in DP fathead minnows could have been due to the presence of MFT in that site. As MFO's metabolize certain hormones, the higher levels of MFO's in DP males could have worked to break down hormones, such as 11-ketotesterone, which regulate such processes as the appearance of secondary sexual characteristics or testicular development. In the aforementioned studies which determined that BKME significantly reduced reproductive output in the white sucker, both studies found elevated MFO activity in exposed populations (Munkittrick et al., 1991; Gagnon et al., 1995).

As mentioned in the previous section, the minnows in DP also suffered predation from perch and this threat may have influenced spawning activity and success. Abrahams (1995) determined that fathead minnows actively avoided areas inhabited by yellow perch and Jones and Paszkowski (1997) determined that fathead minnows altered their nest defense behaviours in the presence of northern pike. Although behavioural adaptations can be energetically costly and possibly affect offspring (Wootton, 1990), there is a paucity of research on the potential for predators to impact the timing of reproduction in forage fish. Wootton (1990) does cite examples in which bluegill sunfish and threespined sticklebacks (*Gasterosteus aculeatus*) spawn in groups or "clumps", possibly in

order to better defend their territories against nest predation. Wootton (1990) also cites the example of the relatively short spawning interval of Pacific herring (*Clupea harengus pallasi*) which may be a strategy to swamp predators, thereby improving the chances of some eggs and larvae.

In conclusion, there is little evidence that the water capping technique used in DP negatively impacted the fathead minnow population. Size and reproduction differences observed in this study could have been the result of waste water exposure, predation pressure, prey availability and other factors. The results of the laboratory bioassays, however, did indicate a significant effect of tailings waste water (Chapter. 2 and Chapter. 3); hence, further controlled field experiments (i.e. mesocosm studies) could prove valuable.

Table 4.2 Population estimates of fathead minnow populations in the monitored ponds. All sites with the exception of Kearl Lake (Kearl) and Thickwood Beaver Dam (TWBD) were constructed by SynCrude Canada Ltd. South Bison (SB) did not contain tailings material while Test Pit 3 (TP3) and Demonstration Pond (DP) contained a layer of MFT capped with water. Population size was estimated using Peterson's mark-recapture technique. All confidence intervals (CI) were calculated using a Poisson distribution with the exception of TP3 1996, and Thickwood 1996, in which case binomial confidence limits were used as the fraction of marked animals was more than 0.10 (Krebs, 1989).

Site	Year	population estimate	upper CI	lower CI
Kearl	1996	43027	74853	25401
Kearl	1997	11377	19244	6932
TWBD	1996	150	305	95
TWBD	1997	1827	3347	1028
SB	1996	2764	4079	2015
SB	1997	5827	11772	2871
TP3	1996	444	600	355
DP	1996	1914	4211	840
DP	1997	1569	2986	496

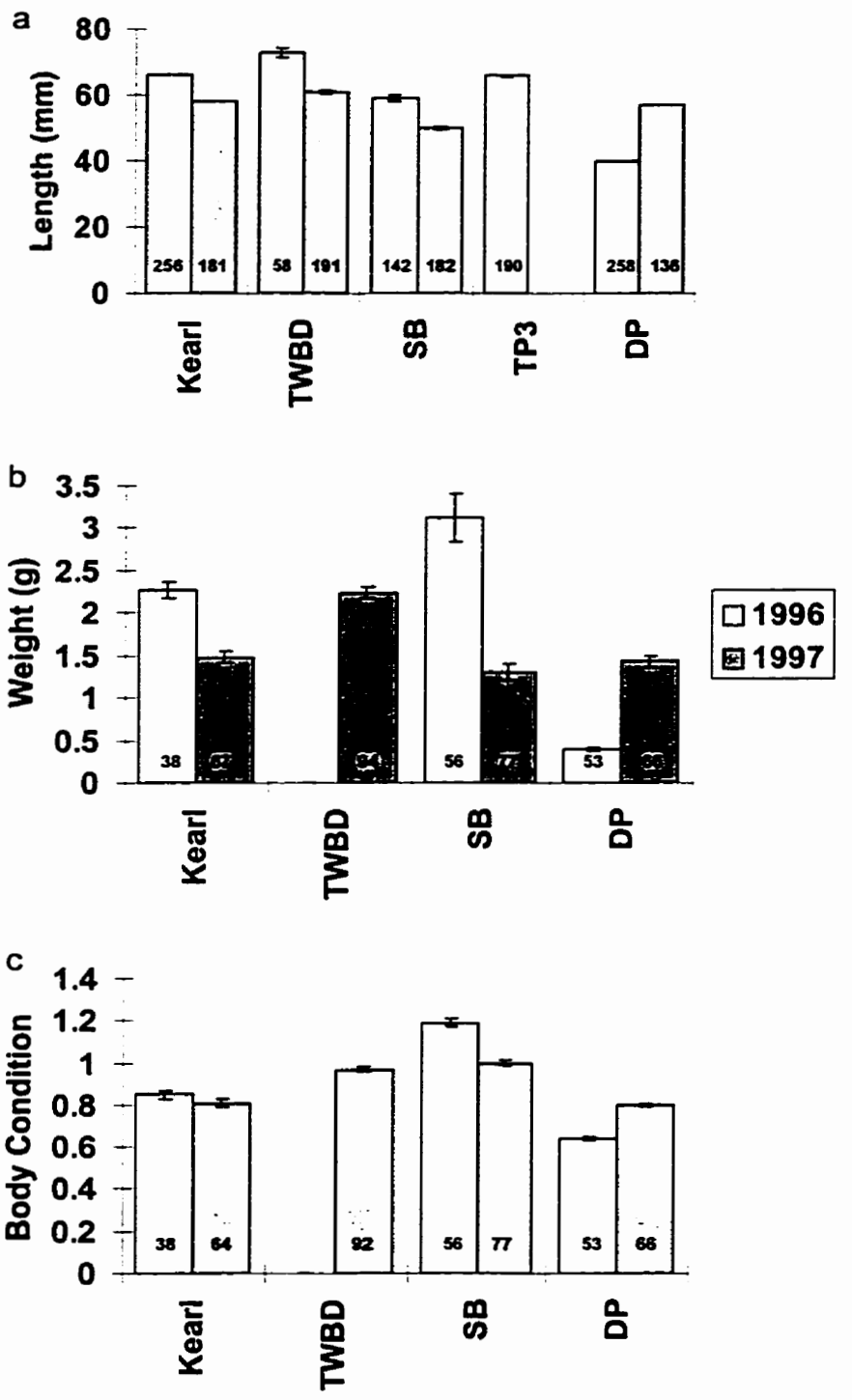

Figure 4.1 Length (a; mean + standard error), weight (b; mean + standard error) and body condition (c; mean + standard error) of fish sampled in May 1996 and 1997. Kearl Lake (Kearl) and Thickwood Beaver Dam (TWBD) are natural reference sites. South Bison (SB) is a constructed site which did not contain MFT while Test Pit 3 (TP3) and Demonstration Pond (DP) contained MFT capped with water. Numbers in the bars represent sample size.

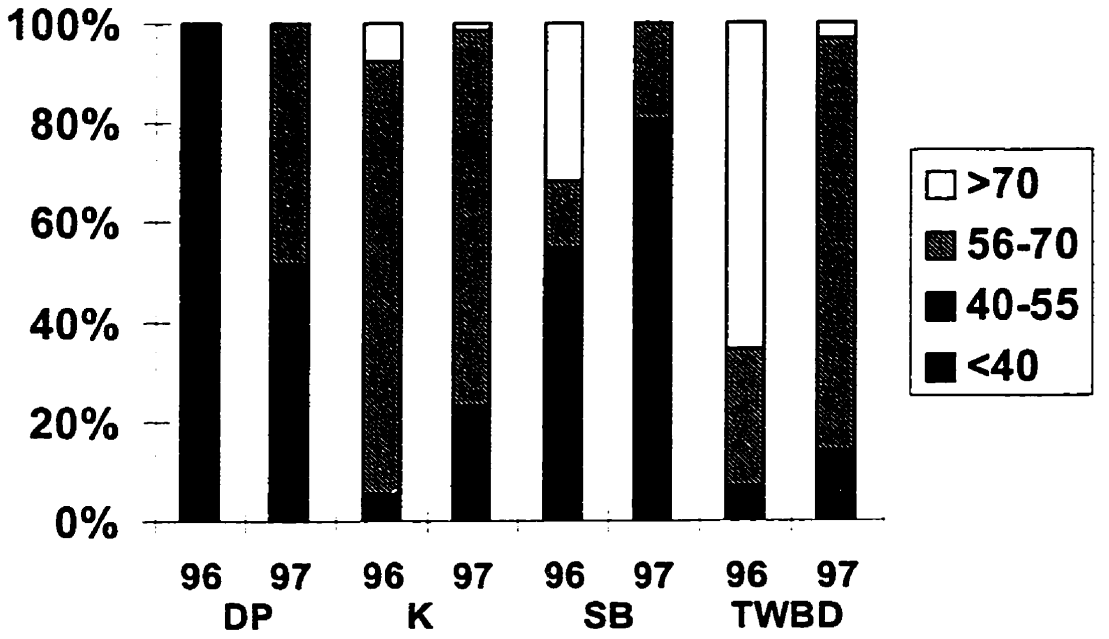

Figure 4.2 Proportion of specific size ranges of fish caught in ponds on and around SynCrude site in May 1996 and 1997. Based on the presence of secondary sexual characteristics, it is probable that all fish in the 56-70 mm size class would spawn the year they were sampled while those in the 40-55 mm size class would not. The first and last size category (< 40 and >70) represent population extremes. Sites are grouped annually to highlight fluctuations in population size from 1996 to 1997. Demonstration Pond (DP) and South Bison (SB) are both located on the SynCrude Canada Ltd. Mildred lake mine site while Kearl Lake (K) and Thickwood (TWBD) are natural reference populations.

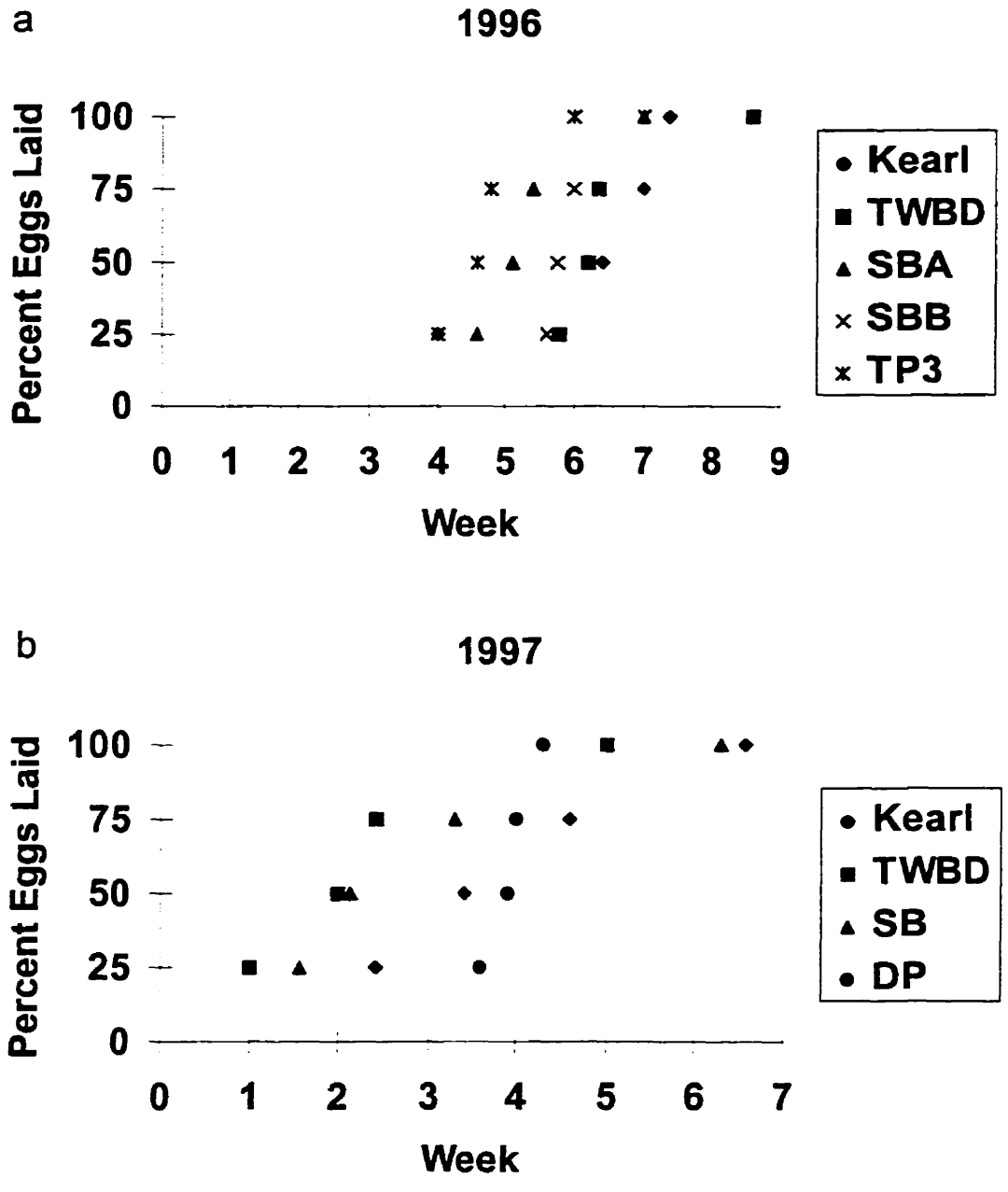

Figure 4.3 Spawning trends in 1996 (a) and 1997 (b). Spawning was monitored by estimating the number of eggs on each board three times a week. Five to ten boards were placed in each site. Each point represents the mean of boards in a site. In 1996, a barrier was erected in SB, isolating some fathead minnows and eliminating predation (SB-A). The boards on both sides were therefore treated differently. Thickwood Beaver Dam (TWBD) and Kearl Lake (Kearl) were natural reference sites. The remaining sites were constructed by SynCrude; South Bison (SB) did not contain any MFT while Test Pit 3 (TP3), and Demonstration Pond (DP) contained MFT capped with water.

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Chapter 5

General Synthesis and Conclusions

Assessing chronic toxicity of a substance can be difficult, as effects range from immediately obvious (i.e. reductions in growth) to very subtle (i.e. changes in behaviour; Cairns et al. 1996). Endpoints associated with reproduction, and larval survival and growth have been shown to be consistently more sensitive than other organism-based endpoints (Woltering, 1984; McKim, 1985, Suter et al., 1987). Although scientists have developed some quick, relatively sensitive, laboratory bioassays for detecting chronic and acute toxicity, no single test has been found that can assess the impact of every chemical on every life stage of an organism (Genoni, 1997), and some argue that bioassays must include more than one life stage and/or generation in order to be effective (Cairns et al. 1996). The ability of laboratory-based bioassays to predict effects in the natural environment, where numerous biotic and abiotic conditions modify toxicity (Sprague, 1985) should also be considered when establishing water quality guidelines.

In attempts to fully assess the potential chronic toxicity of MFT and TPW on growth and reproduction of fathead minnows, I measured numerous endpoints in both the laboratory and in populations of fathead minnows in the field. Results from standard laboratory growth bioassays, a partial-life cycle test, a mesocosm growth bioassay, and from field fish provided a comprehensive evaluation of possible chronic effects, and highlighted some areas which should be further investigated. The ponds used in my study were selected according to

the relative volume of tailings waste water and age of tailings in each site as both of these factors related to overall toxicity (Verbeek, 1995; FTFC, 1995).

I carried out two 7 day laboratory growth bioassays on waters from ponds which ranged in potential toxicity (Chapter 2). The bioassays did not yield any significant differences in larval fish growth to 7 days. Extending the length of the bioassays to reflect a longer exposure period (56 days), did not change the outcome of the bioassay (Chapter 2). There was, however, in both the 7 and 56 day bioassays, a trend towards larger size of fish reared in sites with higher volumes of SynCrude waste product. This growth trend was seen in the partial-life cycle test as offspring of fish reared in SynCrude waste water were all significantly larger than offspring of fish raised in control water (Chapter 3). This trend to increased growth, however, was reversed once larval fish were allowed to grow in field mesocosms (Chapter 2), where growth rates of larval fish decreased in sites containing MFT and/or TPW. This may indicate that water quality conditions besides chemical composition could be affecting fish growth in the field. Abiotic factors (temperature, low oxygen) and/or biotic factors (prey density, predation pressure) also likely had significant influence on the growth of fish in the field populations. One reference site (Thickwood Beaver Dam) did have consistently larger fish than all other sites in both 1996 and 1997, but fish from other natural and constructed reference sites did not show consistent trends (Chapter 4).

There is evidence, both in the laboratory and field, that MFT and TPW could impact reproduction. During the partial life-cycle laboratory bioassay, pairs

reared in TPW took significantly longer to produce nests, and produced fewer nests than pairs reared in other waters (Chapter 3). This delay in spawning was reflected in the population of fathead minnows inhabiting a pond containing MFT, referred to as Demonstration Pond (DP) (Chapter 4). Fathead minnows in DP a) took significantly longer to lay 25% of their eggs than did all other sites in 1997, but b) stopped spawning by the middle of July, taking significantly less time to lay 100% of their eggs.

The results of this study indicate that a population of fathead minnows could possibly be maintained in a watercapped site but further work should be carried out determining a) if there are additional abiotic factors associated with MFT and TPW which could impede fish growth, b) the cause of the delay in spawning in both the DP field population and lab reared TPW fish and c) what this (reduced growth or delayed/reduced spawning) could mean in terms of the long term viability of the fathead minnow populations in any watercapped site. Additional controlled laboratory and mesocosm studies manipulating certain abiotic conditions (i.e. light, temperature, dissolved oxygen, turbidity) may prove useful in understanding growth patterns. Focusing on sex hormones, multi-function oxidases, or behavioural observations for potential disruptions in territoriality or courtship behaviour may provide insight into reproductive effects.

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Appendix A - site descriptions

Kearl Lake : location - north of Fort McKay; sec. 4, twp. 96, rge. 8 (57° 17' 43 " 110° 14' 26"). Open system; drained by North Muskeg Creek, and Lyinimin Creek discharges through muskeg into Kearl Lake (Golder, 1996; refer to Chapter 4). Max. depth of 2.0 m, and 5.22 km^2 in area (Alberta Environment Report 1996). Fish species found in the past include lake chub (*Couesius plumbeus*), white sucker (*Catostomus commersoni*), longnose sucker (*Catostomus catostomus*), fathead minnows (*Pimephales promelas*), brook stickleback (*Culaea inconstans*), pearl dace (*Margariscus margarita*). Natural field site referred to in Chapter 4 and source of adults for mesocosm study in Chapter 2.

Thickwood Beaver Dam Pond (TWBD) : location - east of Fort McMurray city limits, along Tower Rd., sec. 31, twp. 89, rge. 10. Open dynamic system as local resident reports periodically eliminating beavers and dam thereby draining site. Depth and area varied with year but max. depth was 5m and surface area of ~3 ha. Fish species include fathead minnows (*Pimephales promelas*), white suckers (*Catostomus commersoni*), lake chub (*Couesius plumbeus*), brook stickleback (*Culaea inconstans*), pearl dace(*Margariscus margarita*) and red bellied dace (*Phoxinus eos*). Natural field site referred to in Chapter 4.

Deep Wetland (DWL) : location - Syncrude Canada Ltd. Mildred Lake site, sec. 2, twp. 93, rge. 11. Closed system, constructed in 1993. DWL contains ~ 50000 m^3 West Interceptor Ditch water, but no MFT or TPW. Most of it is ~1m in depth except for a stretch which is ~3 m. 5 ha surface area. Fathead minnows (*Pimephales promelas*) present since 1993 and yellow perch (*Perca flavescens*) introduced in 1995, but a winterkill in 1995/6 resulted in 100% death of perch and partial kill of fathead minnows. Used in Chapter 2 as the clean water site for the mesocosm experiment and in SGS1. Also referred to in Chapter 4 discussion.

South Bison (SB) : location - Syncrude Canada Ltd. Mildred Lake site, sec. 24, twp. 92, rge. 11. Closed system, constructed in 1980's in the overburden dumps. Filled primarily with surface rain run off and melt water. Dynamic site as erosion and leaching has altered the site from year to year. Relatively large littoral zone. Max. depth ~4 m and surface area ~ 8 ha, but has never been accurately determined. 600 fathead minnows (*Pimephales promelas*) introduced in 1995 and 850 yellow perch (*Perca flavescens*) added from 1995 to 1997. One of the sites used in SGS1 (Chapter 2) and a field site sampled for Chapter 4.

Demonstration Pond (DP) : location - Syncrude Canada Ltd. Mildred Lake site, sec. 2, twp. 93, rge., 11. Closed system, constructed in 1993 as a model for Base Mine Lake. Filled with 75000 m^3 MFT plus 75000 m^3 West Interceptor Ditch water. Depth of water phase is 2.9 m and a surface area of 4 ha. Fathead minnows (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) introduced during filling in 1993, and 850 perch added from 1995 to 1997. Used in short and long term bioassays (Chapter 2), life cycle bioassay (Chapter 3), mesocosm study (Chapter 2) and as a field site (Chapter 4).

Tailings Pond Water Pond (TPW) : location - Syncrude Canada Ltd. Mildred Lake site, sec. 2, twp. 93, rge 11. Closed system, constructed in 1993. Contains ~ 50000 m^3 of tailings pond water. Maximum depth of ~ 4 m and surface area of ~ 4 ha. Contains no fish. Used in short and long term bioassays (Chapter 2) and in the life cycle bioassay (Chapter 3).

Storage Cell 10 (SC10) : location - Syncrude Canada Ltd. Mildred Lake Site, sec. 2, twp. 93, rge. 11. Closed system, constructed in 1993. Contains 6,300 m^3 tailings pond water and 29000m^3 of MFT. Depth of water phase ~ 1m overlying 9 m of MFT. Contains no fish. Used in long term growth bioassay (LGS2) and in mesocosm study (Chapter 2).

Pit 3 (TP3) : location - Syncrude Canada Ltd. Mildred Lake site, sec. 2, twp. 93, rge. 11. Closed system, constructed in 1989. Filled with 1000 m^3 MFT + 1000 m^3 West Interceptor Ditch water. Depth of water phase is 2.5 m and total surface area of 1 ha. Lake chub (*Couesius plumbeus)*, fathead minnows (*Pimephales promelas*), and brook sticklebacks (*Culaea inconstans*) introduced in 1984 but experienced partial winterkill 1995/96, and total fathead minnow/lake chub winterkill 1996/97. Used in short and long term bioassays (Chapter 2), life cycle test (Chapter 3) and as a field site in 1996 (Chapter 4)

Pit 5 (TP5) : location - Syncrude Canada Ltd. Mildred Lake site, sec. 2, twp. 93, rge. 11. Closed system, constructed in 1989. Filled with 1000 m^3 TPW and 1000 m^3 MFT with a maximum water phase depth of 2.5 m and surface area of 1 ha. Fathead minnows (*Pimephales promelas*), lake chub (*Couesius plumbeus)* and brook sticklebacks (*Culaea inconstans*) introduced in 1984, but winterkilled. 250 fatheads (and 1 accidental perch) introduced into pit in 1996. Used in short and long term growth bioassays (Chapter 2) and life cycle test (Chapter 3).

Pit 7 (TP7): location - Syncrude Canada Ltd. Mildred Lake site, sec. 2, twp. 93, rge. 11. Closed system, constructed in 1989. Filled with 2000 m^3 of MFT but currently has a water layer (>1 m) comprised of rain runoff, melt water and water expressed from the MFT. Contains no fish. Used in SGS2 (Chapter 2).

Appendix A - Water Quality terms of reference

Dep : depth at sampling site (m)

pH : pH of sample at 20° C

Cond: conductivity of sample at 20° C (μS/cm)

Temp: ambient temperature of sample (° C)

DO : dissolved oxygen (ppm)

DS : dissolved solids (ppm)

TS: total solids (ppm)

Turbidity: suspended solids (ppm)

Nap acids: naphthenic acids (ppm)

CHL: chloride (mg/L)

SO_4: sulfate (mg/L)

CHL'a': chlorophyll a (mg/L)

DOC: dissolved organic carbon (mgC/L)

NH_3: ammonia (mgN/L)

NO_2+NO_3: nitrite + nitrate (mgN/L)

TN: total nitrogen (mgN/L)

o-PO_4: orthophosphate (mgP/L)

TP: total phosphorus (mgP/L)

Loc	Date	Dep (m)	pH	Cond (uS /cm)	Temp (°C)	DO	DS (ppm)	TS (ppm)	Turbidity (mg/L) (ppm)	Nap Acids (ppm)	CHL	SO4	CHL'a'	DOC	NH3	NO2+NO3	TN	o-PO4	TP
Deep Wet Lands: On Lease 17, muskeg water drainage																			
DWL	960318	0			0.6	3.1													
DWL	960318	1	7	619	1.5	1.5	489	498	9		10	55.3		40	0.16	0.03	2.1	0.05	0.1
DWL	960516	0			9.6	12													
DWL	960516	0.5	8	437	9.5	11	321	326	5		6.6	41.4	0.02	31	0.1	0.03	1.2		0.1
DWL	960617	0	8	425	18.1	10	312	317	5	0.89	6.5	37.3	0.001	36	0.05	0.03	1.4	0.03	0
DWL	960821	0			17.9	12													
DWL	960821	1	9	385	17.7	11	279	284	5		5.7	39.1	0.007	29	0.01	0.035	1.4		0.1
DWL	960917	0			16	10													
DWL	960917	1	8	439	15.9	9.9	279	284	5	1.65	6.4	38.8	0.001	23	0.01	0.003	1.1		0
DWL	961017		8	421	4	12	297	301	4	1.53	5.7	41.3		25.5	0.05	0.03	1.3		0
DWL	970618	0			22	13													
DWL	970618	1	8	435	21.8	13													
Kearl Lake: AURORA MINE AREA																			
KEARLK	960710		7	138							0.4	3.1		22	0.1	0.03	1.2		0
KEARLK	970820									0.46	0.1	2.5		20	0.01				0
South Bison Pond: South of Base Mine																			
SB	960517		8	1430				1144		3.92	62.8	578		24	0.1	0.03	1		0
SB	960615		8	1875	19.5	9.8	1582	1595	13	9.4	82	771		37	0.05	0.03	0.9	0.03	0
SB	960710		8	1969							83	800	0.001	32.5	0.1	0.03	1.2		0
SB	960819	0	8	1902	19.2	11													
SB	960819	1	8	1900	19.1	11													
SB	960819	2	8	1897	18.3	11													
SB	960819	3	8	1901	18.1	11													
SB	960819	4	8	2590	17.2	13													
SB	960819	5	8	2760	14.6	18													
SB	960819		8	2030			1577	1582	5		80	777		31.5	0.04	0.038	0.7		0.1
SB	960917	0	8	1829	14.7	11				14									
SB	960917	1	8	1804	14.5	11													
SB	960917	2	8	1825	14.4	11	1624	1633	9		85	811		28.5	0.01	0.051	0.7		0.1

Loc	Date	Dep (m)	pH	Cond (uS /cm)	Temp (°C)	DO	DS (ppm)	TS (ppm)	Turbidity (mg/L) (ppm)	Nap Acids (ppm)	CHL	SO4	CHL'a'	DOC	NH3	NO2+NO3	TN	o-PO4	TP
SB	960917	3	8	1816	14.4	10													
SB	960917	4	8	1841	14.1	9.8													
SB	960917	5	8	1914	13.2	9.6													
SB	970225		8	2620			209	221	12	14.17	120	1100							
SB	970514		8	1668						10.57	75	734		28	0.01	0.004	1.7	0.013	0.1
SB	970618	0	8	1835	20.9	12													
SB	970618	1	8	1902	20.9	11													
SB	970618	2	8	1731	19.9	11													
SB	970618	3	8	1813	15.9	6.8													
SB	970618	4	8	1994	11.4	0.9													
SB	970810	0	8	1960	19.8	9.6													
SB	970810	1	8	1961	19.8	9.4													
SB	970810	2	8	1957	19.7	9.3				7.01	86.7	782		31.5	0.01				0
SB	970810	3	8	1962	19.1	9													
SB	970810	4	8	2010	17.8	1													
SB	970810	5	7	2040	16.5	0													
Thickwood Pond: Thickwood community in Fort McMurray																			
TWBD	960709	0			22.2	8.4													
TWBD	960709	1			22	7.4													
TWBD	960709	2	9	2070	21.5	6.7					0.1	0.5		30	0.1	0.03			
TWBD	960810		7	125			156	161	5		0.1	0.5		33	0.02	0.024	1.2		0.1
TWBD	960820		7	160															
Storage Pond	**(SC10)**																		
SC10	960617		9	2240			1665	1689			236	215		71	0.05				
SC10	970224		9	3070	0.2	3.3	2175	2218		83	342	324		62	0.04	0.116	1.5		0
SC10	970514		9	1990						44.56	220	198		44	0.01				
SC10	970618	0			20.9	8.5													
SC10	970618	1			20.9	8.6													
SC10	970618	2	9	2240	18.3	7.6													
SC10	970818	0	9	2240	20.6	9.1	1535	1570		50.76	234	196		54	0.01				0

Loc	Date	Dep (m)	pH	Cond (uS /cm)	Temp (°C)	DO	DS (ppm)	TS (ppm)	Turbidity (mg/L) (ppm)	Nap Acids (ppm)	CHL	SO4	CHL'a'	DOC	NH3	NO2+NO3	TN	o-PO4	TP
Tailings Pond Water Pond (TPW)																			
TPW	960516	0	8	1360	8.9	11		1032			153	164		44	0.1				
TPW	960516	1			8.7	12													
TPW	960516	2	8	2890	7.2	4.6	2287	2586		74	360	372	0.027	77.5	0.1	0.07	1.4		0
TPW	960617	0	9	1920	18	9.5	1371	1384		33	205	229		51	0.05				
TPW	960617	1			18.1	9.4													
TPW	960617	2	9	1900	18.1	9.3	1370	1375		27	208	223	0.001	51	0.05	0.03	1.3	0.03	0
TPW	960709	0			22.2	8.4													
TPW	960709	1			22	7.4													
TPW	960709	2	9	2070	21.5	6.7	1380	1384			220	245	0.001	47	0.1	0.03	1.6		0
TPW	960821	0			18.8	9.9													
TPW	960821	1			18.2	10													
TPW	960821	2	9	2010	17	9.5	1356	1396			207	233	0.001	50	0.01	0.01	1.4		0.1
TPW	960906																		
TPW	960917	0			15	9.6													
TPW	960917	0.5			14.9	9.6													
TPW	960917	1	9	1980	14.7	9.6	1421	1451		50	213	226	0.001	47.5	0.01	0.059	1		0
TPW	961017	0			3.5	12													
TPW	961017	1	9	2020	3.5	12	1417	1439		48.82	185	214	0.008	50.5	0.05	0.03	1		0
TPW	961206		9	2360	1.6	13	1585	1678			241	268		52.5	0.21	0.03	0.5		0
TPW	970224	0.5			0.6	6.1													
TPW	970224	2	8	2690	1.8	5.1	1863	1889		60	319	340		59	0.07	0.069	1.2		0
TPW	970514	0	9	1690	10.6	10													
TPW	970514	1	9	1684	10.2	10				33.18	188	201		37.5	0.01	0.003	2.4		0.2
TPW	970618	0			20.9	8.5													
TPW	970618	1	9	1994	20.6	8.6													
TPW	970818	0			18.9	7.9													
TPW	970818	1	9	2000	16.6	7.4	1367	1411		39.07	215	217	0.001	49.5	0.01				0
Pit 3 (TP3)																			
Pit 3	960516	0	8.2	523	10.1	12	357	369	12		17.2	65.3		30					
Pit 3	960516	1			8.8	13													
Pit 3	960516	2	8.2	518	8.4	13	374	384	10		16.4	65.4	0.001	29	0.100	0.080			

Loc	Date	Dep (m)	pH	Cond (uS /cm)	Temp (°C)	DO	DS (ppm)	TS (ppm)	Turbidity (mg/L) (ppm)	Nap Acids (ppm)	CHL	SO4	CHL'a'	DOC	NH3	NO2+NO3	TN	o-PO4	TP
Pit 3	960617	0			18.4	11													
Pit 3	960617	1			18.5	11													
Pit 3	960617	2	8.6	530	18.4	11	393	402	9	1.2	18.0	69.0	0.001	35.5		0.030			
Pit 3	960821	0			18	9.9													
Pit 3	960821	1			17.7	10													
Pit 3	960821	2	8.3	512	17.5	10	358	363	5		17.3	66.8	0.001	26.5	0.010	0.031			
Pit 3	960906																		
Pit 3	960918																		
Pit 3	961017	0			4.1	11													
Pit 3	961017	1	8.3	522	4.1	12	370	379	9	4.1	16.3	63.2	0.009	25.5		0.030	0		0
Pit 3	961206		7.9	621	3.9	8.6	429	430	1	3.1	19.6	76.0	0.002	22		0.030	0.6		0
Pit 3	970224	1			2.8	1.2													
Pit 3	970224	2	7.4	674	4.3	0.4	453	458	5	4.8	26.3	85.7		27.5		0.011	1.1		0
Pit 3	970224	2.5			4.8	0.2													
Pit 3	970514	0	8.0	495	11.6	11													
Pit 3	970514	1																	
Pit 3	970514	2	8.0	496	11.6	11				3.1	17.7	58.1	0.002	18.5		0.003	2.7	0.003	0
Pit 3	970618	0			21.4	12													
Pit 3	970618	1			21.4	12													
Pit 3	970618	2	8.2	545	20.9	12													
Pit 3	970818	0			19.2	8.2													
Pit 3	970818	1			17.4	8.1													
Pit 3	970818	2	8.4	519	17.4	8.2	337	342	5	3.2	17.5	56.5	0.001	26	0.010				0
Pit 5 (TP5)																			
Pit 5	960516	0	8.6	1179	10.2	12	890	895	5		69.5			32					
Pit 5	960516	1			8.7	13													
Pit 5	960516	2	8.6	2170	9.8	16	1638	1643	5	25.0	128.0	510.0	0.001	51	0.100	0.030			
Pit 5	960617	0	8.7	1925	18.1	7.5	1451	1452	1	21.0	116.0			48.5					
Pit 5	960617	1			18.1	9.6													
Pit 5	960617	2	8.6	2270	17.2	11	1696	1702	6	27.0	137.0		0.001	53		0.030			
Pit 5	960709	0			22.5	9.4			5										
Pit 5	960709	1			21.4	9.1													

Loc	Date	Dep (m)	pH	Cond (uS /cm)	Temp (°C)	DO	DS (ppm)	TS (ppm)	Turbidity (mg/L) (ppm)	Nap Acids (ppm)	CHL	SO4	CHL'a'	DOC	NH3	NO2+NO3	TN	o-PO4	TP
Pit 5	960709	2	8.8	2110	19.8	6.9	1489	1494	5		121.0		0.001	44	0.100	0.030			
Pit 5	960821	0			18.3	9.5													
Pit 5	960821	1			17.5	9.5													
Pit 5	960821	2	8.7	2110	17.4	9.6	1505	1510	5		115.0		0.001	47		0.033			
Pit 5	960906																		
Pit 5	960918	0			15.1	9.4													
Pit 5	960918	1	8.7	2050	14.6	9.3	1506	1524	18	32.0	117.0		0.001	39		0.094			
Pit 5	961002	1			5.7	10													
Pit 5	961017	0			3.5	11													
Pit 5	961017	1	8.7	2060	4	11	1488	1494	5	31.2	108.0		0.025	45.5	0.150	0.030	1		0
Pit 5	961206		8.6	2320	2.2	8.9	1717	1747	5	29.8	131.0		0.001	42.5		0.050	0.5		0
Pit 5	970224	1			1.2	1.9													
Pit 5	970224	2	8.2	2390	2.9	1.9	1888	1898	10	37.0	171.0			47	0.130	0.028	1.2		0
Pit 5	970514	0	8.5	1658	11.7	10													
Pit 5	970514	1																	
Pit 5	970514	2	8.5	1673	11.4	11				21.9	103.0		0.001	30	0.010	0.003	6.1	0.003	0
Pit 5	970618	0			21.6	11													
Pit 5	970618	1			21.2	11													
Pit 5	970618	2	8.6	1990	19.6	8.9													
Pit 5	970818	0			18	9													
Pit 5	970818	1			17.1	9													
Pit 5	970818	2	9.1	2080	16.7	8.8	1472	1478	6	26.5	116.0		0.001	41	0.010				0
Pit 7 (TP7)																			
Pit 7	960516	0			10.1	12													
Pit 7	960516	0.5	8.2	983	8.2	12	681	727	46	10.0	61.6	45.7	0.001	45	0.100	0.030			
Pit 7	960617	0																	
Pit 7	960617	0.5	8.4	1256	17.6	8.8	952	964	12	15.0	50.0	171.0	0.001	55.5		0.030			
Pit 7	960821	0			18.3	7.9													
Pit 7	960821	0.5	8.2	1263	18.1	7.9	869	894	25		77.9	52.2	0.016	42		0.022			
Pit 7	960906																		
Pit 7	960918																		
Pit 7	961017	0			2.7	11													

Loc	Date	Dep (m)	pH	Cond (uS /cm)	Temp (°C)	DO	DS (ppm)	TS (ppm)	Turbidity (mg/L) (ppm)	Nap Acids (ppm)	CHL	SO4	CHL'a'	DOC	NH3	NO2+NO3	TN	o-PO4	TP
Pit 7	961017	0.5	8.2	1219	4	10	901	951	50	23.9	73.6	45.0	0.014	44		0.030	1		0
Pit 7	961206		7.7	1628	0.8	3													
Water Capped Fine Tails Demonstartion Pond (DP)																			
DP	960318	0			0.5	13													
DP	960318	1	8	915	0.7	12	684	730			42	124	0.001	45	0.1			0.01	
DP	960318	2	8	904	3.1	10	724	729		9	42.8	129		43	0.1	0.03	1.9	0.008	0
DP	960516	0	8	766	8.2	11	559	598			34.1	94.5		37.5	0.1				
DP	960516	1			8.1	12													
DP	960516	2	8	745	8	12	562	576		4	33.8	96.1	0.001	38	0.1	0.06	0.8		0
DP	960607	0			18	10													
DP	960607	1			18	9.8													
DP	960607	2	9	784	17.5	9.6	588	589		6	34.5	99.1		39.5	0.06	0.03	4.1	0.03	0
DP	960617	0	9	798	18.2	9.3	588	593			40	99		39	0.05	0.03			
DP	960617	1			18.2	9.4													
DP	960617	2	9	814	18	9.3	581	593		5	42	100	0.001	39	0.05	0.03	1.5	0.03	0
DP	960617	2.5			17.7	4.7													
DP	960709	0			20.8	9.4													
DP	960709	1			20.8	9.3													
DP	960709	2	9	814	20.4	9.2	555	567			36	102	0.001	32	0.1	0.03	1.4		0
DP	960821	0			17.5	9.3													
DP	960821	1			17.7	9.3													
DP	960821	2	8	807	17.6	9.3	550	555			36.1	100	0.001	36.5	0.02	0.039	1		0.1
DP	960821	2.7			17.5	9.3	540	552											
DP	960821	2.85			17.5	9.3	559	1681											
DP	960821	3						180703											
DP	960821	3.15						330369											
DP	960821	3.3						328428											
DP	960906																		
DP	960917	0			15.5	9.8					35.1	93.8	0.001	27	0.09	0.01	0.7		0.1
DP	960917	0.5			15.3	9.7													
DP	960917	1			15.1	9.7	568	587											

Loc	Date	Dep (m)	pH	Cond (uS /cm)	Temp (°C)	DO	DS (ppm)	TS (ppm)	Turbidity (mg/L) (ppm)	Nap Acids (ppm)	CHL	SO4	CHL'a'	DOC	NH3	NO2+NO3	TN	o-PO4	TP
DP	960917	2	8	791	14.5	9.6	544	556		5									
DP	960917	2.5			14.5	9.6													
DP	960917	3.5			14			293491			156	16.7		51	3 1	0.032	4.1		0.1
DP	960917	4			14			248673		76	127	30.5		47.5	2.55	0.026	3.4		0.1
DP	960917	5			12.5			319106			172	5.4		61.5		0.08	4.5		0.3
DP	960917	6			11 5			319473			167	6.5		58.5	4.42				
DP	960917	7			11			317380		102	167	5.3		57.5	3.91	0.113	4.6		0.2
DP	960917	8			11			319742			164	5.2		65.5	4.29	0.076			
DP	960917	9			10.5			318607			161	7.2		62.5	4.98	0.075	5.1		0.2
DP	960917	10			10.5			326565		91	164	3.2		68.5	4.66	0.01			
DP	960917	11			10.5			336291			154	41.3		50.5	5.1	0.148	3.9		0.1
DP	961017	0			4.2	12													
DP	961017	1			4.3	12													
DP	961017	2	8	776	4.3	12	558	568		8	33.4	97.2	0.002	33.5	0.1	0.03	7.8		0.2
DP	961205		8	891	1.9	12	635	648		7.58	42.2	116	0.001	31.5	0.5	0.03	0.9		0
DP	970224	1			0.6	9.1													
DP	970224	2	8	990	1.3	9.2	682	687		9	54.3	147		34.5	0.07	0.02	1.1		0
DP	970224	2.5			2.8	9.4													
DP	970514	0	8	757	11.1	11													
DP	970514	1			11	10													
DP	970514	2	8	754	11	10				6.34	37.7	94.2	0.001	26	0.01	0.004	2.2	0.003	0.2
DP	970618	0			20.8	8.9													
DP	970618	1			20.6	8.9													
DP	970618	2	8	837	20.5	8.6													
DP	970618	2.5			16.6	8.2													
DP	970818	0			18.5	9													
DP	970818	1			18.3	8.7													
DP	970818	2	9	793	17	8.6	0.054	0.056		6.5	39.1	94.2	0.001	32	0.01				0
DP	970818	2.5			16.9	8.6													
DP	970818	2.8	9	766			0.054	0.0573											
DP	970818	2.95					0.1358	5.8475											
DP	970818	2.96			17	3.5													

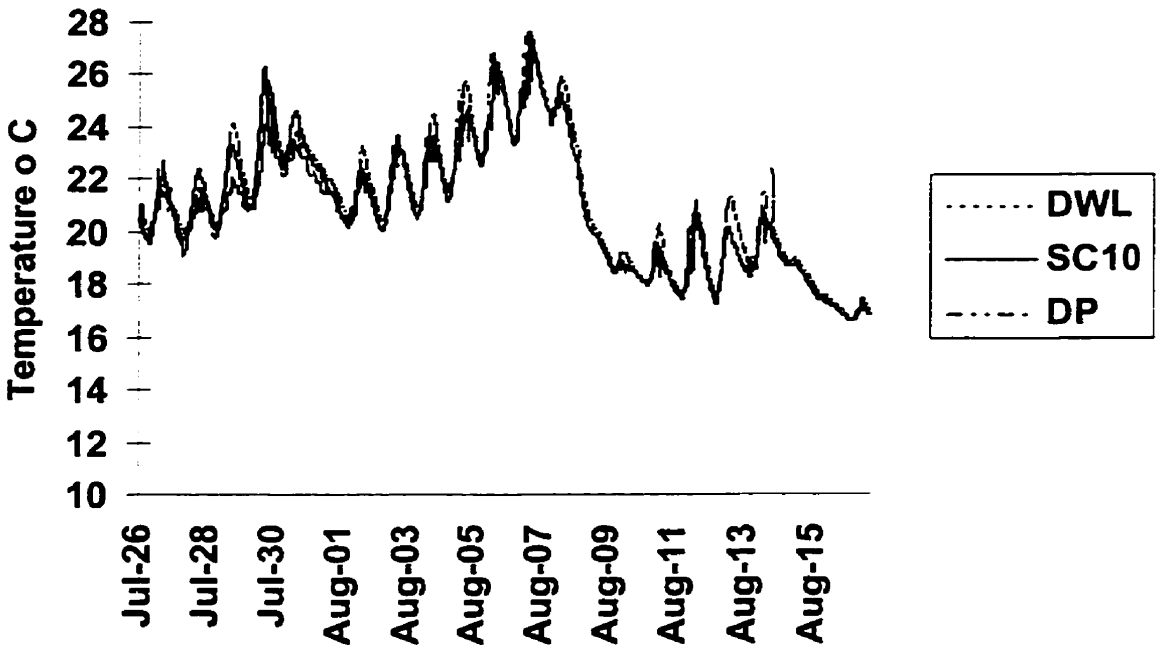
Loc	Date	Dep (m)	pH	Cond (uS /cm)	Temp (°C)	DO	DS (ppm)	TS (ppm)	Turbidity (mg/L) (ppm)	Nap Acids (ppm)	CHL	SO4	CHL'a'	DOC	NH3	NO2+NO3	TN	o-PO4	TP
DP	970818	3.1						28.9284											
DP	970818	3.25						29.6902											
DP	970818	3.4						31.4397											
DP	970818	4			15.9			31.5875		71.84	166	9		53.5	4.3				0.1
DP	970818	5			15.4			32.4168		80.83	180	4.1		55	4.85				0.1
DP	970818	6			14.9			32.8625											
DP	970818	7			11.6			31.8219		80.76	173	5.5		51.5	5.55				0.1
DP	970818	8			12.2			31.3509											
DP	970818	9			10.8			32.9654		77.54	179	7.6		54	5.7				0.1
DP	970818	10			11.4			33.2498		77.09	179	6.5		54	5.55				0.1

Appendix B

LGS bioassay weekly food and water volume; values are volume per fish.

Week	vol. of water (liters)	vol. of food (twice daily)
week 1 (day 1-7)	0.18	12 µl
week 2 (day 8-14)	0.21	18 µl
week 3 (day 15-21)	0.21	18 µl
week 4 (day 22-28)	0.29	25 µl
week 5 (day 29-35)	0.35	31 µl
week 6 (day 36 - 42)	0.40	36 µl
week 7 (day 43 - 49)	0.40	44 µl
week 8 (day 50 - 56)	0.40	89 µl

Appendix C: Daily temperatures, recorded by dataloggers, of sites used in mesocosm experiment



Temperature (°C) of water in sites used for the mesocosm experiment. Temperatures were taken on a weekly basis with a hand-held probe.

Date	DWL	DP	SC10
July 17	21.3	20.8	20.8
July 23	21.5	21.5	21.4
July 31	22.5	22.1	21.4
August 6	23.6	23.5	23.4
August 13	18.9	18.7	18.3
August 20	18.3	17.9	17.6

Appendix D

Suspended solids (mg/L) of sites containing TPW, MFT + water and no waste (natural water only). Means ranged from 14.33 to 28.33 mg/L in the TPW group, 11.0 to 12.50 mg/L in the MFT + water group and 5.71 to 5.80 mg/L in the no waste, or natural water group. Each point on the graph represents one reading from one site at each of the dates. Data were obtained from SynCrude Canada Ltd.

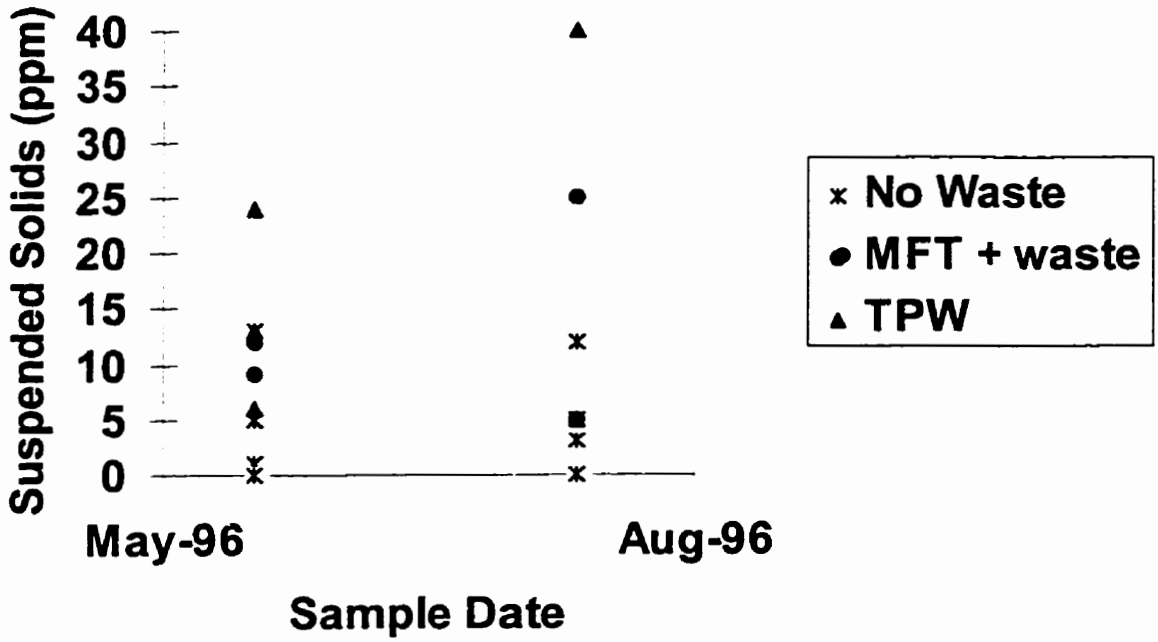
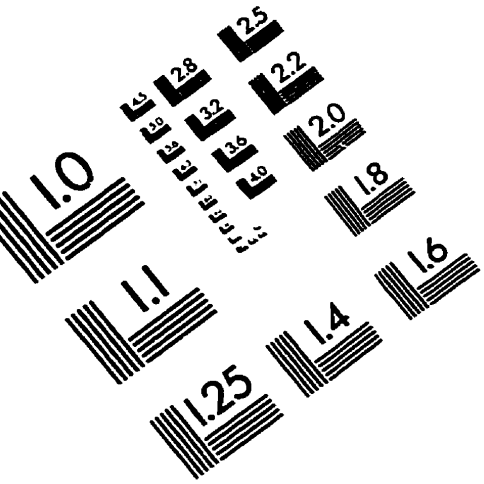
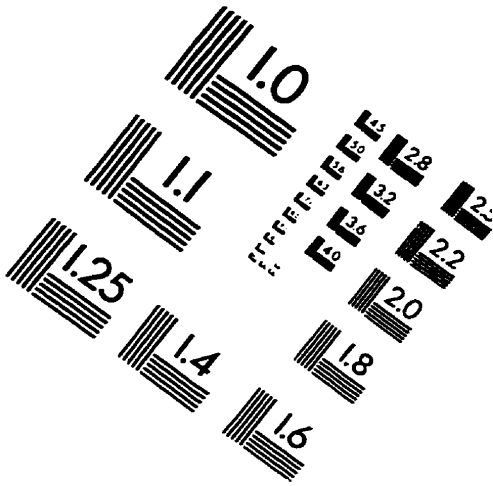
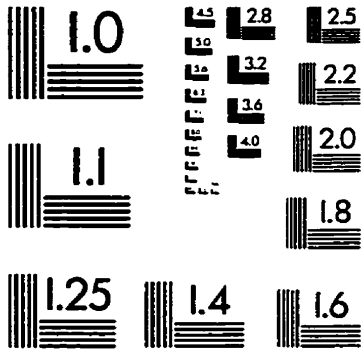
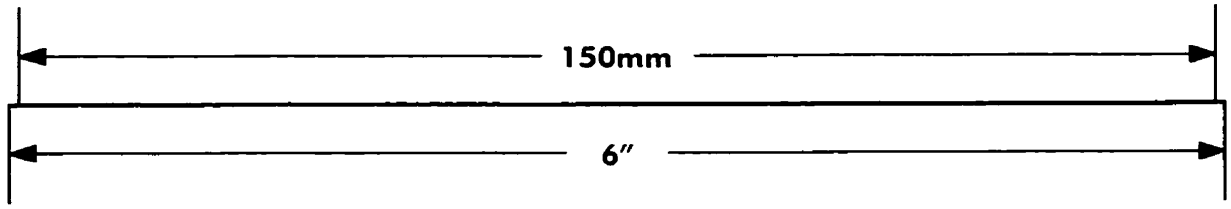


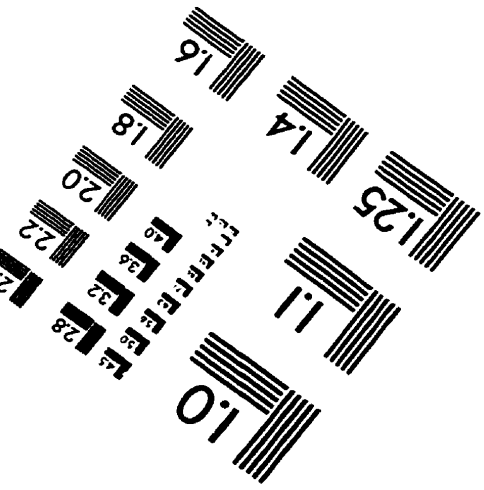
IMAGE EVALUATION
TEST TARGET (QA–3)

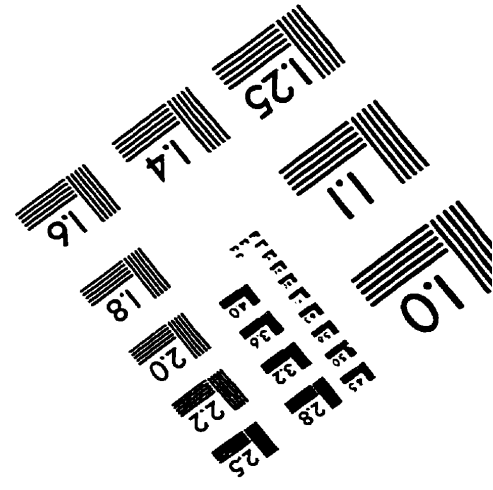












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