Effects of Contaminants on Aquatic Organisms in the Peace, Athabsaca and Slave River Basins

2

Synthesis Report

Canada Alberta Northwest Territories

Northern River Basins Study

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Synthesis Report

**Cover Photo: Commercial Fishing on Lake Athabasca.
Courtesy of Leonard Flett.**

NORTHERN RIVER BASINS STUDY
SYNTHESIS REPORT NO. 2

EFFECTS OF CONTAMINANTS ON AQUATIC ORGANISMS IN THE PEACE, ATHABASCA AND SLAVE RIVER BASINS

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SUMMARY

To determine whether contaminants in the NRBS study area were causing harmful effects on biota of the systems and identify methodologies for monitoring biological effects, the Contaminants Component developed a work plan with four primary objectives. These objectives were:

- to conduct a basin-wide survey of biochemical responses to contaminants in at least one major resident fish species;
- to conduct a basin-wide survey of toxicity in bottom and suspended sediments;
- to assess the utility of semi-permeable membrane devices as substitutes for wild fish in long term biological effects monitoring;
- to assess the feasibility of using small, locally-resident fish species as alternates to large adult fish in long term biological effects monitoring.

Burbot were chosen as the primary species for biomonitoring in the basin-wide fish survey. Northern pike, longnose sucker and flathead chub were identified as other potential species for monitoring.

Four types of physiological response were selected. Three of the responses were selected because they either were known to be caused by pulp mill effluents or by organochlorines or both. These responses were:

- the induction of liver detoxification enzymes;
- reproductive hormone levels and gonad morphology;
- effects on liver vitamins;
- induction of metallothionein activity.

The main null hypothesis tested was: for three classes of sites (reference, near-field and far-field relative to pulp mills) there are no differences among classes in the physiological parameters measured. The data were also examined for any site anomalies within the classes to flag these as possible indications of site-specific effects.

The main conclusions of the basin-wide survey were:

- no effects on the liver detoxification enzymes of burbot from pulp mill effluents are evident;
- mild induction of liver detoxification enzymes was detected in burbot from the Athabasca River near the oil sands plants and in burbot from the Wabasca River;
- evidence exists for differences in sex hormones in both male and female burbot associated with pulp mill effluents but site related differences in oocyte size or fecundity corresponding to low female hormone levels were not apparent;
- concentrations of the liver vitamins retinol, dehydroretinol, and retinyl and dehydroretinyl esters were comparable to those reported elsewhere for uncontaminated locations and were greater than those reported in fish exposed to contaminants in polluted locations;

- hypothesized induction of metallothioneins in response to effluents from pulp mills was not supported by the results of the metallothionein survey although there were several sites where metallothioneins were elevated.

One unusual feature of the fish distributions in the basin-wide survey was the high proportion of immature adult size fish in the near-field and far-field regions. This data is suggestive of a pulp mill-related effect on age-to-maturity in fish. Unfortunately, the distribution of mature adult fish in this survey was also anomalous since there was a higher proportion of mature females in the reference areas relative to males while the reverse was true in other areas. The possibility that burbot utilize different parts of the river basins at different stages in their lives complicates the interpretation of the immature fish distributions as an effluent-related effect.

In the sediment toxicity studies, four species of benthic invertebrates were exposed in chronic laboratory toxicity tests to depositional and suspended sediments collected from throughout the NRBS study area. The results indicated that whole sediments from some locations had detrimental effects on at least one of the species. However, the sites with the greatest effects were the furthest upstream site and the farthest downstream site in the Athabasca River. Neither of these sites is close to any of the industries and neither is in the oil sands area. Therefore, there was no indication in these toxicity assays that pollution from industrial development has led to toxicity problems in the sediment.

Two new approaches to biological monitoring were evaluated in the study. These were the use of semi-permeable membrane devices (SPMDs) and the suitability of small fish species for monitoring. SPMDs are thin polyethylene tubes containing fish lipid that are suspended in water bodies and effluents after which the recovered lipid can be analysed chemically or used in biological testing. In the NRBS study, river water and effluents from major industries were sampled and tested for their ability to induce detoxification enzymes in cultured fish liver cells. The study demonstrated that the levels of inducers in effluent from all the pulp mills in the NRBS basins are low compared to elsewhere. Thus, the SPMDs provided complementary evidence that enhanced the interpretation of the results of the basin-wide survey. The geographical distribution of MFO inducers in the SPMDs was consistent with that found in the basin-wide burbot survey, drawing attention to the oil sands area of the basins. The site specific character of the technique allowed the identification of tributary hot spots that were not detected in the wild fish survey.

Suitability of smaller fish species for monitoring receiving environments was evaluated by determining the degree to which physiological responses of small fish were similar to large fish and the degree to which the responses were reflective of localized environmental conditions. Two study areas from the upper Athabasca River immediately downstream from pulp mills were intensively studied. The species chosen as sentinels were spoonhead sculpin for the Hinton study area and lake chub in the Whitecourt study area. Based on the results of the surveys, the potential to use small fish species is very high. In particular, the species were abundant, well distributed and relatively stationary suggesting that the observed fish responses reflected the local environment. The typical biochemical measurements made on larger fish species were also possible using smaller species.

These included: body and organ metrics, reproductive parameters, age estimates, liver detoxification enzyme activity, and reproductive steroid production.

In summary, based on the basin-wide survey of physiological parameters in wild fish, on the sediment toxicity studies, and on both of the newer approaches to biological monitoring, there was limited evidence of harmful effects of pulp mill effluents on biota in the basins. Although there was suggestive evidence of physiological responses in fish downstream from the pulp mill examined in the preliminary studies, by the end of the study most of these responses were not evident. This apparent improvement was presumably due to process modifications that occurred at the mills during the study.

An unexpected finding was that the highest liver enzyme responses in the basin-wide survey were observed in areas of the lower Athabasca River far downstream from the pulp mills and in a Peace River tributary that originated in the oil sands area and that did not receive pulp mill effluent. There were also unexplained responses in both the metallothionein and liver vitamin parts of the basin-wide survey. For the most part, these responses occurred mostly in the downstream areas and appeared to be unrelated to pulp mills or other obvious discharge sources.

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1.0 INTRODUCTION

The Northern River Basins Study (NRBS) was established in 1991 by the governments of Canada, Alberta and the Northwest Territories to assess the cumulative effects of industrial, municipal, agricultural and other development on the Peace, Athabasca and Slave river basins and to provide recommendations to the governments on their future management. To complete this assessment, the NRBS has conducted a series of scientific studies to gather and assess information on water and ecosystem quality, fish and fish habitat, vegetation, wildlife, hydrology and use of aquatic resources in the northern river basins.

Eight scientific component groups were established to design and carry out the study's science program. To provide guidance for the design of the scientific program, the Northern River Basins Study Board developed a vision statement identifying 16 guiding questions. As the study progressed, the Contaminants Component, originally given responsibility for determining the identity, concentrations, distribution and trends of contaminants in the study area, was also asked to address questions on the effects of contaminants on the Peace, Athabasca and Slave ecosystems and on the design of a long term program to monitor for them. This report summarizes the activities of the Contaminants Component to address these latter questions.

> *Question #1a. How has the aquatic ecosystem, including fish and/or other aquatic organisms, been affected by exposure to organochlorines or other toxic compounds?*

> *Question #14. What long term monitoring programs and predictive models are required to provide an ongoing assessment of the state of the aquatic ecosystems? These programs must ensure that all stakeholders have the opportunity for input.*

In developing a work plan to address question #1a, the members of the Contaminants Component focused on the level of stressor specificity required by the question. The question did not ask what changes had occurred in the NRBS ecosystems due to development in general, but rather whether there had been effects attributable to chemical contamination by organochlorines and other toxic compounds. Thus, to address question #1a, the component had to develop a work plan that would produce information specific to toxic substances.

In addition to identifying the sources of contaminants, another key consideration was the target ecological level examined. When toxic substances are released into ecosystems, effects may be observed at various ecological levels beginning at the individual organism level and progressing through population, community and ecosystem with increasing severity of exposure.

Whether effects will be observed and the ecological level at which they will be observed depends on the susceptibility of various individuals and species, the toxicity of the chemical(s) and the exposure concentrations and conditions. This relationship between level of exposure and level of impact is shown schematically in Figure 1. Ecosystems are integrators of effects from various stressors. When present at sufficiently high concentrations, toxic substances can be a stressor. But any resulting effects on populations, when they occur, are generally indistinguishable from effects on populations due to other stressors. Even when the substances or effluents discharged into a system are known to be capable of causing certain effects, attributing effects observed in field studies to those substances without considering the specificity of the response can be misleading. For example, several field studies in the 1980s observed changes in blood chemistry in fish in the receiving waters around pulp mills and attributed the effects to substances present in the effluent (Södergren 1989). These same biological responses can be caused by habitat changes in the receiving waters related to the discharge and to stresses on the fish due to sampling. Thus, contaminants were only one possible cause for the effects studied and the occurrence of the biological responses in wild populations could not be taken as evidence of contaminant effects.

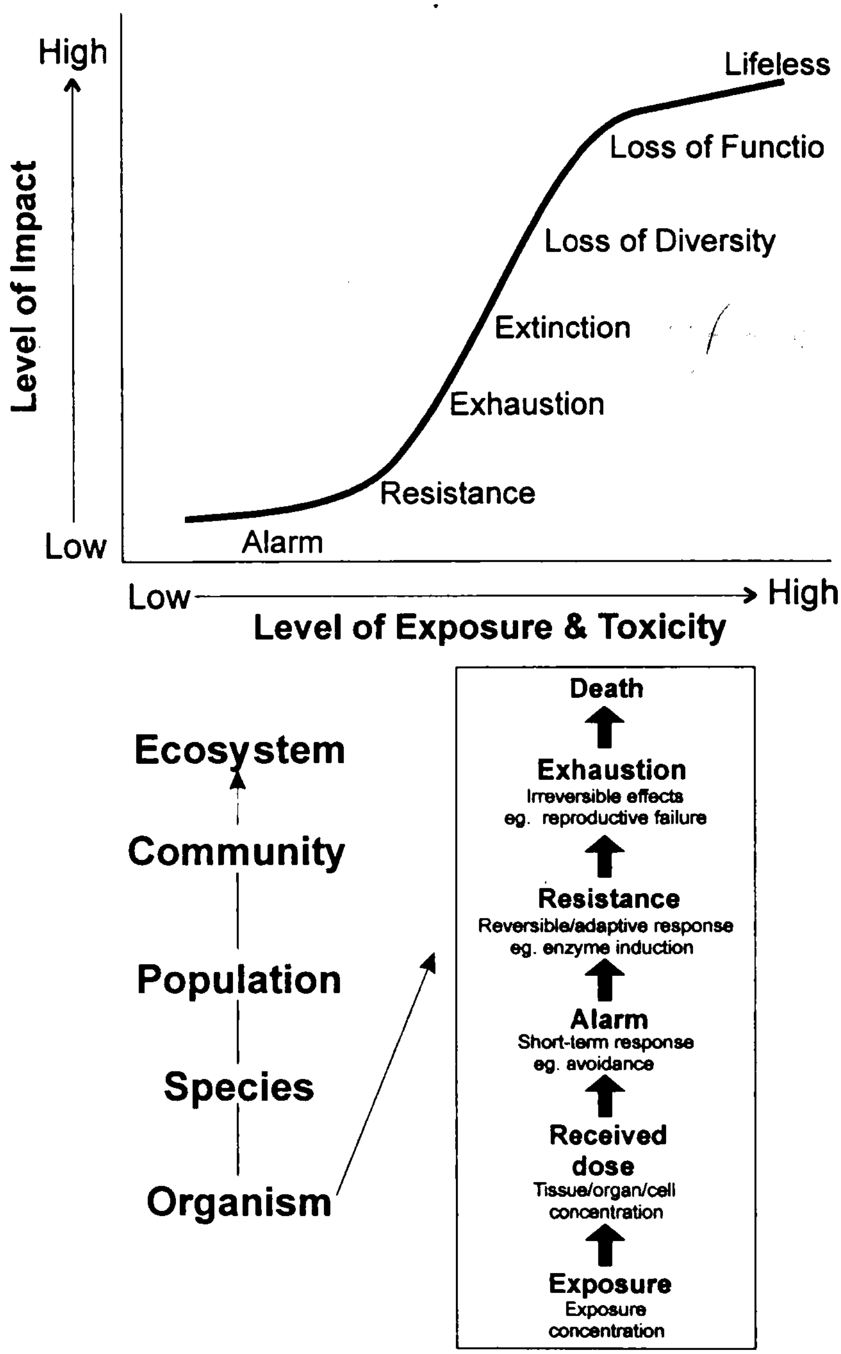

Figure 1. Schematic relationship between severity of exposure, degree of impact, type of effect and level in ecosystems where effects of toxic substances are observed. The box contains a representation of the levels of response of individual organisms to contaminant exposure and examples of the type of observations or measurements made at each level.

In the work plan developed by the component to address the effects issue, two primary research approaches were identified: one targeted at the level of the individual organism based on a basin-wide survey of biochemical indicators in wild fish, the other focussed on toxicity to benthic invertebrate communities in sediments.

In developing a plan to address the biological monitoring part of Question #14, the Contaminants Component members initially favoured monitoring based on the same wild adult fish species used in the basin-wide study. This approach is already employed in monitoring programs in many locations, including the Slave River Monitoring Program carried out by the Government of the Northwest Territories. However, during the basin-wide fish survey, it became apparent that there were concerns about the availability and mobility of large mature fish that affected the utility of a monitoring strategy based on large fish. To be most useful, a fish species should be locally-resident and easily captured in sufficient numbers at predictable times of the year. It appeared from the fish sampling conducted in the early years of the NRBS that there were few large fish species that were widely distributed throughout the system. Two widely distributed species available in sufficient numbers were burbot (*Lota lota*) and longnose sucker (*Catostomus catostomus*). However, traditional knowledge from native fishermen and information developed by the NRBS and by corollary studies indicated that both these species were migratory at certain times of the year. In addition, during the fish survey itself, difficulties were encountered in obtaining enough fish in the time period allowed at each site. Furthermore, the fish collected during the survey were not homogeneously distributed by sex or age. There was possibly some unexplained ecological variable influencing fish distributions. This made it difficult to obtain comparable samples from sites throughout the basin. Because of these difficulties, the component initiated research to examine two alternate approaches to long term biological monitoring. These were the use of semi-permeable membrane devices (SPMDs) and the use of smaller, less mobile fish species.

Thus, the Contaminants Component work plan to address questions #1a and #14, had four primary objectives. These objectives were:

1. to conduct a basin-wide survey of biochemical responses to organochlorines and other contaminants in at least one major resident fish species,

2. to conduct a basin-wide survey of the toxicity in bottom and suspended sediments for use in the Sediment Quality Triad approach

3. to assess the utility of semi-permeable membrane devices as potential substitutes for wild fish in a long term monitoring program

4. to assess the feasibility of using small, locally-resident fish species as alternates to large adult fish in a long term biological effects monitoring program

This document provides a summary of the research undertaken to address these objectives.

2.0 BASIN-WIDE SURVEY OF BIOCHEMICAL INDICATORS IN WILD FISH

2.1 INTRODUCTION

The rationale behind the survey of fish physiological indicators is that among the levels of responses of individual organisms like fish to contaminants, there are some responses that can be very specific to contaminants. When coupled with contaminant exposure information, such biochemical responses have proven very useful in providing an indication of whether exposures to toxic substances have been sufficiently serious to cause physiological responses in the organism. The important initial decisions in this approach involve choice of indicator species and choice of biochemical responses.

In our studies, the key criteria regarding choice of biomonitor species were sensitivity, distribution, and mobility. Since there are differences between species in the degree of their physiological response to toxic substances, it is important to chose a biomonitor that responds strongly to the stressor. In addition, the species should be non-mobile so that they will be representative of the area in which they are captured and should be widely distributed within the ecosystem under study to allow the geographical distribution of the responses to be determined.

The NRBS study of physiological effects in fish consisted of a preliminary survey of mixed function oxygenase (MFO) induction, gonad morphology and reproductive hormones in fish collected in 1992, followed by selection of an indicator fish species, implementation of a basin-wide collection of that species, and analysis of the full suite of biochemical indicators in the indicator species.

The four physiological responses chosen for the NRBS studies were: induction of MFOs, the enzyme system responsible for detoxification of contaminants in the liver and other organs; alterations in levels of the reproductive hormones estradiol and testosterone and altered gonad morphology; induction of metallothionein proteins; and alteration in the levels and forms of A- and E-type vitamins in the fish livers. These bioindicators are discussed in detail below. Although a variety of contaminants may cause the responses, all of the responses chosen are specific to contaminant exposure.

2.1.1 Induction of Mixed Function Oxygenase Enzymes

Mixed-function oxygenases are multi-enzyme complexes, consisting of cytochrome P450 and NADPH-cytochrome P450 reductase. They catalyse the oxidation of a variety of low molecular weight naturally occurring substrates such as steroids and fatty acids and the four gene families of P450 are the main enzyme pathway responsible for biotransformation of manmade organic chemicals by the liver and other organs. Although measurable MFO activity can be found in the livers of virtually all higher organisms, synthesis of many of the cytochromes P450 is induced by different compounds. Polychlorodibenzo-p-dioxins (PCDDs) and polychlorodibenzo-furans (PCDFs) are especially potent inducers of the P450 1A type, cytochromes known typically to be inducible by planar polycyclic aromatic compounds such as methylcholanthene and benzo(a)pyrene. This induction is thought to occur due to binding of the planar polycyclic aromatic to an intracellular receptor known as the aryl hydrocarbon receptor, or AhR (Figure 2) (Safe 1990). Although an endogeneous ligand for the AhR receptor has not been identified, the high affinity of the AhR for many planar aromatic compounds, including PCDDs, is well documented. The increase in hepatic MFO activity is among the most sensitive cellular responses to small doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Giesy *et al.* 1994).

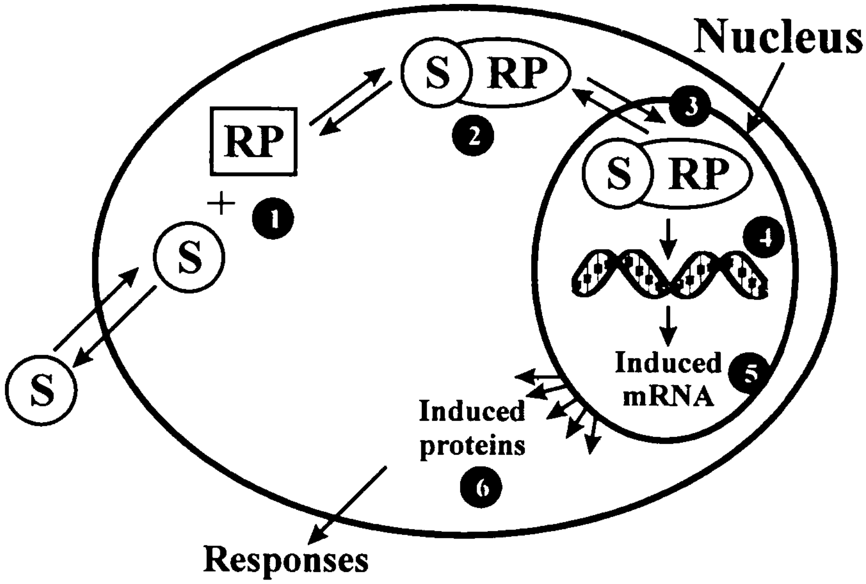

Figure 2. Proposed model for the mechanism of action of chlorinated aromatic hydrocarbons like TCDD and related substances. The steps involved in the proposed mechanism are 1. reaction of the substrate (S) and the receptor protein (RP) formation of the cytosolic receptor complex; 2. activation of the complex; 3. formation of nuclear receptor complexes; 4. interaction with specific DNA sequences; 5. enhanced gene expression; and 6. protein synthesis.

The measurement of MFO induction in organisms is thought to be a useful bioindicator since the toxicity of several classes of organochlorines, including PCDDs/Fs and planar polychlorobiphenyls (PCBs), may be mediated through the AhR. Apparent AhR-mediated responses include lethality, reproductive and developmental toxicity, immunotoxicity and cancer (Okey *et al.* 1994). Members of many common families of contaminants possess the correct structural conformation for binding to the AhR including: polycyclic aromatic hydrocarbons (PAHs); PCBs; PCDDs, PCDFs; polychlorodiphenylmethanes; polychloronaphthalenes; polychlorinated azoanthracenes; polychlorobenzothiophenes; and polychloroterphenyls.

In environmental surveys involving wild organisms like fish, the activity of hepatic monooxygenases is assessed using an *in vitro* approach involving microsomes isolated from tissue samples that have been fast-frozen in the field at very low temperatures (dry ice or liquid nitrogen). In the laboratory, isolated liver microsomes are exposed to test substrates. MFO activity is determined from the rate of reaction of these substrates with the liver microsomes. Two common assays for expressing MFO activity are ethoxyresorufin-O-deethylase or EROD, which is based on the reaction of ethoxyresorufin as substrate, and aryl hydrocarbon hydroxylase or AHH, which is usually based on the oxidation of the substrate benzo-a-pyrene. Since a correlation exists between EROD and AHH, either can be used to determine MFO activities. Cytochrome P450 difference spectra are used for some samples to confirm that the enzymes have not become denatured. We employed all three of these measurements in the NRBS basin-wide survey.

There may be a difference in the severity of the effects produced by PAHs compared to PCDDs and other chlorinated substrates. Although PAHs react via the AhR, they have a high rate of metabolic clearance and if the exposure is not sustained, their effect is not sustained. The full range of responses may be exclusively caused by chlorinated organic compounds like PCDDs that are resistant to metabolism and interact with the AhR in a sustained fashion. This has implications on the use of MFO induction as a bioindicator of AhR-mediated toxicity since binding to the AhR is necessary but not sufficient to cause the whole range of effects.

Thus, for the NRBS, the EROD and AHH measurements were used as a bioindicator of the potential for toxicity due to organochlorines. Since EROD induction is among the most sensitive responses caused by small doses of TCDD and other toxic organochlorines, the absence of significant MFO induction can be taken as good evidence that these substances are not present at levels expected to cause toxic effects, unless levels of contaminants are so high that liver toxicity is occurring. Conversely, an observation of significant MFO induction can only be taken as a possible indicator of toxicity until the inducers are identified and the strength of their interaction with the AhR is determined. In addition, no induction of activity can be assumed to be toxicologically relevant if the normal activity is only minimally changed.

2.1.2 Reproductive Steroids/Gonad Morphology

Of the various fish physiological responses that have been the subject of field and laboratory studies, the most serious whole organism responses seen in wild fish and life cycle laboratory studies are related to reproduction. Diminished reproductive performance related to changes in maturity and fecundity have the potential to result in significant changes at the population level.

One of the most widely documented sources of reproduction-related responses is to pulp mill effluent. Studies conducted at several Scandinavian and North American sites during the late 1980s and early 1990s found that fish exposed to effluent from some bleached kraft pulp mills exhibited an increased age to maturity, smaller gonads, and lower fecundity with age in both males and females, as well as an absence of secondary sex characteristics in males, and females failed to show an increase in egg size with age (Sandström *et al.* 1988; Södergren 1989; McMaster *et al.* 1991, 1992b; Munkittrick *et al.* 1992b; Munkittrick 1992; Adams *et al.* 1992; Hodson *et al.* 1992; Friesen *et al.* 1994; Gagnon *et al.* 1994a,b; Sandström 1994). One of the major disruptions seen was a change in the steroid synthetic pathway that appeared to prevent the production of normal steroid hormone levels by the gonads.

The field observations have been confirmed by laboratory studies. In one study, fathead minnows (*Pimephales promelas)* exposed throughout their life cycle to secondary-treated bleached kraft mill effluent demonstrated delays in sexual maturity, reduction in egg production, depression of secondary sexual characteristics, and depressions of hormone production at exposure concentrations greater than 20% (Robinson, 1994). The reproductive responses were also confirmed by Kovacs *et al.* (1995), in which the threshold for reproductive responses for effluent from a bleached kraft mill was found to be less than 2% effluent during chronic exposures.

These reproductive responses have not been observed at all pulp mill sites. Studies conducted near a secondary-treated bleached kraft pulp and paper mill discharge in Ontario found increased MFO levels but minimal changes in reproductive steroids (Servos *et al.* 1992, 1995; Munkittrick *et al.* 1994a). Within the NRBS study area, a series of studies which followed the installation of chlorine dioxide substitution at the bleached kraft mill on the Wapiti River concluded that, apart from MFO induction, there were few consistent changes in a large number of physiological parameters (Swanson *et al.* 1993, 1994; Kloepper-Sams and Benton, 1994; Kloepper-Sams *et al.* 1994; Owens *et al.* 1994 a, b; Pryke personal communication). The absence of significant changes in physiological parameters in these studies could have been related to a number of factors, including the dramatic seasonal changes in dilution, the high mobility in that system of the fish species studied (i.e. longnose sucker and mountain whitefish), in addition to the pulping process and water treatment employed by the mill.

A number of physiological parameters have been evaluated for their ability to predict the reproductive responses in fish exposed to pulp mill effluent. Many studies have focused on the levels of the steroid hormones testosterone, estradiol, 17α,20ß-dihydroxy-4-pregnen-3-one, and 11-ketotestosterone (McMaster *et al.* 1991; Munkittrick *et al.* 1992b). The measurement of these steroid hormone levels appears to offer the best potential to predict the impacts of sexual maturity and fecundity. Therefore, we included measurement of these steroid hormones as part of the NRBS basin-wide survey. Life cycle studies showed a very good correlation between depressions in circulating steroid levels and altered secondary sexual characteristics with reproductive changes similar to those seen in wild fish (Robinson 1994). It is commonly assumed that changes in steroid hormone levels are predictive of changes in reproductive performance, although different species respond differently to exposures, and reduced steroid levels in some species are not translated into responses at the whole organism level.

Species in which steroid effects have been observed in pulp mill field studies other than NRBS include white sucker (*Catostomus commersoni*) (McMaster *et al.* 1991; Friesen *et al.* 1994), longnose sucker (Munkittrick *et al.* 1992a), lake whitefish (*Coregonus clupeaformis*) (Munkittrick *et al.* 1992b), peamouth chub (*Mylocheilus caurinus*) (Gibbons *et al.* 1994), mummichog (*Fundulus heteroclitus*) (McCarthy, unpubl. data), and brown bullhead (*Ictalurus nebulosus*) (McMaster *et al.* 1995), as well as fathead minnow (Robinson, 1994) and goldfish (*Carassius auratus*) (McMaster *et al.* 1996) in laboratory exposures. Even for species for which whole organism effects were not observed after changes in steroid hormone levels, the steroid changes may be predictive of reproductive effects in more sensitive species.

In addition to the depressed steroid hormones seen near pulp mills, depressed steroid hormones have been seen after exposure to other chemicals, for example: PCBs (Freeman and Idler, 1975; Sivarajah *et al.* 1978; Thomas, 1988; Kidd *et al.* 1993), PAHs (Truscott *et al.* 1983; Thomas, 1988; Singh, 1989; McMaster *et al.* 1995), sitosterol (MacLatchy and Van Der Kraak, 1995), fatty acids (Wade *et al.* 1994; Mercure and Van Der Kraak, 1995), nonylphenol (White *et al.* 1994) and contaminants in certain pesticide formulations (Munkittrick *et al.* 1994b; Hewitt *et al.* 1995).

The substances in pulp mill effluents responsible for reproductive hormone effects have not been identified. PCDDs and PCDFs are present in some effluents and are also capable of producing impacts on reproduction and MFOs. They may be exerting an influence at some sites since correlations have been seen between dioxin concentrations and some physiological responses, particularly MFO induction (Kloepper-Sams and Benton, 1994; Hodson *et al.* 1992; Servizi *et al.* 1993). However, the fast recovery time of fish moved to clean water (Munkittrick *et al.* 1992a), the appearance of responses at mills not using chlorine (Munkittrick *et al.* 1994a), the failure of secondary treatment to alleviate reproductive changes, and the rapid recovery in wild fish during shutdown (Munkittrick *et al.* 1992a) suggests that dioxins, or dioxin-like compounds are not likely responsible for these biochemical responses at many sites (Munkittrick *et al.* 1994a; Servos *et al.* 1994). Persistent organochlorines exert much more persistent biochemical disruptions (Muir *et al.* 1990a).

2.1.3 <u>Metallothionein</u>

Metallothioneins (MTs) are low molecular weight sulfhydryl-rich proteins that are the major metal binding proteins in animals and also occur in plants, algae and bacteria. Typically, cysteine comprises about one third of the 60-odd amino acids in MTs (Hamer 1986; Engel and Brouwer 1989). They appear to play a role in homeostatic control of the essential metals zinc and copper. They also appear to act to sequester certain heavy metals like mercury and cadmium and prevent their interaction with critical cellular components like enzymes. Of particular importance to the use of MT as a biochemical indicator of metal exposure is that MT synthesis is induced in animals by exposure to copper, zinc, cadmium, silver, mercury and other heavy metals, and a capacity for the inducible synthesis of MTs has been demonstrated for many diverse tissues and organisms (Klaverkamp and Baron, 1996). Synthesis of metallothionein takes place primarily in the liver and kidney in proportion to the stressor. Thus, concentrations of MT in tissue of organisms from different parts of a watershed has been used as a bioindicator of heavy metal pollution in that system (Klaverkamp and Baron, 1996). However, more information regarding the specificity of the induction response is needed since it has been suggested that factors such as stress, cold, and hypoxia also induce MT (Benson *et al.* 1988, 1990).

2.1.4 <u>Hepatic Vitamins</u>

Retinol (vitamin A_1), dehydroretinol (vitamin A_2) and tocopherol (vitamin E) (Figure 3) have been suggested as sensitive biomarkers of exposure to certain organic pollutants, including some organochlorines, as well as nutritional status of organisms (Spear *et al.* 1989, 1994). Since animals are incapable of *de novo* synthesis of retinoids, all Vitamin A isoprenoidal retinoids come from dietary carotenoid precursors. Under normal physiological conditions, retinoids are stored in the liver as esters of long-chain fatty acids. The dominant forms in fish are retinyl palmitate (RP) and likely dehydroretinyl palmitate (DRP). Retinol and dehydroretinol are hydrolysed from the storage esters and the free retinoids are transported to where they are needed by a highly regulated process involving the retinol/retinol-binding-protein/transthyretin (retinol/RBP/TTR) complex. It is well established that chlorinated organics can affect vitamin A in organisms. Various coplanar PCBs

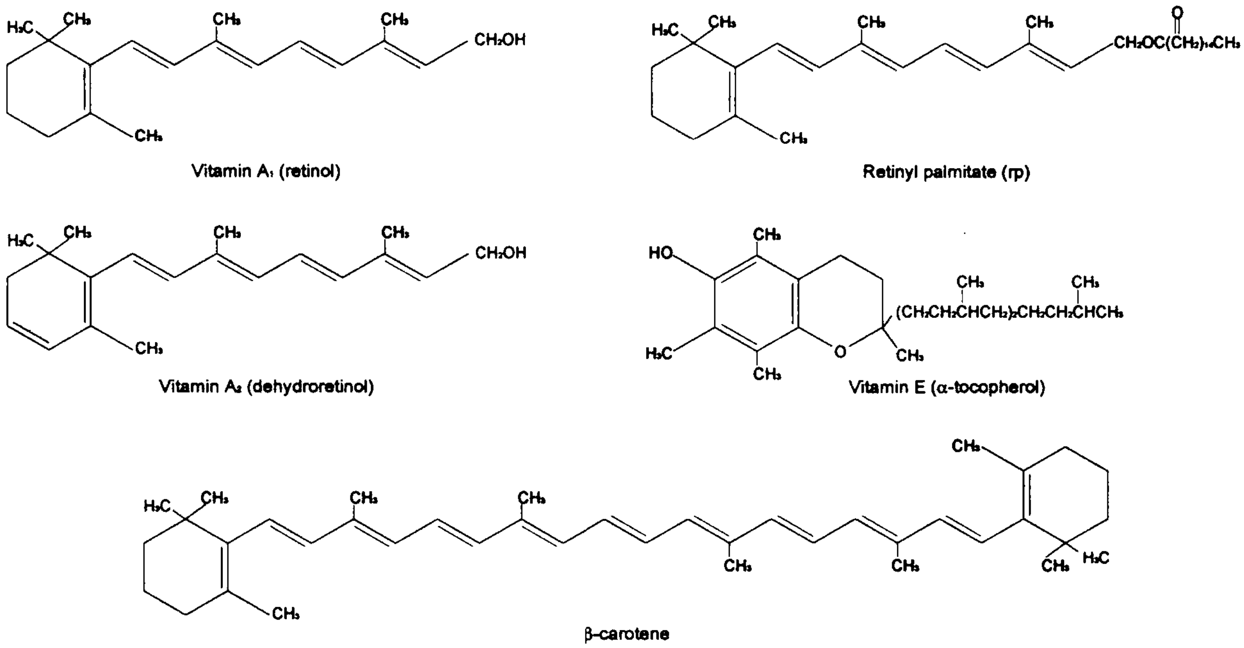

Figure 3. Structures of retinoid vitamins, the main dietary precursors and one of the main storage forms.

have been shown to decrease hepatic vitamin A stores in rat (Azais *et al.* 1987; Spear *et al.* 1988), mouse (Brouwer *et al.* 1985), ring dove (Spear *et al.* 1986, 1989) and lake trout (Palace and Brown 1994). Since a variety of physiological processes including vision, growth, reproduction and immunocompetence depend on a tightly regulated supply of retinoids, depletion of vitamin A compounds can have serious consequences for organisms. Vitamin A deficiency has been shown to cause growth retardation, edema and canal lesions.

Biological effects of PCBs in lab rodents resemble those seen during Vitamin A deficiency (Azais *et al.* 1987; Innami *et al.* 1974; Brouwer and Van den Berg 1984). This has led to the hypothesis that imbalances in Vitamin A homeostasis may be an integral part of the mechanism of action of chlorinated aromatic contaminants. Mechanisms may include catabolism of the retinoids by induced Cytochrome P450 1A1 since a decrease in liver retinoids following exposure to P450 1A1 inducers is well known and accelerated retinoid metabolism has been demonstrated (Spear *et al.* 1989; Bank *et al.* 1989). Other possible non-Cytochrome P450 1A1 mechanisms include alterations of circulating levels of blood retinol by interfering with retinol binding to the retinol/RBP/TTR complex (Brouwer and Van den Berg 1986) and decreased dietary supply due to loss of appetite (Spear *et al.* 1994).

Tocopherol is involved in cellular defence against oxidative damage by free radicals. Exposure to dietary PCBs has been shown to cause a decrease in hepatic tocopherol in fish (Palace and Brown 1994).

Although information about retinoids and tocopherol levels in wild fish exposed to organic contaminants is limited, alterations of vitamin A levels have frequently, but not always, been correlated to mixed function oxygenase (MFO) induction in the liver. Recent unpublished studies also show that retinoid levels are altered in fish exposed to pulp mill effluents (S.B. Brown, personal communication). Low retinoid stores have been observed in white sucker and lake sturgeon (*Acipenser fluvescens*) from the St. Lawrence River system (Spear *et al.* 1992) and Great Lakes birds (Spear *et al.* 1986).

2.2 RESULTS

2.2.1 Preliminary Survey

Fish for the preliminary survey of biochemical responses were collected in the spring and fall of 1992 from the upper Athabasca River between Jasper National Park and the Windfall Bridge, upstream from Whitecourt. There were six sites for the collections (Figure 4), one site upstream and five sites downstream of Hinton. Details of the collection are presented in Barton *et al.* (1993a, b).

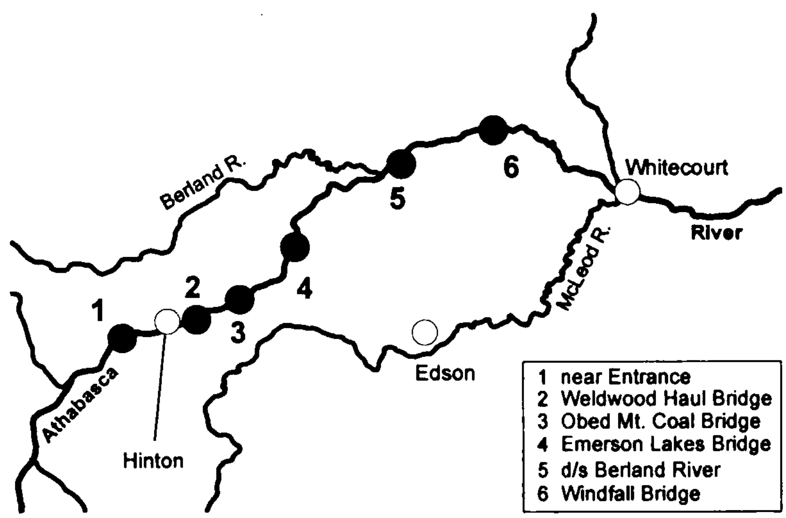

Figure 4. Map of the Upper Athabasca River, showing the sites of fish collection in the spring and fall special fish collections, 1992. (Barton *et al.* 1993a, b).

In the spring collection, the original intention to collect 10 fish of the largest size class for each species was thwarted by low fish numbers throughout the system. Therefore, sampling focused on collecting mature adults. For the most numerous benthivorous fish species, the mountain whitefish (*Prosopium williamsoni*), 10 adult fish were collected at each site. For the most numerous piscivore, northern pike (*Esox lucius*), the desired number was obtained at only the two most downstream sites. Numbers at the other sites varied from 0 to 2 for the downstream sites to 6 at the upstream site. Extra effort to obtain more fish was unsuccessful. At least part of this nonhomogeneous distribution may have been due to differences in habitat (Barton *et al.* 1993 a, b). Other species collected in lesser numbers included white sucker, longnose sucker, bull trout (*Salvelinus confluentus*), rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), Arctic grayling (*Thymallus arcticus*), lake whitefish and burbot.

In the fall of 1992, fish were collected from the same sites as the spring survey, ie. one site upstream and five sites downstream from Hinton. The results parallelled the spring survey. Fish were not sufficiently numerous at any of the sites to follow the original intent to obtain 10 samples of the largest size or age class. Sampling again focused on collecting 10 samples of adult fish of each species. Sufficient numbers of mountain whitefish were collected at all six sites but sufficient numbers of northern pike were caught only at the sites farthest downstream. In the spring survey, it was noted that white sucker and longnose sucker were abundant in the study reach. Therefore, in the fall survey, an attempt was also made to collect a sufficient number of suckers as a second benthivore species. The results of these two fish collections are summarized in Table 1.

Liver samples from mountain whitefish and northern pike collected in the spring and mountain whitefish, northern pike, longnose sucker and white sucker collected in the fall were analysed for

Table 1. Summary of sample numbers, sex, and age range (yrs) for major species sampled during the spring and fall special fish collection.

Species	Site	Spring 1992		Fall 1992	
		Number/Sex	Age Range	Number/Sex	Age Range
Mountain whitefish	Entrance	3M/7F	6-10	5M/5F	6-10
	Weldwood Haul Br.	1M/11F	6-12	2M/9F	5-10
	Obed Mtn. Coal Br.	2M/8F	6-10	2M/8F	5-9
	Emerson Lks. Br.	2M/8F	6-11	2M/9F	6-10
	d/s Berland R.	4M/6F	6-11	1M/9F	6-9
	Windfall Br.	4M/6F	8-12	5M/5F	5-13
Northern pike	Entrance	2M/3F/1U	3-5	-	-
	Weldwood Haul Br.	1M/1F	3	1F	4
	Obed Mtn. Coal Br.	-	-	-	-
	Emerson Lks. Br.	1F	6	1M/1F	4-5
	d/s Berland R.	5M/5F	2-7	6M/4F	3-8
	Windfall Br.	6M/4F	4-8	4M/6F	4-10
White sucker	Entrance			-	-
	Weldwood Haul Br.			1M/1F	6-7
	Obed Mtn. Coal Br.			-	-
	Emerson Lks. Br.			3F/1U	6-8
	d/s Berland R.			3M/7F	6-11
	Windfall Br.			2M/8F	6-11
Longnose sucker	Entrance			3M/7F	7-10
	Weldwood Haul Br.			6M/4F	8-10
	Obed Mtn. Coal Br.			6M/4F	6-10
	Emerson Lks. Br.			7M/3F	6-10
	d/s Berland R.			2M/8F	8-10
	Windfall Br.			1M/6F	7-10

MFO activities using assays for ethoxyresorufin-O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) (Lockhart *et al.* 1996). In addition, difference spectra were taken to allow calculation of cytochrome P450 content.

The difference spectra indicated that most of the samples had experienced some deterioration in sample quality. This deterioration may have compromised the EROD and AHH values. Despite this complication, Lockhart *et al.* (1996) completed the examination of the data for differences between sites. Although the deterioration may have compromised the absolute values of enzyme activity, there was no evidence of selective deterioration between sites, so we hoped that the data could still be used to reveal qualitative differences between sites. Results for mountain whitefish in the spring and white sucker in the fall showed a pattern of EROD and AHH activity consistent with a source of inducing compounds downstream from Hinton (Figure 5).

Although degradation of EROD enzymes in mountain whitefish samples had previously been observed in other field studies (Kloepper-Sams *et al.* 1994), the fact that it occurred in this study was disappointing since it meant that the absolute values for the EROD and AHH induction could not be relied upon. However, the suggested overall geographical pattern in the levels of degraded EROD was consistent with an MFO inducing substance entering the river at Hinton. The observations were also consistent with current knowledge since the presence of MFO inducers in pulp mill effluent has been observed in many locations.

The fish from this preliminary survey were also analysed for steroid hormones, 17ß-estradiol and testosterone in females and testosterone and 11-ketotestosterone in males. Estimates of maturity and fecundity were also made. The results are presented in Brown *et al.* (1993). Levels of 17ß-estradiol were significantly reduced in fall female longnose sucker downstream from Hinton (Figure 6). There were no site differences in ovarian development so these depressions did not appear to be affecting gonadal growth. For fall female mountain whitefish, fish were sampled at a critical point in their spawning cycle. This meant that differences between sites were complicated by the timing of sampling and other changes occurring during spawning. Nevertheless, the pattern of 17ß-estradiol in females downstream from Hinton mirrored the depression in levels observed for female longnose sucker. No conclusions between sites could be drawn for white sucker and pike because the samples came mostly from the sites farthest downstream and no upstream reference fish were available. Similarly, too few male fish of any species were collected for comparisons to be drawn. In summary, depressed levels of circulating steroid hormones were observed in female longnose suckers and possibly in mountain whitefish collected downstream from the mill at Hinton. The observations were also consistent with current knowledge since the presence of hormone effects in fish from waters receiving pulp mill effluent has been observed in many locations, as discussed above.

Later in the study, livers of female longnose sucker from this survey were analysed for retinoid vitamins. The results, reported in Brown and Vandenbyllaardt (1996) indicated a statistically significant decrease in the retinyl palmitate, the storage form of Vitamin A, at the two most downstream sites.

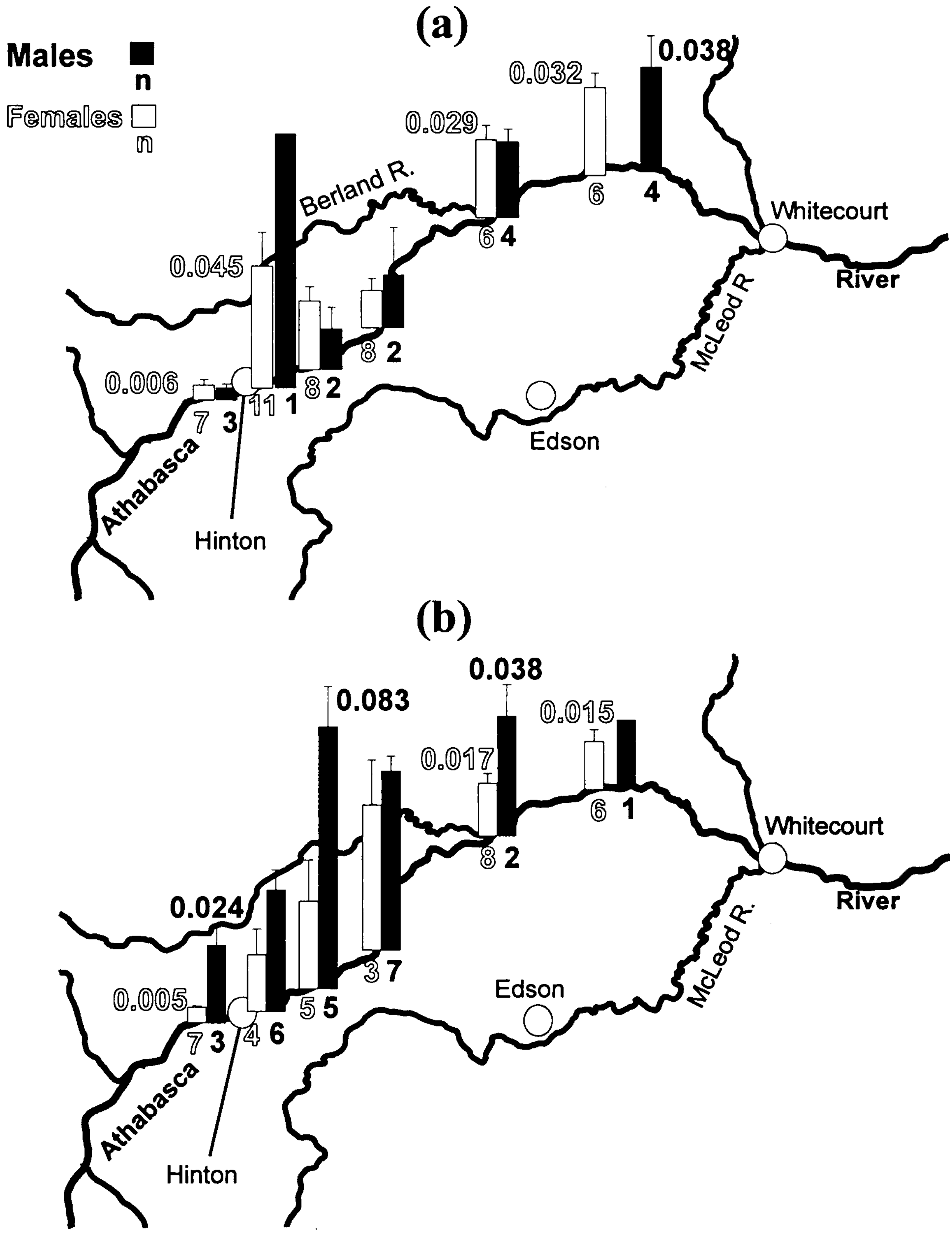

Figure 5. Liver microsomal EROD in fish from the upper Athabasca River; (a) mountain whitefish, spring, 1992; (b) longnose sucker, fall, 1992.

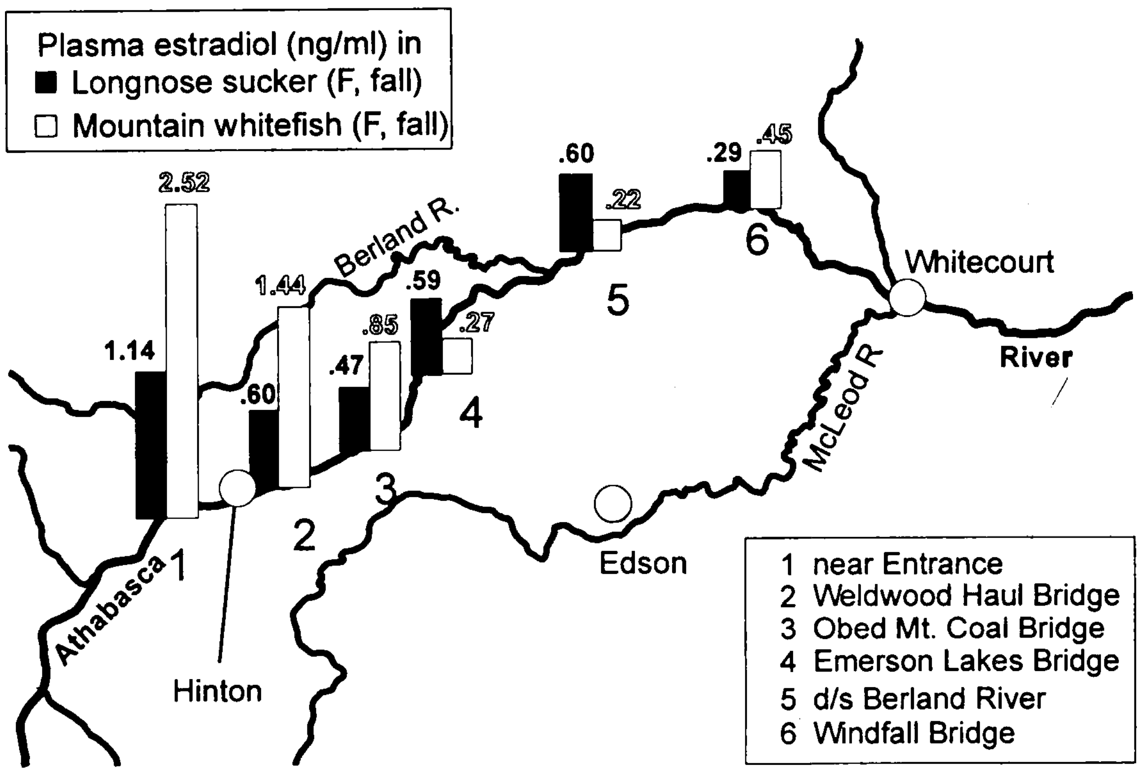

Figure 6. Relative levels of serum 17ß-estradiol in mountain whitefish and longnose sucker at sites in the upper Athabasca River, 1992 (Brown *et al.* 1995).

2.2.2 Choice of biomonitor species

The preliminary survey focused attention on the critical importance of having a sufficient number of fish from reference sites upon which comparisons of downstream impacts could be based. This had a direct bearing on the choice of fish as biomonitor species in the basin-wide survey. Ideally, a fish species used in a monitoring program should have certain characteristics that permit its use as a biomonitor. Individuals of that species should be available at all sites in the study area in appropriate ages and sexes. They should be non-mobile and therefore representative of local conditions. They should be readily captured, preferably with a selective capture technique that would not also capture large numbers of unwanted and in some cases rare species. They should be a species that is known to respond to stressors but should not be so sensitive that they are unavailable in areas receiving low or moderate levels of anthropogenic stress. Finally, there should be some indication that they are good predictors for the monitored parameters (e.g. biomarker responses such as MFO induction and EROD), so that the monitoring program will be based on sensitive data.

Unfortunately, based on the information available during the study, such a fish species did not occur within the basin. The results of the fish surveys described above indicated that for the Athabasca River, a selected fish species was often not available at many sites. There was a change in major species between the upper and the lower reaches of the river (Boag 1993; R.L.&L. Environmental 1994a, b, 1995). Adding to that complexity, the mix of species available in the Peace River appeared to be somewhat different from either the upper or lower Athabasca.

After extensive discussion with the members of the other component groups, particularly the Food Chain Component, we decided that the species most closely meeting the criteria described above was the burbot. This species was relatively widely distributed and relatively easy to catch in the NRBS surveys (Hvenegaard and Boag 1993; R.L. & L. 1994a, b). The preferred method of capture, set lines, was reasonably selective for burbot; not many other fish species were inadvertently captured. Although they were known to have short spawning runs in midwinter, there was no indication of high mobility so they were expected to be reasonably indicative of local or, at worst, regional conditions. They could be found in some upstream and tributary locations, so they offered the possibility of a choice of reference locations. Burbot are voracious predators at the top of the food chain so they should be good general indicators of contaminant bioaccumulation. Their large fatty livers tend to accumulate high concentrations of nonpolar contaminants like certain chlorinated organics (Muir *et al.* 1990a), so they offer good sensitivity for detecting contaminants. Burbot have been used as a biomonitor species in northern regions and were known to exhibit some of the physiological indicators used in the NRBS program (Lockhart *et al.* 1996). Finally, they had some social significance, because both the flesh and livers are consumed by people in the basin (Balagus *et al.* 1993).

Based on these characteristics, burbot were chosen as the primary biomonitor species for the subsequent basin-wide fish survey conducted in 1994. Several alternate species were identified as potential back-ups. They were northern pike, longnose sucker and flathead chub . These species had more restricted ranges or other characteristics that made them less desirable than burbot, but were available in some areas where burbot had not been reported.

2.2.3 Basin-wide Survey of Biochemical Indicators in Fish

In designing the basin-wide survey, the group were faced with a problem common to many of the NRBS components, the geographical region to be covered was very large compared to other field studies. Had it been desired to study the impact of a single known stressor, an individual pulp mill for instance, the study design would have been a variant of that used in the preliminary study, ie. to intensively sample a limited number of sites over a restricted geographical region. However, while that would have provided reasonably detailed information about that individual site, it would not have provided information useful in assessing cumulative impacts at the basin-scale. If the geographical scale had been smaller, a limited number of intensive representative areas could have been considered. However, to conduct a basin-wide study at that level of detail would have required many more years and much more resources than were available for the NRBS.

The group decided that a study could be designed that addressed the question at a regional scale and that addressed a limited number of testable hypotheses. Since much of the public concern centred around pulp mills and their impact on the river, the study design focused on determining whether regional effects of pulp mills could be observed in aquatic biota. The group selected sites in the Peace-Athabasca-Slave system that could be grouped into three regional classes. These were: reference sites on tributaries or on the mainstems upstream from any known sources; near-field sites, within 100 km of pulp mill discharges; and, far-field sites, more than 100 km from a pulp mill discharge. In carrying out the eventual sampling, we found that fish of the right species and size were not available at all these sites. Three sites were re-sampled after the basin-wide survey was complete. Several others were dropped for logistical reasons. The sites where fish were collected and their designations in the regional scheme are given in Table 2.

Four types of physiological response were selected. They have been described above. Three of the responses were selected because they either were known to be caused by pulp mills or by organochlorines or both. One of these, the induction of MFO enzymes, is likely the most widely and consistently observed physiological response of fish to pulp mill effluent. It has been observed for effluents from mills of different pulping processes (Robinson *et al.* 1994), including mills with no bleaching (Pesonen and Anderson 1992; Lindstron-Seppa *et al.* 1992; Williams *et al.* 1996). It is also a sensitive measure of the presence of sufficient levels of TCDD, TCDF (tetrachlorodibenzo-p-furan) and other substances whose toxicity is expressed through the Ah receptor. As such, it is an excellent cumulative indicator of this whole group of toxic substances with a common route of toxicity. The second response, effects on sex hormones, is the only whole organism response observed to date around pulp mills with the potential to directly affect populations. This response can also be caused by persistent organochlorine substances. The third response, effects on liver vitamins, is known to be related to organochlorine exposure. Its link to pulp mill effluents has not been well studied. Finally, the fourth bioindicator, levels of metallothionein was chosen as an indicator of exposure to metals. It is less well documented in field studies and the NRBS was viewed as an excellent opportunity to evaluate its utility in a major field study. Taken together, these physiological indicators should provide an excellent assessment of whether impacts are occurring due to pulp mill effluents and/or persistent organochlorine contaminants in these basins.

The basic plan of the study was to test several null hypotheses. The most general null hypothesis was: for three classes of sites, reference, near-field and far-field, there are no differences in the measured response of mature fish of each sex. A second null hypothesis that it was intended to address was: for three classes of sites, reference, near-field and far-field, there are no differences in distribution of immature fish as a function of site within the basins. A second type of data examination was also performed. Although sample numbers would be considered small for a site-by-site comparison, we felt that important information might be lost in lumping all sites within a class together. Therefore, we decided to examine the data for any site anomalies within the classes and to flag these as possible indications of site specific effects that would require more intensive study.

Burbot, northern pike, longnose sucker, and flathead chub were collected from mid-September to late October 1994 and in mid-December from 23 sites (Figure 7) in the NRBS study area (Jacobson and Boag 1995). Fishing techniques included baited setlines, electrofishing, gill nets, and angling.

Table 2. Location of fish collection sites in the NRBS basin-wide survey indicating fields used in the statistical analysis and possible discharge sources for each site.

River	Site	Field	General Location	Potential Effluent Exposure
Athabasca Basin				
Athabasca	A1a	NEAR	near highway 947 crossing	d/s from Hinton pulp mill
	A1b		near Berland River	
	A2	REF	u/s Hinton	d/s from Jasper
	A3	NEAR	near Fort Assiniboine	d/s Whitecourt pulp mills
	A4	NEAR	near the Calling River	d/s from ALPAC pulp mill
	A5	FAR	near Fort Mackay	d/s from Suncor
McLeod	MR	REF	near Whitecourt	d/s from town of Edson
	MR2	REF	u/s from town of Edson	tributary reference, d/s from coal mines
Pembina	P	REF	near town of Jarvie	d/s town of Barrhead, tributary reference
Lesser Slave	LSV	NEAR	near town of Slave Lake	d/s Slave Lake Pulp
Clearwater	CW	REF	u/s from Fort McMurray	tributary reference
Peace Basin				
Peace	PR1	FAR	near Many Islands Prov. Park	d/s from B.C. pulp mills and old refinery at Taylor
	PR2	NEAR	near the Notikewin River	d/s from Daishowa mill and Smoky River confluence
	PR3	FAR	u/s from Fort Vermilion	further d/s from Daishowa mill and Smoky River confluence
Wapiti	WR1	REF	near Pipestone Creek Prov. Park	20 km u/s from Grande Prairie mill
	WR2	REF	near O'Brien Prov. Park	5 km u/s from Grande Prairie mill
Smoky	SR1	NEAR	near Watino	d/s Grande Prairie mill
	SR2	REF	near Grande Cache	u/s reference, nearby coal mine and power plant
	SR3	REF	u/s from Wapati confluence	u/s reference
Little Smoky	LSR1	REF	near highway 744 crossing	Tributary reference
	LSR2		3 km d/s LSR1	
Wabasca	WB	REF	near highway 67 crossing	Tributary reference
Peace-Athabasca Delta				
Delta	JV1	FAR	near Jackfish Lake village	
	JV2		near Big Eddy	
Slave Basin				
Delta	SRD1	FAR	u/s from Nagle Channel	d/s from Fort Smith
	SRD2		at mouth of Nagle Channel	

There were nine sampling sites in the Peace River drainage visited during the fall. These included sites on the Peace, Smoky, Little Smoky, Wapiti, and Wabasca rivers. Two sites were re-sampled in mid-December, one on the Smoky River and one on the Little Smoky River, to try to increase the

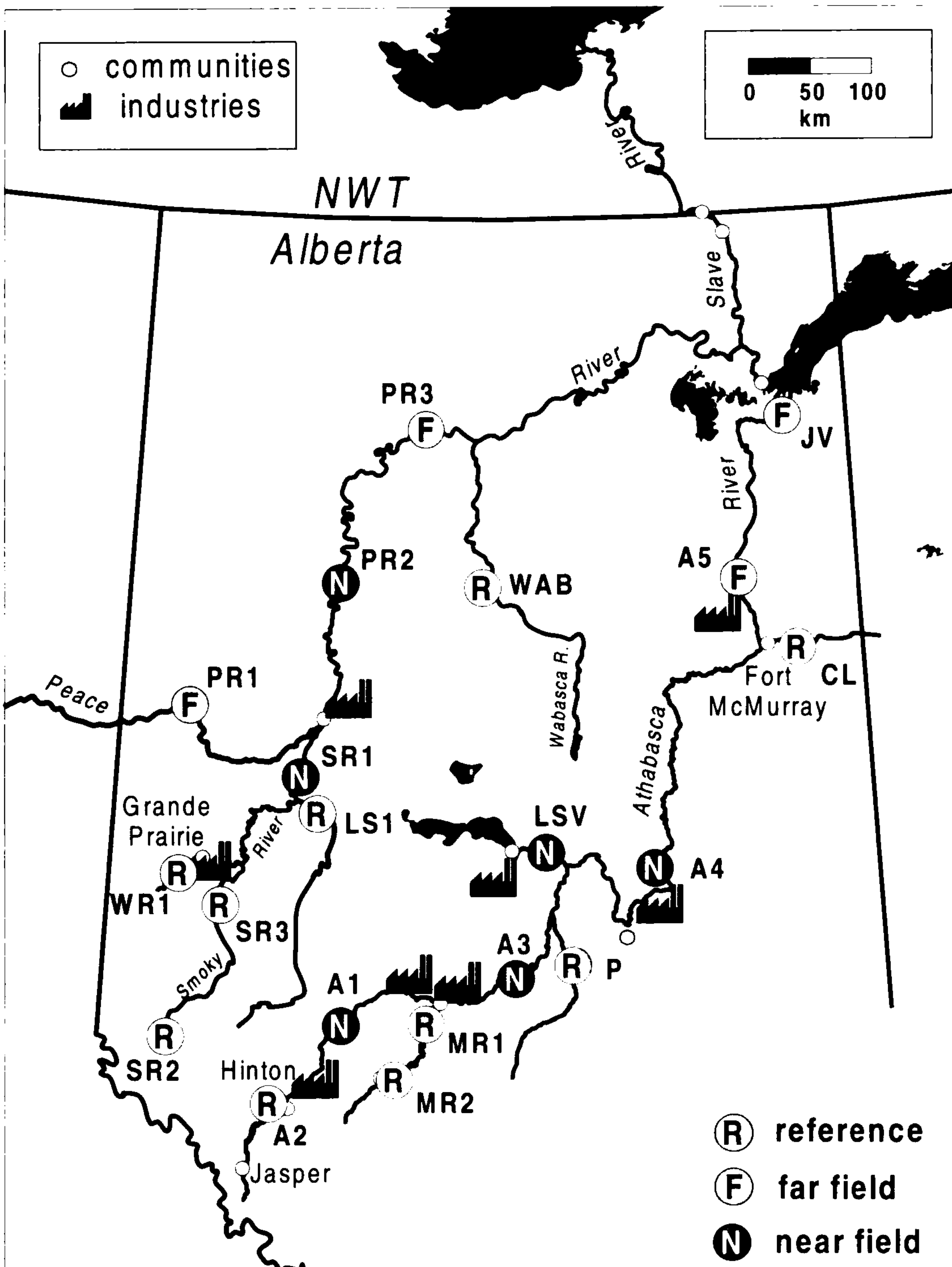

Figure 7. Site location and designation for the fish collection for the basin-wide survey, 1994.

sample size. Nine sites were sampled in the Athabasca River drainage: five Athabasca River sites, and one on each on the McLeod, Pembina, Lesser Slave, and Clearwater rivers. The McLeod River was re-sampled in December. Fish were also collected from the Peace-Athabasca Delta and the Slave River Delta.

In total, 535 fish were caught consisting of 222 burbot, 50 northern pike, 88 longnose sucker, and 24 flathead chub. Burbot were caught at all sites sampled except the Smoky River near Grande Cache and the Peace-Athabasca Delta near Jackfish Village. Northern pike were not collected at the Wabasca, McLeod, Lesser Slave rivers, or four of the five Athabasca River sites and were relatively scarce at the other sites sampled. Most longnose sucker were caught in the Peace, Smoky, Little Smoky, and Wapiti rivers. In the Athabasca River basin, the set line fishing method produced longnose suckers at only one site on the Athabasca River and in the McLeod River. Flathead chub were found in the Peace, Smoky, Little Smoky, and Wapiti rivers.

Induction of Mixed Function Oxygenases

Microsomal mixed-function oxygenase enzyme activities were determined in livers of burbot, longnose sucker and flathead chub collected in the basin-wide survey. The results are discussed in Lockhart and Metner (1996). For both EROD and AHH, there was no evidence of a relationship between MFO activity and the pulp mills. The EROD results for burbot, segregated by gender and maturity are shown in Figure 8. The data for the other fish species, longnose sucker and northern pike were similar. There is no indication in these data that the cytochrome P450 system was induced in fish from the near-field region relative to the other regions. In several cases, EROD and AHH activities for fish in the near-field region were lower than those for the reference or far-field sites. The site comparison revealed that the much of this difference was due to the A5 and the WAB site. On an individual fish basis, the highest EROD activities were found in fish from reference or far-field regions. On a site basis, the highest activities were found at the A5 site, a far-field location downstream from Fort McMurray, and at the Wabasca River site, a reference location not associated with

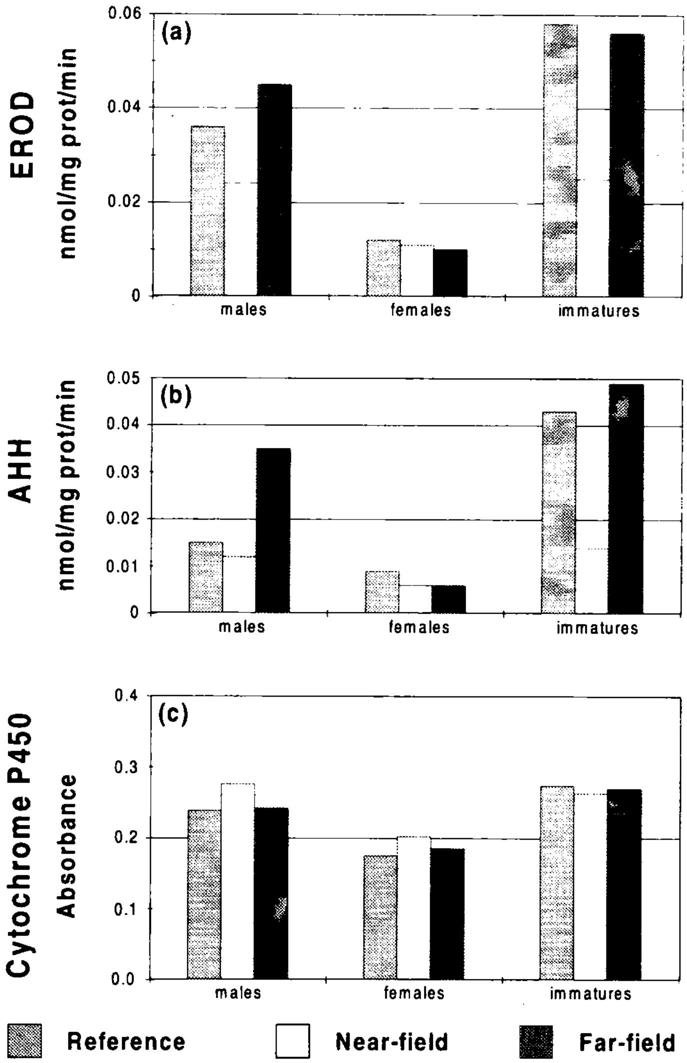

Figure 8. MFO enzyme activity between regions in the basin-wide survey: (a) EROD activity; (b) AHH activity; (c) cytochrome P450.

any known point source of MFO inducers. This induction must have been associated with a source other than pulp mills. One potential source of MFO inducers is oil seeps. Some polycyclic hydrocarbons have the correct structural features to cause MFO induction. If they were present in the seeps in the oil sands areas, they could potentially contribute to the measured values. The highest values were observed in mature males. The basin-wide distribution of MFO induction in mature burbot males is presented in Figure 9.

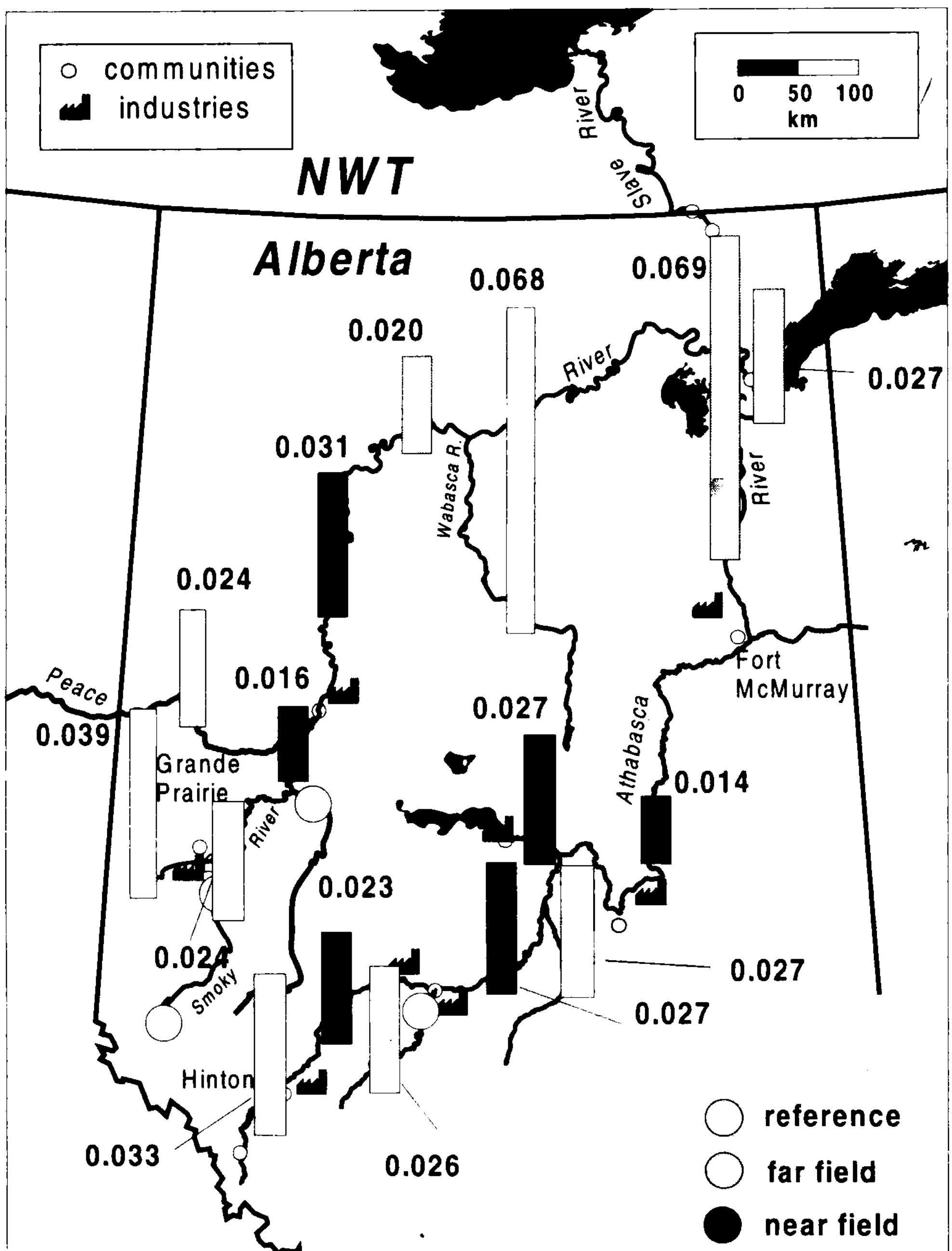

Figure 9. Site distribution of EROD activity in male burbot from the basin-wide survey, 1994.

Figure 10 presents a comparison of site data for induction of MFO enzymes in this study with that obtained for burbot in other northern locations. Apart from the Fort McMurray area on the Athabasca River and the Wabasca River site, the values observed in this study are comparable with those observed in remote locations elsewhere and show no indication of point source related MFO induction in the NRBS study area.

The main conclusions of this part of the study were:

- effects of pulp mill effluents on the mixed-function oxygenase enzymes of burbot are not evident in these studies. There are sufficient numbers of samples of burbot available to have allowed detection of effects if they had caused effects a few fold above controls, but they were not detected in a geographic pattern consistent with known sources of pulp mill effluents.
- mild induction of mixed-function oxygenases was detected in burbot from the Athabasca River near the oil sands plants and in burbot from the Wabasca River. This is the second such indication for fish from the oil sands of the Athabasca River and the first indication for fish from the Wabasca River. Attribution of the induction effect to oil sands is consistent with the chemistry of these materials in the Athabasca River, but is speculative in the Wabasca River.

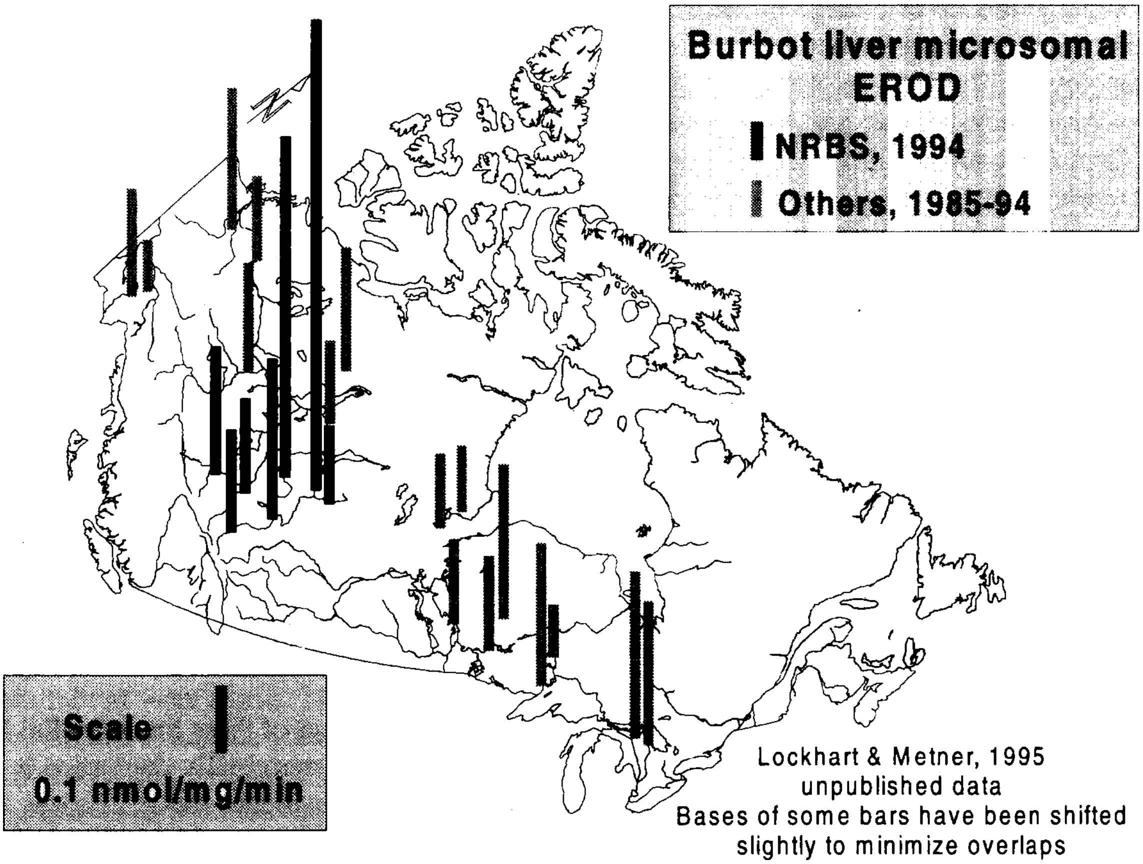

Figure 10. Comparison of site data for induction of MFO enzymes in male burbot from the basin-wide survey with burbot in other northern locations.

Reproductive Steroids/Gonad Morphology

The fish from the basin-wide survey were analysed for steroid hormones; 17ß-estradiol and testosterone in females; testosterone and 11-ketotestosterone in males. In addition, estimates of maturity, gonad morphology and fecundity were also made. The results were reported by Brown *et al.* (1996). In female fish, in the near-field sites from the Peace and Athabasca basins, plasma 17ß-estradiol was significantly reduced in female burbot and longnose sucker relative to the reference sites (Figure 11). Since 17ß-estradiol is produced in the ovary and its function is to stimulate production of yolk proteins for incorporation into developing clutch oocytes, prolonged reductions in its circulating level could adversely affect ovary development. There was also some evidence of change in measures of ovarian development in burbot from some sites, so the lower 17ß-estradiol levels may have impacted gonadal growth and development in fish from these areas. Burbot from the Slave River were in the best condition with the largest oocytes but fecundity estimates were lowest. As previously reported for longnose sucker collected in the upper Athabasca River (Brown *et al.* 1993), site related differences in oocyte size or fecundity estimates corresponding to low 17ß-estradiol levels were not apparent in fish collected on the Peace River. As discussed above, the lowered steroid hormone levels and altered gonadal parameters are consistent with observations on female fish in many other species downstream from pulp mill effluent discharges. The long-term consequences of low levels of circulating estradiol are not known.

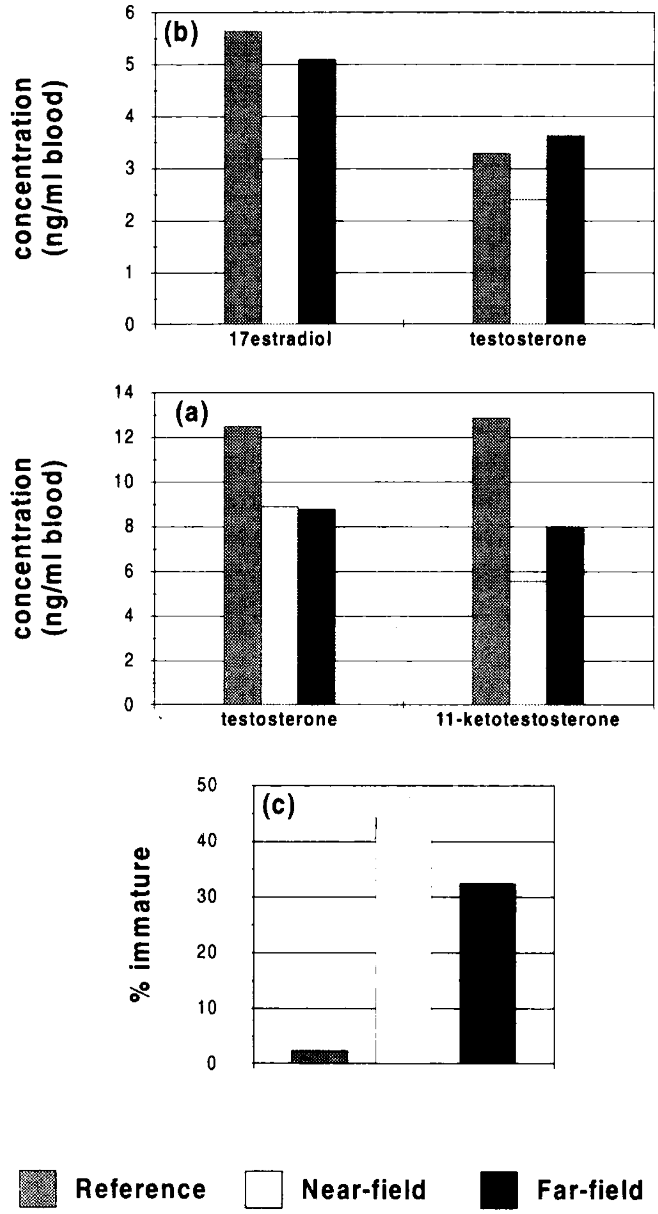

Figure 11. Regional analysis of steroid hormone levels and maturity in burbot from the basin-wide survey: (a) mature males; (b) mature females; (c) % immatures.

Studies to verify the present findings and to determine the consequences of these low estradiol levels are required. In male burbot, plasma 11-ketotestosterone appeared marginally depressed in most samples collected from near-pulp mill regions. Generally, there was evidence for differences in sex hormones in both male and female burbot in this survey that were consistent with effects found in fish collected from waters receiving pulp mill effluents.

One unusual feature of the fish distributions was the high proportion of immature adult size fish in the near-field and far-field regions (Figure11). Normally a small proportion, ~10-15%, of the fish in adult burbot surveys are adult size fish that are still sexually immature. In the near-field fish collected in this survey, the percentage of adult-sized immature fish was greater than 40% (Brown *et al.* 1996). In other areas, eg. Scandinavia, where such a phenomenon was observed, it was regarded as a reproductive disorder. In Scandinavia, substantial numbers of immature adult burbot have been located in waters affected by loading from metal industries or pulp mills (Brown *et al.* 1996). In Canada, delayed maturation has been observed for sucker species in the receiving waters of one pulp mill (McMaster *et al.* 1991; Munkittrick *et al.* 1992a,b) and a laboratory study confirmed that effluent from one pulp mill in concentrations greater than 20% caused a delay in maturation of fathead minnows (Robinson 1994). These observations need to be investigated in more detail. If the high percentage of immature fish downstream is confirmed as an effluent effect, it is of concern. Unfortunately, the distribution of mature adult fish in this survey was also anomalous. There was a higher proportion of mature females in the reference areas relative to males while the reverse was true in other areas. This raises the possibility that distributions of burbot within the study region may not be homogeneous and that burbot utilize different parts of the river basins at different stages in their lives. More investigations of burbot behaviour, particularly migratory behaviour, are essential to understanding these distributions.

Hepatic Vitamins

The results of the analysis of hepatic retinoid levels and tocopherol in burbot and longnose sucker from the basin-wide survey are discussed in Brown and Vandenbyllaardt (1996). Analysis of the results is complex because it was discovered that, contrary to expectations, dehydroretinyl palmitate is apparently not the predominant storage form in all species of freshwater fish. In this survey, there were species differences between burbot, longnose sucker and pike in the storage forms of hepatic Vitamin A. Longnose sucker livers contained predominantly retinyl esters while northern pike contained predominantly dehydroretinyl esters. Burbot livers contained approximately equivalent amounts of retinyl and dehydroretinyl esters. Furthermore, there were several storage forms found in this survey whose identity is unknown and whose concentration was approximated using the retinyl or dehydroretinyl palmitate as a surrogate. Although there were some site exceptions, concentrations of the free forms of retinol and dehydroretinol were low, as expected. As discussed previously, concentrations of these vitamins are highly regulated in organisms and they are produced from storage forms when needed. The concentrations of retinol and dehydroretinol generally fell within the range observed in other studies.

Similarly, although there were some site differences in levels of retinyl and dehydroretinyl esters, concentrations were similar between reference, near-field and far-field groupings. Furthermore, the concentrations of hepatic vitamins found in the basin-wide survey were comparable to those reported elsewhere for uncontaminated locations and were greater than those reported in fish exposed to contaminants in polluted locations. Thus, there was no evidence of a cumulative regional scale impact of pulp mills on hepatic vitamin stores.

The results of the tocopherol analysis also did not reveal significant tocopherol stress on a regional basis. In fact, fish from the near-field group had the highest levels of tocopherol. This may have been due to better nutrition. Fish in the near-field regions generally had higher condition factors than other fish in the other groupings.

One significant exception to the above summary was for the PR3 (Peace River near Fort Vermilion) site. Male burbot, and to a lesser extent immature burbot, from this site contained high levels of free retinol and dehydroretinol. Averages in male burbot were 20-fold higher than any other site in the survey. Tocopherol levels in these fish were the lowest of the survey. Taken together, this may indicate symptoms of tocopherol deficiency. The effect was not observed in longnose sucker from this site. Since the burbot sample numbers at this site were low, this observation should be replicated. If it can be replicated, it should be investigated in more detail.

In general, the results of the hepatic vitamin analyses did not support the suggestion that fish in the Athabasca River were subject to high enough concentrations of organochlorines or other substances to cause liver responses. Some specific sites were identified where retinoid and tocopherol levels were anomalous. These sites did not seem to be related to pulp mills. Further studies should be done at these locations to replicate the observations and investigate the causative factors, if warranted.

Metallothionein

Metallothionein concentrations were measured in liver, kidney, gill and intestine from burbot, longnose sucker and northern pike from the basin-wide survey (Klaverkamp and Baron 1996). There were large ranges, as high as 30-fold, between sites for metallothionein levels. In general, the regional analysis did not detect effects indicative of metal-induced stress in the near-field regions. This is not altogether surprising, since pulp mills are not generally regarded as sources of metal pollution. In general, metallothionein levels in several tissues in the far-field grouping were elevated relative to the reference group. On an individual site basis, site PR3 again separated from the other sites. For several tissues, metallothionein levels in burbot and longnose sucker were highest at this site. Metallothionein levels in kidney of burbot from the Slave River delta were 7- to 26-fold higher than burbot from any of the other sites. These fish may have been exposed to metals from mining in the Great Slave Lake area. However, fish from the delta had the highest condition factor, so it does not appear that the elevations of metallothionein were indicative of health effects. Metallothionein may also have been induced somewhat in fish from the Pembina and Wabasca Rivers, two of the tributary reference sites in the survey. In several cases, a downstream trend of increasing metallothionein concentration in fish tissues was observed. This may be indicative of cumulative effects.

In general, the results of the metallothionein survey did not support the hypothesis that induction of metallothioneins occurred in response to effluents from pulp mills. Several sites where metallothioneins were elevated were identified. These sites should be the subject of more intensive investigation to replicate and explain these observations.

3.0 SEDIMENT TOXICITY TESTING

3.1 INTRODUCTION

In addition to the basin-wide fish survey, another approach chosen by the Contaminants Component to assess the NRBS study area for contaminant-related effects was based on sediment dwelling organisms. The presence and persistence of contaminants in sediments may constitute a source of toxicity to organisms that live in or near the sediment such as epibenthic and burrowing invertebrates. Such toxicity may have direct detrimental effects on these species as well as indirect effects on the other organisms like fish, amphibians or shore-birds that use them as food. The use of benthic invertebrates in chronic sediment toxicity tests is well documented (Burton *et al.* 1992). Several species of invertebrates have been recommended as suitable organisms for the acute and chronic toxicological testing of sediments. The freshwater amphipod, *Hyalella azteca*, and the chironomids, *Chironomus tentans* and *Chironomus riparius*, have received the most attention but other organisms have also been used such as mayfly, *Hexagenia* spp. and the oligochaete worms, *Lumbriculus variegatus* or *Tubifex tubifex*. Benthic invertebrates, as a group, represent a wide range of life histories and feedings habits, and therefore can be potentially effective indicators of contamination through their various forms of contact with contaminants. For example, *Hyalella azteca* and *Chironomus riparius* are grazers on the surface sediments, while the oligochaete worm, *Tubifex tubifex*, is a burrower and ingests sediment particles. These different modes of feeding and movement offer biological indications of contamination through exposure of chemicals bound to surface particulates (grazers), and to contaminants buried in the sediments as well as that component found in interstitial water (burrowers).

One method of determining sediment quality is the Sediment Quality Triad Approach. This approach involves a three-part assessment comprised of the chemical measurement of contaminant concentrations in sediment, laboratory bioassays to determine the toxicity of the sediment and analysis of benthic infaunal community structure to determine whether benthic communities have been altered (Chapman 1990). Together, these three measures provide the strongest evidence presently available for determining whether environmental degradation has occurred due to toxic substances in sediments. The sediment chemistry provides information on extent of possible contamination but cannot be relied on to provide measures of possible toxicity because the limitations of chemical analysis. For example, chemical measures alone do not provide estimates of the bioavailability of the contaminants and spatial heterogeneity is lost when sediments are homogenized for analysis. However, the level of contamination is an important factor for the evaluation of the strength of association between the contaminant and the effect and consistency of that relationship. Sediment bioassays provide direct evidence of potential toxicity but the species used in the tests and the laboratory conditions may not be representative of conditions in the field. However, the results of laboratory tests are useful in testing the coherence with existing knowledge of effects observed in the field. The field measurements of benthic communities provides evidence of pollutant-related effects only if other non-pollution-related stressors are not important. The possible combinations of responses and the information that they provide are summarized in Table 3.

Table 3. **Possible combinations of responses in the Sediment Quality Triad Approach and possible conclusions from each combination of responses (after Chapman 1990).**

Chemical Contamination	Toxicity	In-situ Alteration	Possible Conclusions
+	+	+	strong evidence for toxic chemical induced degradation
-	-	-	strong evidence for no toxic chemical induced degradation
+	-	-	chemical contaminants not bioavailable
-	+	-	unmeasured toxic chemicals present
-	-	+	alteration not due to toxic chemicals
+	+	-	toxic chemicals may be stressing the ecosystem
-	+	+	unmeasured toxic chemicals likely causing degradation
+	-	+	chemicals not bioavailable and degradation is not due to toxic chemicals

Positive (+) and negative (-) responses indicate whether or not measurable, statistically significant differences exist between responses measured for the test site relative to control or reference measurements.

Chapman (1992) lists examples of the use of this approach in marine and estuarine waters in: Vancouver Harbour, BC; Puget Sound, WA; San Francisco Bay, CA; the Gulf of Mexico; the North Sea; and in Chesapeake Bay; and in freshwater studies in: Oklahoma, South Dakota, Ohio and the Great Lakes.

The objective for this project was to expose four species of benthic invertebrates (the amphipod, *Hyalella azteca*, the chironomid, *C. riparius*, the mayfly, *Hexagenia* spp. and the oligochaete worm, *T. tubifex*) in chronic laboratory toxicity tests to depositional and suspended sediments collected from the NRBS study area. The testing was conducted in two phases. In the first phase, sediments from the Athabasca River in the reach from just upstream from Hinton to Whitecourt were tested. In the second phase, a survey of sediment toxicity throughout the Peace-Athabasca system was carried out.

3.2 RESULTS

Depositional sediments were collected in October 1993 from two sites upstream and five sites downstream from Hinton (Figure 12). Four chronic toxicity tests were utilised to test all sediments collected from the Athabasca River as well as a clean control sediment from Long Point, Lake Erie, for biological quality assurance (Day and Reynoldson 1995). These tests involved four types of benthic invertebrates: the amphipod, *Hyalella azteca*; the chironomid, *Chironomus riparius*; the mayfly, *Hexagenia* spp. and the oligochaete tubificid worm, *Tubifex tubifex*. The tests lasted from 7 to 28 days. Endpoints measured in the tests were: *H. azteca*, survival and growth; *C. riparius*, survival and growth, *Hexagenia* spp., survival and growth; and *T. tubifex*, survival and reproduction (total number of cocoons and young).

The results of the bioassays are summarized in Table 3. In general, there was no indication of toxicity at any of the sites for three of four species and for seven of eight chronic endpoints. For example, survival of *C. riparius*, *Hexagenia* and *H. azteca* in sediments collected downstream from Hinton was equal to or greater than survival of these species in sediments collected either upstream from Hinton (control sites), in the reference Lake Erie sediment used for QA/QC or the mean survival measured in >196 uncontaminated sediments from the nearshore areas of the Great Lakes. Growth of all three species was similarly not reduced at any of the sites.

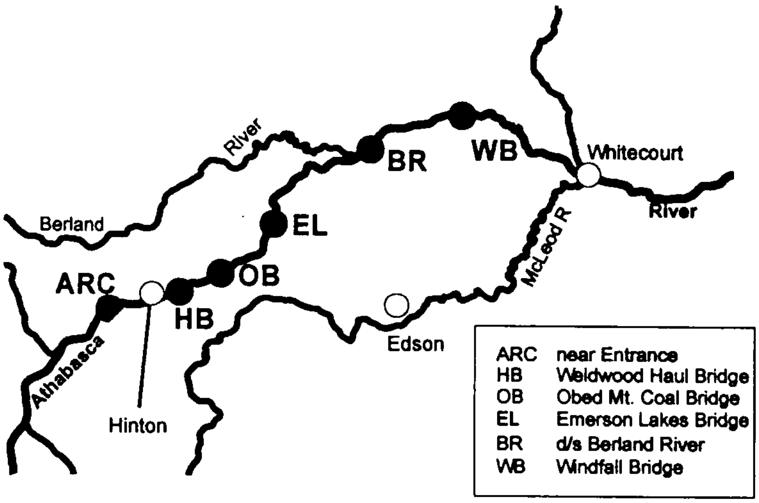

Figure 12. Site locations and designation for the sediment collections on the upper Athabasca River for toxicity testing, 1993.

Table 4. Results of the four bioassays applied to sediments from the Upper Athabasca River. Effects are based on results for >196 clean reference sediments from the nearshore areas of the Great Lakes.

	Hyalella azteca		*Chironomus riparius*		*Hexagenia Spp.*		*Tubifex tubifex*	
	% survival	Growth	% survival	Growth	% survival	Growth	Tot. # cocoons	# live young
ARC	-	-	-	-	-	-	-	-
HB	-	-	-	-	-	-	-	-
OB	-	-	-	-	-	-	-	-
EL	-	-	-	-	-	-	-	-
BR	-	-	-	-	-	-	-	+
WB	-	-	-	-	-	-	-	(+)

Positive (+) and negative (-) responses indicate whether or not measurable, statistically significant differences exist between responses measured for the test site relative to control or reference measurements.

Only one toxicity test endpoint demonstrated a response to sediments collected downstream from Hinton. Production of live young by the tubificid worm, *T. tubifex*, was significantly reduced in sediments collected above the Berland River and near Windfall Bridge. Production of cocoons by these organisms was not similarly affected. In the case of the Berland River site sediment, production of live young was significantly below the reference value of >196 Great Lakes sediments. In the case of the Windfall Bridge site, the live young produced were near the Great Lakes reference value but significantly below all other sites, except the Berland River site.

The reasons for this reduced reproduction are not clear. These sites are the farthest downstream from the pulp mill, so there is no obvious effluent effect. One of these sites, Berland River, had a very high concentration of sand, which would not have been conducive to the burrowing behaviour of the organism. At the other site, Windfall Bridge, the sand content was the lowest of the collection and silt was very high. There were downstream differences in the phosphorus and nitrogen content of the sediments, but the differences were not striking and appeared unlikely as an explanation for the toxicity. Analysis of the sediments for metals revealed that most metals either remained the same or increased slightly downstream. One exception was arsenic, that was less than detection at all sites but Berland River and Windfall Bridge. At these latter sites, concentrations exceeded the low effects criterium for the province of Ontario (6 ppm; Persaud *et al.* 1992).

Based on these results, sediments located downstream from Hinton in the Athabasca River do not appear to be toxic to benthic invertebrates living in or near the sediment. Two exceptions are the sediments collected upstream from the Berland River and possibly near Windfall bridge where reduced reproduction of tubificid worms was noted.

The second phase of the project involved collection of sediment throughout the basins and subjecting them to the same toxicity tests. In addition, suspended sediment was collected at some sites. The rationale for adding suspended sediment was that a suspended sediment collected by the NWRI-PERD companion study in the oil sands area in 1994 caused a toxic response in the *Tubifex tubifex* bioassay (B. Browlee - pers. comm.). In the survey, bottom sediment was collected from eight locations in the Athabasca River and four locations in the Smoky and Peace rivers. Suspended solids were collected from nine locations in the Athabasca River and six locations in the Smoky and Peace rivers. The locations of these collections are shown in Figure 13. The toxicity of the bottom sediment samples was assessed in chronic laboratory toxicity tests using the same four test organisms utilised in 1994: the chironomid *Chironomus riparius*, the

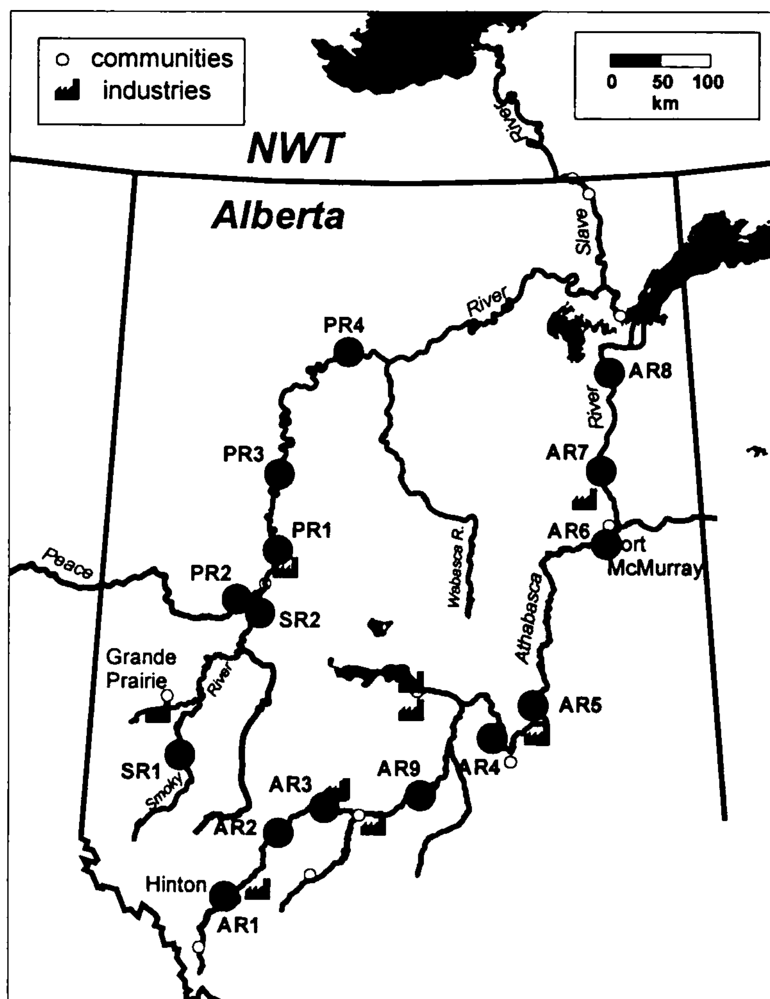

Figure 13. Site locations and designation for the basin-wide survey of sediment toxicity, 1994.

mayfly *Hexagenia* spp. (*H. limbata* and *H. rigida*), the amphipod *Hyalella azteca*, and the oligochaete worm *Tubifex tubifex*. Because of small quantities of suspended sediment obtained, only one toxicity test could be performed. The test organism chosen was the oligochaete worm *Tubifex tubifex* (Dobson *et al.* 1996).

All bioassay results are presented in Table 5. Results from the negative control sediment (Long Point sediment from Lake Erie) met or exceeded acceptable limits for a valid test for all bioassays (i.e., 70% survival for *C. riparius*; 80% survival for *H. azteca* and *Hexagenia* spp.). Groups of sites are compared to each other based on a similar river system. Therefore, comparisons of results are confined to sites within the Athabasca River (AR) and, similarly, sites within the Smoky and Peace Rivers (SR and PR) are grouped. Toxicities were compared to the appropriate reference sediments for each basin.

Table 5. Results of the four bioassays applied to sediments from the basin-wide sediment survey. Responses are relative to average values for >196 Great Lakes clean sediments (Dobson et al. 1996).

	Hyalella azteca		*Chironomus riparius*		*Hexagenia Spp.*		*Tubifex tubifex*	
	% survival	Growth	% survival	Growth	% survival	Growth	Tot. # cocoons	# live young
AR1	+	+	-	-	+	+	+	+
AR2	-	-	-	-	-	-	-	-
AR3	-	-	-	-	-	-	-	-
AR4	-	-	-	-	-	-	-	-
AR5	+	-	-	-	-	-	-	-
AR6			-	-				
AR7	-	-	-	-	-	-	-	-
AR8	+	-	-	-	+	+	+	-
AR9	-	-	-	-	-	-	-	+
SR1	-	-	-	-	-	-	-	-
PR1	-	-	-	-	-	-	-	-
PR2	-	-	-	-	-	-	-	-
SR2								
PR3	-	-	-	-	-	-	-	-
PR4								

Positive (+) and negative (-) responses indicate whether or not measurable, statistically significant differences exist between responses measured for the test site relative to control or reference measurements.

The results indicate that whole sediments from some locations in the three rivers have detrimental effects on *Hyalella*, *Hexagenia*, and *Tubifex*. Effects were particularly noted at sites AR1 and AR8, where they were seen in three species. Figure 14 shows the locations of the sites where toxicity was observed in at least one of the tests. It is uncertain whether these effects can be attributed to

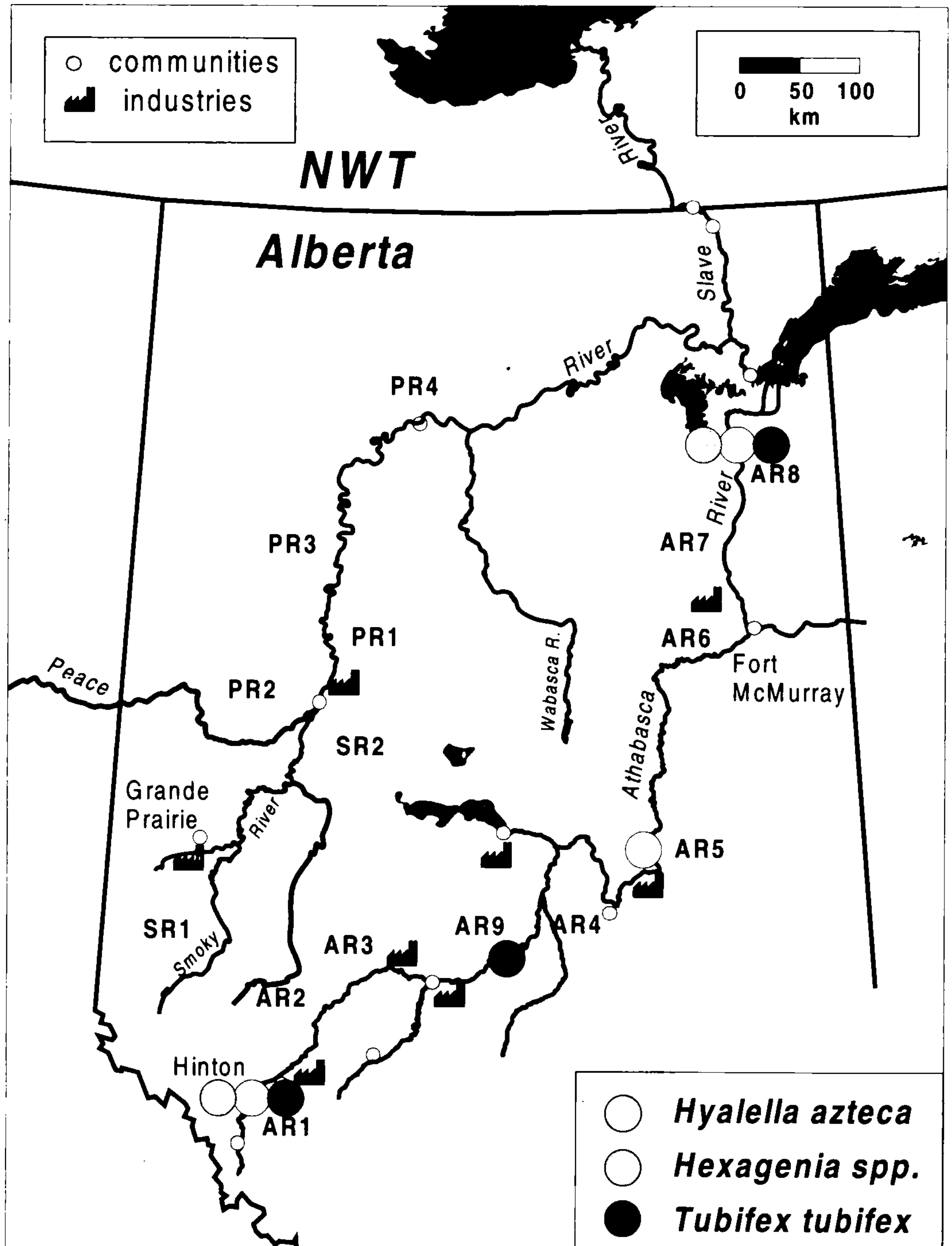

Figure 14. Site locations where sediment toxicity was observed in at least one species in laboratory testing of sediments collected in the basin-wide survey, 1994.

chemical contamination or physical characteristics of the sediment. These two sites were the sandiest of the sites examined. Burrowing animals such as *Tubifex* and *Hexagenia* are negatively affected by sandy conditions because of a physical inability to burrow and/or a low level of food reserves (unpublished data, T.B. Reynoldson and K.E. Day). Although there were some cases where the LEL (Lethal Exposure Limit) was exceeded for some metals, there was no apparent correlation between metal distribution and sediment toxicity. In addition, although the sand fraction has not previously been considered a major repository of organic contaminants, studies of sediment contamination performed under the NRBS found higher contaminant concentrations in the sand fractions than in the silt fractions for sediments from many sites in the basin (Crosley 1996).

Results from bioassays with suspended sediment were highly variable and are not presented here. There were few statistical differences among sites; all sites had a higher mean number of offspring/adult worm than the reference level.

3.3 CONCLUSIONS

With the exception of *Chironomus*, effects on measured endpoints were observed in bioassays with some or all of the animals using whole sediments collected from sites AR1, AR5, AR8, AR9, SR1, and PR2 but the causes of these effects may not be due to elevated levels of some metals at some sites but to the sandy sediments. The sites with the greatest effects were AR1, the furthest upstream site, and AR8, the farthest downstream site, in the Athabasca River. Neither of these sites is close to any of the industries and neither is in the oil sands area. Therefore, these bioassays could not identify toxicity from point-sources. These observations need to be compared to benthic community information obtained as part of the NRBS Nutrients Component to assess whether the toxicity detected at some sites was sufficient to cause impacts at the population level. In addition, the importance of nutrient/contaminant interactions in confounding observed effects needs to be addressed (Lowell *et al.* 1995, Chambers 1996). If effects are confirmed at the population level, more extensive chemistry will be needed as the third part of the triad.

4.0 SEMI-PERMEABLE MEMBRANE DEVICES

4.1 INTRODUCTION

Information on the distribution and fate of chemicals in receiving waters is often used to assess the risks to aquatic fauna of pulp mill effluent, and other types of industrial and municipal discharges. Such chemical information can be misleading since it does not take into account factors that affect exposures, such as bioavailability and rate of depuration, and may lead to lower responses than would be predicted by chemical data alone. To assess responses, biological monitoring is more desirable because if the contaminants can be measured in biota, it follows that they must have been bioavailable. In addition, biological monitoring can make it possible in many cases to assess physiological changes and other sub-lethal responses in the organisms. However, in large systems, biological sampling can pose serious logistical problems, particularly in remote areas. The unavailability of organisms of the desired species, age, physiological state and sex can be a serious problem that jeopardizes the success of a study. In addition, in unproductive systems where the total number of organisms available is low, intensive sampling of organisms for physiological analyses can be detrimental to populations. Finally, for mobile organisms like fish, there is always the issue of lack of control over the exposure history of the organism. Fish movements in and out of impact zones can give rise to big differences in exposure history, even for fish caught at the same location. This results in large ranges in the response data that make it difficult to detect differences between sites.

To address this problem, several groups have been conducting research into the development of passive samplers that would mimic fish in their ability to accumulate contaminants from the water and would permit sampling intervals and exposure conditions to be controlled. One such sampler is the semipermeable membrane device or SPMD (Huckins *et al.* 1990, 1995). SPMDs are thin polyethylene membrane tubes containing a purified fish lipid. The membrane allows fat soluble chemicals to be absorbed via a mechanism similar to the diffusion of compounds across a fish gill membrane, allowing these devices to be used as surrogate fish. Following a specified time period in an ambient water body or effluent, the SPMDs are sent to the laboratory for the lipids to be recovered. The recovered lipid can be analyzed chemically or used in biological testing. The concentrations of accumulated chemicals are often comparable to those in fish from the sample location (Huckins *et al.* 1990, 1995).

Because SPMDs can be strategically located in streams and the exposure times controlled, they offer many practical benefits as an initial biodetection tool. SPMDs provide time-integrated samples of effluents and river waters. This allows a representative sample to be gathered that is less vulnerable than a single sample to pulses of chemicals and process changes or upsets. They are preferable to caged organisms since SPMDs are not subject to either starvation or death and can therefore be used for prolonged periods of time or in locations like toxic effluent streams.

SPMDs have some disadvantages. One of these is related to the selectivity of the membrane: only freely dissolved neutral organic compounds are sampled. While this selectivity is similar to that of a fish membrane, the SPMDs lack the active and facilitated transport processes of a living membrane. Charged ions (metals such as Cu^{++} and Zn^{+} or ionized phenols and acids) do not cross the membrane and are not accumulated in the lipid. Another difference between SPMDs and fish is that the SPMDs cannot metabolize the compounds. While this is an advantage for analytical detection, it must be recognized the compounds accumulated by SPMDs may not be accumulated by biological organisms to the same extent if the organisms have the ability to breakdown and excrete the chemicals. Also, SPMDs can mimic only the waterborne uptake of chemicals into an organism. If the food chain is the main route of uptake of a chemical, SPMDs will not predict bioaccumulation.

To evaluate the potential for SPMDs as a monitoring device in the NRBS study area, a two year feasibility study was conducted. River water and effluents from major industries were sampled and the samples were tested for MFO inducing ability with cell culture techniques using a fish cell line. Cell line bioassays have proven to be of utility due to their reproducibility relative to live fish (Tillit *et al.* 1991). As discussed in Section 2 of this report, increased MFO activity is frequently observed in fish sampled from waters containing pulp mill effluent and is often associated with other changes in reproduction, growth, pathology and physiology. The cell line bioassay has proven useful in identifying the presence of bioactive compounds in environmental samples; see for example, Ankley *et al.* (1991). For expressing the potency of SPMD extracts as inducers in fish cells, MFO induction in cells exposed to SPMD extracts was compared with MFO induction in cells exposed to a known potent inducer, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), using the EROD bioassay discussed above. MFO induction was expressed as "EROD potency equivalents in pg/g" based on the activity of TCDD. This does not imply that the SPMD extracts contained TCDD or any other dioxin or furan, only that the extracts contained chemicals that were equivalent in MFO-inducing potency to a certain amount of TCDD.

4.2 RESULTS

In 1994, SPMD sampling on the Athabasca River took place at one major town and five industrial wastewater sites, four pulp mills and an oil sands facility, and one pulp mill on the Lesser Slave River (Parrott *et al.* 1996a). The devices were deployed for 2 weeks in waters of the Athabasca and Lesser Slave Rivers, in effluents from the pulp mills and in wastewater from the oil sands mining and upgrading facility. A total of 19 sites were sampled (Figure 15). One deployment device containing two SPMDs was used at upstream sites, one deployment device containing three SPMDs was used inside the pulp mills and oil sands facility, and three replicate deployment devices containing two SPMDs each were used at downstream locations. At each site, 2 SPMDs were used as trip blanks, and were exposed to air, handled as if deployed, and returned to the laboratory. Success of recovery of the SPMDs was 66%, with some of the losses caused by high water velocity and shifting channels and sediments. Most losses of SPMDs did not affect the design of the experiment, but reduced the number of replicate samples. One serious loss was that of the SPMDs from the effluent ponds of Alberta-Pacific Forest Industries. This reduced the number of pulp mills examined in the study from five to four.

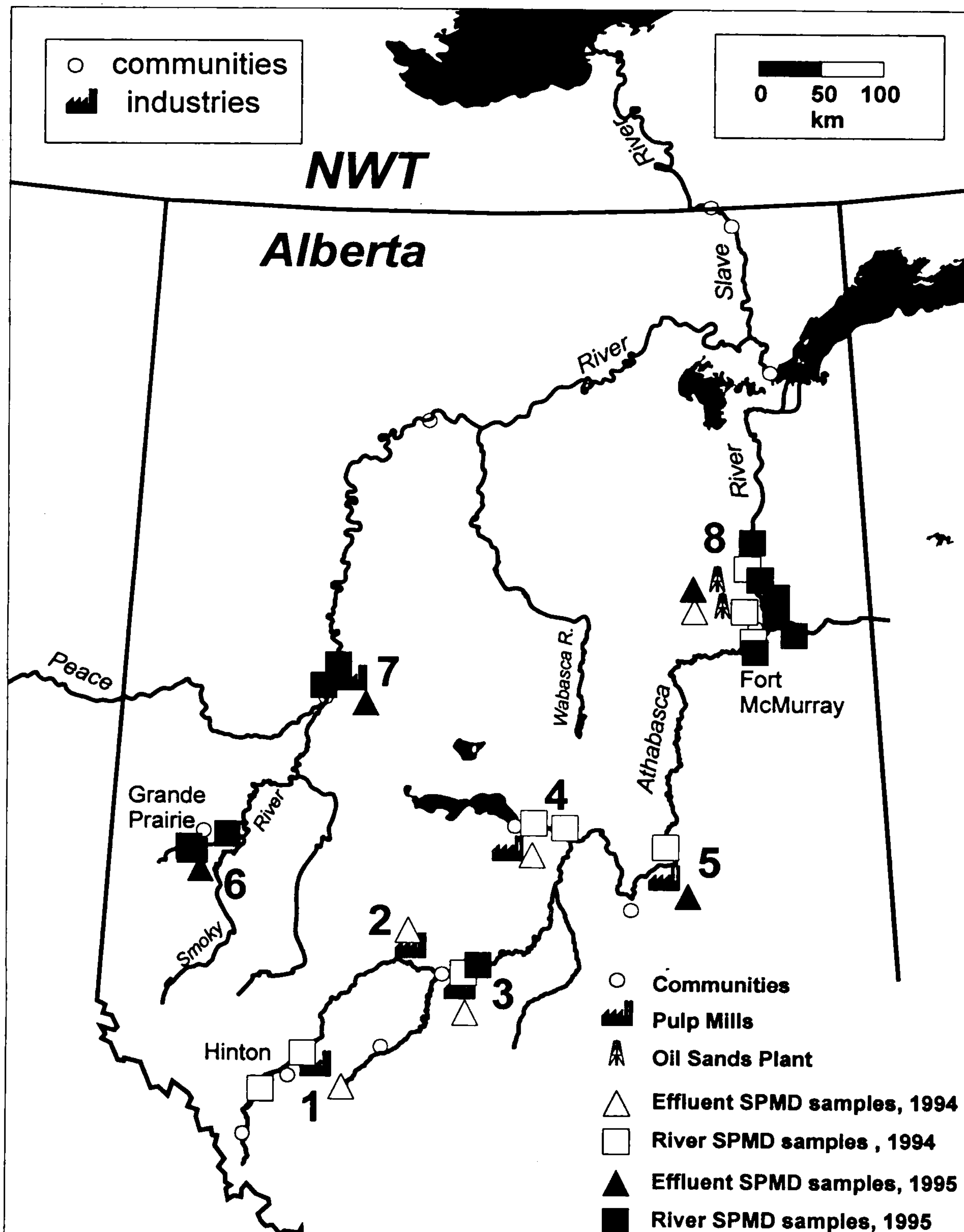

Figure 15. Map showing the locations of SPMD deployment at Weldwood of Canada, Ltd. (1), Alberta Newsprint Company, Ltd. (2), Millar Western Pulp Ltd. (3), Slave Lake Pulp Corporation (4), Alberta Pacific (5), Weyerhaeuser Canada Ltd. (6), Daishowa Canada Company, Ltd. (7) and Suncor, Inc. (8).

All SPMD extracts induced EROD in a fish liver cell line (Figure 16). Levels of inducers in trip blanks were low, usually about 6 pg EROD potency-EQ/g. Levels of inducers in SPMDs from Athabasca River water were fairly consistently low from Hinton to Boyle, averaging about 13 pg EROD potency-EQ/g or only twice that of the trip blanks. The levels of MFO induction in SPMDs exposed to river water increased downstream from Fort McMurray. In this area, SPMDs accumulated inducers from the river at levels ranging from 58 to 720 pg EROD potency-EQ/g. The source of this induction could have been sewage or other outfalls from the town of Fort McMurray or from natural oil seeps from the oil sands. Variability in the potency of SPMD extracts from different locations across the river downstream from Fort McMurray suggested that the source was localised.

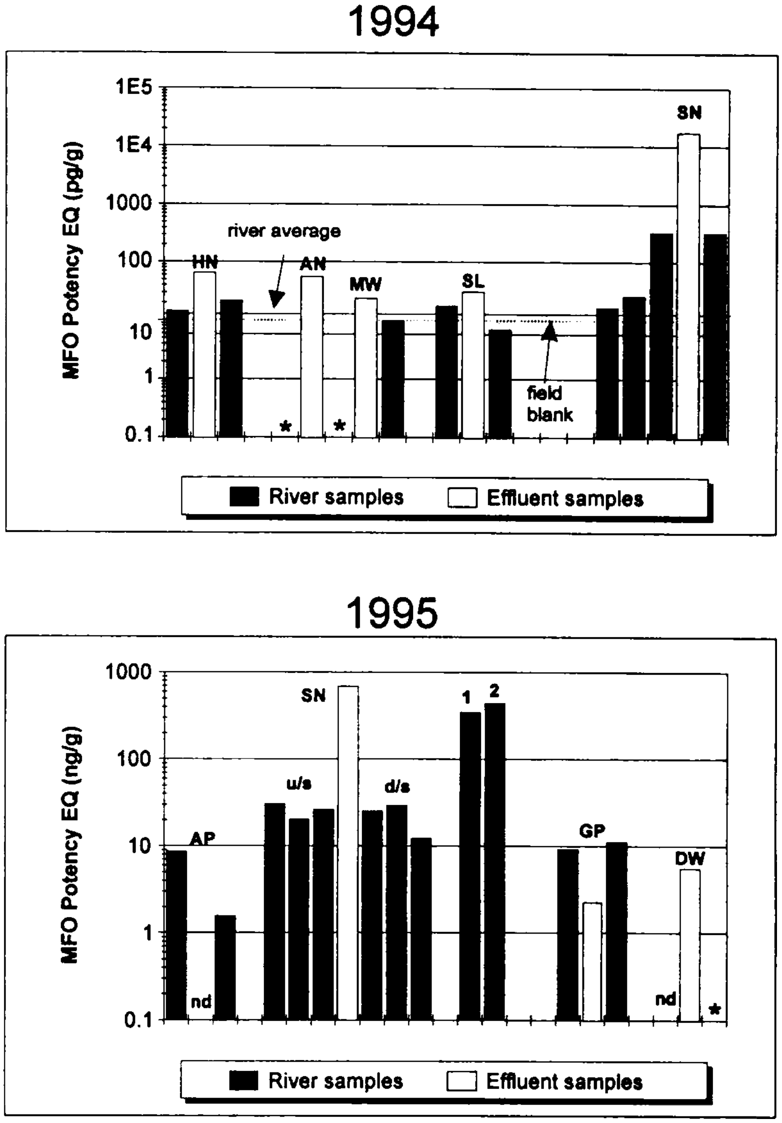

Figure 16. MFO activity in the cell line bioassay caused by exposure to SPMD extracts of effluents and water from sites on the Athabasca and Peace Rivers upstream and downstream from the following industries: Weldwood of Canada, Ltd. (HN), Alberta Newsprint Company, Ltd. (AN), Millar Western Pulp Ltd. (MW), Slave Lake Pulp Corporation (SL), Alberta Pacific (AP), Weyerhaeuser Canada Ltd. (GY), Daishowa Canada Company, Ltd. (DW) and Suncor, Inc. (SN). MFO activity in extracts from two Athabasca River tributaries, the Clearwater (1) and the Steepbank Rivers (2) are also shown.

Of four pulp mills tested, effluent SPMDs from three had induction potencies that were greater than the upper 95 % confidence limit (CL) for background levels of induction in Athabasca River water. Of the pulp mill effluents, the most potent SPMD extracts were from Weldwood, followed by Alberta Newsprint and Slave Lake Pulp. SPMDs from these pulp mills contained two to five times the average levels of inducers in SPMDs deployed in the Athabasca River. SPMDs deployed in Millar Western effluent did not induce MFO above the upper 95% CL for background water. Compared to MFO induction by extracts of SPMDs deployed in two Ontario bleached kraft mill effluents, the pulp mill effluents from the Athabasca River were one third to one twentieth as potent. By contrast, SPMDs deployed for 14 days in wastewater from Suncor had very high levels of MFO inducers: 16,800 pg EROD potency-EQ/g. The potency of extracts of SPMDs from Suncor effluent was over twenty times that of SPMDs from river waters upstream of Suncor.

The results from the 1994 project indicated that SPMDs from the four pulp mill effluents did contain small quantities of MFO inducers but that these small quantities did not result in a measurable increase in MFO inducers in receiving waters downstream from the pulp mills. SPMDs deployed in Athabasca River waters downstream from Fort McMurray also contained inducers, indicating some undetermined anthropogenic or natural source in this area. Despite its high induction, the Suncor effluent also did not measurably increase the inducer concentration downstream from the outfall compared to upstream, presumably because the effluent volume was low compared to the total discharge of the river.

In 1995, field deployments of SPMDs around Fort McMurray and in the Suncor plant were repeated to investigate more closely the variability of inducers around the oil sands area (Figure 15). Deployments were also made at the three pulp mills from which samples were not obtained in 1994 (Figure 15). In addition, small rainbow trout were caged in effluent and river water to compare MFO induction in fish cells exposed to SPMD extracts with whole fish responses (Parrott *et al.* 1996b). A modified method to calculate EROD-EQs was used. This meant that numerical comparisons of EROD-EQs between years could not be made although each year's samples are internally consistent.

A total of 45 devices were deployed in 1995 and 44 were recovered. Unfortunately, the river deployments were made on a falling hydrograph and water levels dropped dramatically between deployment and retrieval. Of the 45 devices deployed, 7 were exposed to air when the water level dropped and were not useable. Several others were the victim of tampering. Total recovery of useable devices was 35 out of 45.

Extracts of SPMDs deployed in river waters of the upper Athabasca, Peace and Wapiti rivers were low in MFO induction potency, while the potency of Athabasca River SPMDs increased in the vicinity of Fort McMurray and the oil sands (Figure 16). This trend was similar to that seen in the 1994 survey. In the 1995 survey, the increased SPMD deployments in the oil sands area detected two hot spots where EROD potencies were high; the south side of the Clearwater River and the north side of the Steepbank River. The source of these inducers is presumably natural. SPMDs deployed in Suncor wastewater contained high quantities of EROD inducers, similar to the 1994 results. The cell line bioassay dose- response curves for SPMD extracts from the oil sands area were peculiar, but internally consistent. Their interpretation will require further study.

Effluents from the three pulp mills (Alberta Pacific, Weyerhaeuser and Daishowa) tested in the 1995 survey had low potencies of EROD inducers. This result was similar to that seen in the 1994 Athabasca survey (Parrott *et al.* 1996a). SPMDs from the Suncor ponds were the most potent, with 358 to 860 ng EROD-EQ/g, although the range for the three replicate samples of 2 SPMDs each overlapped the potencies of the Clearwater and Steepbank samples.

In the whole-fish exposures, a total of 131 fish were exposed to Suncor wastewater. For controls, 25 fish were exposed to lab water (negative controls) and 24 fish were exposed to 10 µg/L of the known MFO inducer ß-naphthoflavone (BNF) (positive controls). Fish showed dose responsive increases in EROD activity with increasing concentrations of wastewater. The induction in small fish exposed in the lab to the wastewaters mirrored the induction in the fish cell bioassay. Both small fish and fish cells responded to the Suncor wastewater with EROD induction. Exposure of small rainbow trout to Suncor wastewater for 4 days induced hepatic EROD activity. Induction was the greatest at 32% effluent, and was up to three times higher than EROD activity in fish exposed to BNF. Induction at 100% was slightly less than at 32%, suggesting possible inhibition or toxicity of the wastewater. Regressions comparing log EROD activity to log wastewater exposure concentration showed the threshold for induction of EROD significantly above control activities was 1.6 to 1.7% wastewater. After dilution, concentration of the wastewater in the Athabasca River is less than 1% in the period of the study, suggesting if rainbow trout have comparable sensitivities to the inducing chemicals as wild fish, the wild fish should not have elevated EROD from exposure to waterborne inducers.

The fish cell bioassay was very sensitive, with cells responding to extremely small amounts (0.02%) of the SPMD extract. However, the fish cell bioassays did not achieve very high induction maxima, only one tenth of the control TCDD induction maxima. This was similar to the live fish, which were induced as high as the ß-naphthoflavone positive control but not as high as trout exposed to TCDD (500 pmol/mg/min, Parrott *et al.* 1996a).

The detection of two induction hotspots in the oil sands area in tributaries of the Athabasca River, the Clearwater and Steepbank Rivers, suggests natural erosion of the oil sands contributes potent MFO inducers in these areas. The Clearwater River SPMDs detected a localized source of inducers, as SPMDs deployed on the south side were high while SPMDs on the north side were similar to the background induction potency for Athabasca River waters in the oil sands area.

4.3 CONCLUSIONS

The deployment of SPMDs in this study proved a valuable tool capable of providing insight into local sources of biologically active substances that would have been overlooked or missed in such broad surveys as the basin-wide survey. The study demonstrated that the level of MFO inducers in effluent from all the pulp mills in the NRBS basins are low compared to elsewhere. In this respect, the study is in agreement with the results of the basin-wide fish survey. However, since the SPMDs were not mobile in the river, they answer the criticism that the fish sampled in the basin-wide survey could have been mobile and that the reason that their activity was low was due to movement out of the exposure zone, either into tributaries or upstream. Thus, the SPMDs provided critical complementary evidence that enhanced the interpretation of the results of the basin-wide survey.

Although the identity and in-mill sources of the inducers is not known, it has been demonstrated elsewhere that bleaching liquor contributes most of the induction in untreated final mill effluent (Schnell *et al.* unpublished observations), that induction is dependent on wood type (Martel *et al.*

1996) and the amount of organics released from wood during bleaching (Williams et al. 1996). In addition, cooking liquors from the chemical pulping process contain potent inducers. The general low level of inducers in the effluents of the Alberta mills was unanticipated. It presumably results from a combination of factors, including: good control of pulping liquor losses to the treatment system; removing more of the lignin in the cooking process thereby reducing the amount of organics that need to be removed from the pulp in bleaching and that end up in the effluent; and the use of hardwood in the wood furnish.

In general, although there were procedural difficulties that need to be solved, for example the continuing loss of devices due to tampering, the SPMD feasibility study proved to be a success. The technique clearly is useful in sorting out localised distributions of bioactive chemicals in receiving waters. In this respect, again, the results are noteworthy. Based on the results of this study, more effort into further refinement of the technique is justified. In particular, attention should be devoted to characterising the influence of environmental parameters like water velocity and temperature on mass transfer of contaminants into the SPMD and also to the stability of the lipids in the devices over time.

The geographical distribution of MFO inducers found in this study was consistent with that found in the basin-wide burbot survey. The site specific character of the technique allowed the identification of tributary hot spots that were not detected in the wild fish survey. The devices will prove useful in tracking down the actual sources of inducing substances. It is presumed that in the Fort McMurray area they are natural because of the general lack of development in the area where the inducing activity was detected.

5.0 SUITABILITY OF SMALL FISH SPECIES FOR ENVIRONMENTAL EFFECTS MONITORING

5.1 INTRODUCTION

An assumption underlying the use of sentinel species as a basis for a biological monitoring program is that organisms collected at a site reflect the local environment. Most successful uses of sentinel species have been in situations where their movement was restricted by habitat or barriers rather than in open river systems. There has been concern that larger fish species, such as those used in the NRBS basin-wide survey, may not be suitable as monitors of localized environments in open river systems like the NRBS area because they are too mobile. Their movement in and out of effluent exposure areas imparts an unacceptable degree of variability to the biological measurements. Before they can be used as sentinel species in open rivers, there is a need to evaluate the suitability of fish species for monitoring riverine environments.

Smaller fish species, such as cyprinids and cottids, have been advocated as possible sentinel species because they are thought to have a limited mobility relative to larger species and may possess a small home range. If this is true, these characteristics should make them ideal as sentinels since they should be more reflective of the localized conditions than larger species. The objective of this study was to assess the suitability of smaller fish species for monitoring receiving environments. The work is described in full in Gibbons *et al.* (1996). Suitability was determined by focusing on two criteria: the degree to which physiological responses of the small fish to a pollution source were similar to those of large fish; and, the degree to which the responses were reflective of localized environmental conditions.

Two study areas from the general region of the Upper Athabasca River immediately downstream from pulp mills were intensively studied. The study areas were:

1) Hinton study area centred around the effluent discharge from the Weldwood bleached kraft pulp mill in Hinton, and
2) Whitecourt study area focussing on effluents from two non-kraft mills, the Alberta Newsprint thermo-mechanical pulping (TMP) mill 10 km west of Whitecourt and the Millar Western chemi-thermo-mechanical pulping (CTMP) mill in Whitecourt.

The project was conducted in three field surveys:

1) pilot field survey (spring, 1994);
2) fall field survey (1994); and,
3) spring field survey (1995).

In the spring, 1994 pilot survey the objectives were: to identify the species potentially available as sentinels; to identify the best method of capture of the selected sentinel species; to select reference and exposure sites; and to collect preliminary data on physiological and whole organism characteristics of sentinel fish exposed to pulp mill effluent. The fall 1994 field survey was a continuation of the field work initiated during the spring and addressed the following objectives: final selection of the sentinel species for the Hinton area; collection of greater numbers of sexually mature sentinel fish to facilitate the analyses of whole organism parameters, MFO activity and *in vitro* steroid responses; evaluating physiological responses and whole organism responses of sentinel fish species and verification of the preliminary results observed during the pilot survey; defining exposure zones using water analysis; and, recording general habitat characteristics of each intensive fish collection site.

The final survey was conducted during the spring, 1995. This survey focussed only on the sentinel species in the Hinton area. The survey was initiated to: collect sexually mature sentinel fish at sites within the reference, near-field and far-field zones to investigate the geographical extent of whole organism and physiological responses downstream from the Hinton mill outfall; continue the evaluation of the *in vitro* steroid responses of the sentinel fish species following a prolonged period of high effluent exposure associated with winter low-flow conditions; improve our knowledge of the basic biology and life history of the sentinel species; further assess the mobility of the sentinel species; conduct water analyses for the purpose of confirming exposure zones; and, record general habitat characteristics of each intensive fish collection site.

5.2 RESULTS

A total of eleven sites in the Hinton study area, and nine sites in the Whitecourt study area were sampled for the purpose of collecting potential sentinel species. In the upper Athabasca River system, possible sentinel species considered are listed in Table 6 (Nelson and Paetz, 1992). For each study area, the resident fish were sampled and a sentinel species was selected based on availability, ease of capture, non-migratory behaviour, small size (max. adult total length < 15cm), and capture efficiency.

Specific sites for more intensive study were selected from the numerous sites sampled within a zone during the sentinel species survey according to the following criteria:

- for reference sites, the immediate area was not exposed to mill effluent or other discharges
- for near-field sites, the immediate area was exposed to mill effluent but no other discharges and had similar physical characteristics, e.g. habitat, as the associated reference site
- the selected sentinel species was present in adequate numbers for statistical analyses

In the Hinton study area, seven taxa of fish were collected (Table 6). A total of 1,126 fish were collected at 11 sites in the Hinton study area during the spring, 1994 survey. Immature mountain whitefish were the most abundant and widely distributed fish species, followed by young-of-the-year sucker species. Of the possible sentinel species, small numbers of longnose dace and spoonhead sculpin were found. The majority of longnose dace captured were immature and further daytime and nighttime attempts to collect adults were unsuccessful. Similarly, most spoonhead sculpin captured were immature; however, collection of a few adults (1 male, 6 females) suggested that increased sampling effort could provide greater numbers of mature male and female sculpin.

Table 6.Fish species considered for environmental effects monitoring in the NRBS.

Species	Possible sentinel species	Hinton area	Whitecourt area
Longnose dace (*Rhinichthys cataractae*)	✓	✓	✓
Pearl dace (*Margariscus margarita*)	✓		
Lake chub (*Couesius plumbeus*)	✓		✓
Fathead minnow (*Pimephales promelas*)	✓		
Spottail shiner (*Notropis hudsonius*)	✓	✓	✓
Trout-perch (*Percopsis omiscomaycus*)	✓		✓
Spoonhead sculpin (*Cottus ricei*)	✓	✓	✓
Mountain whitefish (*Prosopium williamsoni*)		✓	✓
Rainbow trout (*Oncorhynchus mykiss*)		✓	
Bull trout (*Salvelinus confluentus*)		✓	
Longnose sucker (*Catostomus catostomus*)		✓	
White sucker (*Catostomus commersoni*)			✓
juveniles, sucker (*Catostomus spp.*			✓
Burbot (*Lota lota*)			✓
Northern pike (*Esox lucius*)			✓

In the Whitecourt study area (including Windfall Junction), ten taxa of fish were (Table 6). A total of 677 fish were collected at 9 sites in the Whitecourt study area during the spring, 1994 survey. Lake chub was both the most abundant of all fish species and the most abundant and widely distributed sentinel species collected. Most chub were found along the quieter margins of the river where there was large cobble and boulder substrates. Backpack electrofishing proved to be the most efficient method of capturing lake chub from these habitats.

Based on these results, the species as sentinels were spoonhead sculpin for the Hinton study area and lake chub in the Whitecourt study area.

To assess whether small fish responded to the effluents in the same way as larger fish, measurements of body and organ metrics, reproductive parameters, age, mixed function oxygenase activity (MFO, EROD activity) and *in vitro* steroid production by gonadal tissues were made. In addition, information on the basic biology of each selected sentinel species was described. Very little life history information is known for these species. The development of sufficient background information will facilitate future assessment of species responses to effluent. During the fall 1994 and spring 1995 surveys water samples were collected at each sampling site for chemical analyses. As well, during each survey, habitat classification was conducted for each sampling site.

5.2.1 Hinton Study Area

In the spring of 1994, numbers of sculpin were too low to support analysis of downstream trends in metrics. However, from the limited data it appeared that the size of mature sculpin was approximately 7-8 cm in total length and 3-5 g in body weight. Mean size of immature sculpin was less than 5.7 cm long and 1.82 g in body weight. Mean gonad weight for female sculpin collected from the reference site was 0.08 g and represented 1.58% of the carcass weight. Prior to these collections, spoonhead sculpin were thought to spawn in April and May after the water temperature reached 6°C (Roberts, 1988). The spent condition of the ovaries from fish collected during this survey suggested that they had already spawned by May 5 (water temperature ≈ 9.5°C).

During the fall 1994 survey, sufficient numbers of mature sculpin were collected at a reference site, near-field site and an additional site located across river from the effluent plume to compare their characteristics. Spoonhead sculpin from the near-field site were older, heavier, fatter and had larger gonad and liver weights relative to reference fish. The increased level of energy expenditure and storage was considered indicative of an increased food supply, or enrichment. Exposed sculpin also exhibited an increase in the production of testosterone and 17ß-estradiol. Slight MFO induction relative to reference fish confirmed that the fish at the near-field site were exposed to effluent.

During the spring 1995 survey, sculpin were collected from three reference sites, as well as two near-field and far-field sites (21 km and 48 km from outfall). Differences in fish measurements between the reference sites were assumed to be representative of the natural variability in reference fish characteristics. In this survey, sculpin from the near-field zone were heavier, fatter and had larger liver weights than reference fish, similar to the fall survey. Exposed female sculpin exhibited higher gonad weight and fecundity. This general response pattern again suggested that the fish were responding to an increase in food resource and were not negatively affected by effluent exposure. Significant EROD induction (4-fold) confirmed that sculpin from near-field site were exposed to mill effluent. However, gonadal tissues of sculpin from the near-field sites exhibited similar *in vitro* production of testosterone and 17ß-estradiol to tissues from reference sculpin.

Sculpin from the far-field zone were compared with sculpin from the near-field and reference sites. Although some whole organism parameters were found to decrease downstream, many of the changes observed between reference and near-field fish had persisted, or become more pronounced. Hepatic EROD results indicated that sculpin at the middle far-field site (21 km) were exposed to sufficient effluent concentrations to cause induction equal to that observed at the near-field site. There was no reduction in EROD activity until the furthest far-field site (48 km); however, EROD activity in male sculpin was still higher than reference levels. With the exception of male sculpin, there were no differences in *in vitro* steroid production among the near-field, far-field and reference sites. For male sculpin, testosterone production was depressed at the middle near-field site and stimulated at furthest far-field site relative to near-field and reference values. Levels at the further far-field site may have been higher because males at that site were guarding nests with fertilized eggs and at a different reproductive stage.

5.2.2 <u>Whitecourt Study Area</u>

In the spring 1994 survey, in the vicinity of Whitecourt, seven taxa of fish were collected by electrofishing: mountain whitefish, lake chub, trout-perch, longnose dace, spoonhead sculpin, burbot and juvenile sucker species. The reference site for this study area was in the Athabasca River upstream from Whitecourt. This site was considered to be far enough downstream from Hinton not to be unduly influenced by the Weldwood effluent. Based on the reference site, mean sizes of mature males were similar to females. Male and female chub were approximately 8-9 cm mean fork length and 5-8 g mean total body weight. Immature chub were found to be less than 6 cm fork length and 2.3 g body weight. Mature male and female chub were similar in age and approximately 2-3 years old. Although the exact time of spawning for lake chub in the Whitecourt area is unknown, it has been suggested that lake chub spawn in late spring/early summer (Brown *et al.* 1970). At the time of sampling (May 6-14, water temperature ≈ 14°C), lake chub had not yet spawned and the gonadosomatic index for prespawning males was approximately 2-2.5%, and 13-15% for prespawning females. As well, the mean fecundity for reference females was 1,848 eggs/female and the mean size of eggs were 0.38 mg in weight and 0.83 mm in diameter.

Sufficient numbers of lake chub were collected to conduct site comparisons of fish metrics, MFO activity and *in vitro* testosterone production. Except for fecundity, there was little evidence to suggest that fish collected below the mill outfalls exhibited characteristics different from the reference fish. Data collected during the fall survey were used to further examine relationships.

During the spring 1994 survey, sufficient numbers of mature chub were collected at the reference site (Windfall Bridge), and the near-field sites to conduct site comparisons. From the preliminary comparisons of body size, organ metrics and age of lake chub, there was little evidence suggesting that fish were responding to effluent from either mill. Only fecundity of exposed chub was found to be different (lower among pooled near-field females). EROD activity in lake chub collected downstream from either outfall was not significantly induced relative to reference chub. The absence of significant EROD induction has also been documented in rainbow trout during laboratory exposures to 100% effluent from both mill facilities (Munkittrick *et al.* unpublished data). As well, *in vitro* production of testosterone by follicles from female chub was not significantly different among fish from the reference and near-field sites.

In the fall of 1994, adult lake chub downstream from the Millar Western mill outfall exhibited increased condition relative to fish from the Alberta Newsprint and reference reaches, without accompanying increases in organ size or individual body size estimates. Immature lake chub from the Alberta Newsprint site exhibited reduced condition relative to fish from the Millar Western site, but were similar to reference fish. As well, the mean liver size of exposed males was larger than reference males. There was a tendency towards increased production of testosterone by follicles of lake chub at the near-field sites. In an *in vitro* test, gonad samples were treated with human chorionic gonadotropin (hCG), a hormone that stimulates the production of reproductive steroids. In gonad samples from fish from the reference site, hCG stimulated production of 17ß-estradiol, but had no effect on follicles from fish exposed to mill effluents. The absence of increased levels of 17ß-estradiol from hCG mediated follicles of exposed females may be considered a response to effluent exposure. The absence of an MFO response suggests that exposure to effluent was low, possibly as a result of low effluent discharge and high effluent dilution.

To determine whether lake chub collected downstream from Millar Western exhibited characteristics different from upstream conditions, comparisons were made with chub collected from the Alberta Newsprint near-field site. Univariate comparisons of body size indicated that there were no significant site differences in total weight and fork length for mature male, female or immature lake chub. As well, there were no differences in mean ages of males and females. Size-at-age relationships were also similar between sites for female chub. There were no significant differences in relationships describing condition, gonad size or liver size of female chub. Fecundity and egg size, weight and diameter were also similar between sites. Comparisons between condition of immature fish indicated a significant difference in the slopes of the regression lines between the Alberta Newsprint and Millar Western site fish. By calculating the separate regression lines for each site, it was apparent that:

1) the size range of immature fish collected at the Alberta Newsprint near-field site included individuals smaller (3-4 cm range) than those collected from the Millar Western near-field site; and,
2) immature fish from below Alberta Newsprint were slightly "fatter" at small lengths, but their weight gain per increase in length was lower than for fish captured below Millar Western until a fork length of 5.8-6.0 cm.

Because there were no statistical differences in body size and organ weights for male and female fish collected below Alberta Newsprint and Millar Western, these data were pooled for comparisons with reference fish. Immature fish were not pooled due to significant differences in condition. There were no differences in the univariate size estimates of total weight or fork length in mature male or female lake chub collected from the reference and exposed sites. As well, there were no differences in mean ages of males and females. Size-at-age relationships were also similar between sites for female chub. There were no significant differences in condition, gonad weight or liver weight between reference and exposed female chub. Fecundity adjusted for body size was significantly higher at the reference site; however, egg weight (adjusted for body size) was not different between sites. Egg diameter was not related to body size and the univariate comparison of egg diameter indicated there was no difference between sites.

Total body weight and fork length of immature chub from either Alberta Newsprint or Millar Western near-field sites were not different from immature fish collected from the reference site (Windfall Bridge). Similarly, there were no differences in condition of immature fish collected from either mill site relative to reference fish.

From the site comparisons of body size, organ metrics and age of lake chub, there was little evidence suggesting alterations in these fish characteristics below Alberta Newsprint or Millar Western outfalls.

Mean hepatic EROD activity in male lake chub collected below Alberta Newsprint was 2.6-fold higher than reference males. However, variability among males from Alberta Newsprint was high and the difference relative to reference males was not found to be significant. EROD activity in male chub downstream of Millar Western was also not significantly different from reference males. Similarly, EROD activity in female lake chub collected downstream from either outfall was not significantly induced relative to reference females from Windfall Bridge. Typically, fish exposed to pulp mill effluent, especially effluent from mills with kraft pulping, show increased levels of hepatic EROD activity relative to unexposed fish. The absence of induction in fish below either of the mills in the Whitecourt study area was likely influenced by the high level of effluent dilution even during low river flow conditions. Laboratory tests investigating the effluent thresholds for MFO responses indicate that induced MFO activity is observed at concentrations of 0.5-0.7% for some kraft mill effluents (Robinson et al. 1994). Maximum effluent concentrations immediately below the Alberta Newsprint outfall during low flow conditions has been estimated to be approximately 0.15% (Sentar, 1994a). Maximum effluent concentrations within 155 m of the Millar Western outfall was estimated at 0.75%, and quickly dropped to 0.4 % approximately 1.5-1.7 km downstream (Sentar, 1994b).

In the fall of 1994, lake chub were collected from two reference sites located approximately 16 km from each other. Observed downstream changes in body and organ metrics of female and immature chub suggested that, in the absence of anthropogenic stressors, it was likely that the observed differences represent the natural variability in fish responses. As such, when comparing fish populations exposed to effluent with reference fish populations, care should be taken to adequately describe the natural variation in the reference zone (e.g. sample multiple reference sites within the reference zone that are as similar as possible to each other and the exposure site characteristics).

In vitro production of testosterone by follicles from lake chub was not significantly different among fish from the reference, Weldwood and Alberta Newsprint sites. In addition, there was no significant difference between basal testosterone production and follicles challenged with hCG. However, based on this first survey, hCG did not appear to be a suitable choice as a chemical stimulus for the production of hormones in lake chub *in vitro* steroid tests. Future surveys should use alternative stimulants in an attempt to find one that is effective for lake chub. As with the MFO results, the high degree of effluent dilution for Alberta Newsprint and Millar Western discharges, even during low flow conditions, minimized the likelihood of a steroid depression response in the exposed fish.

During the fall 1994 survey in the Hinton study area, in addition to the reference site and the near-field site, an additional site located across river from the effluent plume was sampled to investigate the lateral mobility of spoonhead sculpin. Spoonhead sculpin sampled across river from the effluent plume exhibited an intermediate MFO response relative to reference and near-field fish. The results suggested that lateral and upstream/downstream movement of spoonhead sculpin was limited at these sites. Concentrations of chloride, sulphate and sodium were higher at the mill near-field site further suggesting exposure to effluent. In the 1995 spring survey, as in the fall survey, sculpin across river from the plume exhibited responses intermediate between the reference and near-field fish.

In the spring 1995, sculpin collected from sites across river from each other exhibited differences in body/organ metrics, MFO activity and *in vitro* steroid production. Given the close proximity of these sites, these differences suggested that spoonhead sculpin did not undergo extensive lateral movement across river. As in the fall 1994 survey, fish responses across the river from the plume site were intermediate between responses observed at the reference and near-field sites. These results suggested that longitudinal movement up and down the river between sites was also limited.

The North Saskatchewan River has been suggested as a suitable alternative reference site for monitoring receiving environments of NRBS area pulp mill effluents. When data from reference sites of the Athabasca River were compared with data from the North Saskatchewan River, there were significant differences in some body/organ metrics, MFO activity and *in vitro* steroid production between the Athabasca and North Saskatchewan reference sites. The observed differences suggest that sculpin collected at the North Saskatchewan River did not accurately reflect the status of sculpin from reference sites in the Athabasca River. Had the North Saskatchewan site been used as the reference in this survey instead of sites on the Athabasca River, different conclusions regarding exposed fish would have resulted. It was unclear whether the North Saskatchewan River site represented the upper range of the normal performance of spoonhead sculpin, or whether these fish were responding to fluctuations in water levels associated with the Big Horn Dam. Regardless of the cause, had the North Saskatchewan River been used as a reference, the data comparison would have led to conclusion that an effluent-related impact on fish physiology was occurring. As mentioned above, such a conclusion is not supported by the comparison of reference and impacted sites on the Athabasca River.

5.3 CONCLUSIONS

Prior to this survey, little information was available in the literature regarding the responses fish populations of the Athabasca River to pulp mill effluent. In addition, few studies described the general biology of smaller fish species which were not of recreational or commercial value. Consequently, the project also provided the opportunity to determine whether small species such as spoonhead sculpin and lake chub exhibit responses reflective of local environmental conditions, conduct an assessment of the effluent impacts on resident fish species of the Athabasca River, and add to the knowledge base describing the basic biology of each sentinel species.

Based on the three surveys conducted, the potential to use small fish species is very high for the purpose of monitoring downstream from pulp mill outfalls. In particular, the abundance and distribution of resident forage species facilitates sampling a range of sites comprising reference, near-field area and far-field areas. In addition, the relatively stationary behaviour of small species like spoonhead sculpin greatly improves the probability that the observed fish responses will reflect the local environment. The typical biochemical measurements made on larger fish species were also possible using smaller species. These included: body and organ metrics, reproductive parameters, age estimates, mixed function oxygenase activity, and *in vitro* steroid production.

To further improve our understanding of the responses of small sentinel fish species to mill effluents discharged into the Athabasca River, there are several scientific studies that could be conducted both in the field and laboratory:

- Not enough information is available on the growth rates, reproductive strategies, or life history of these species to help plan monitoring programs based on forage fish to monitor impacts of industrial discharges in large rivers. Baseline data on life history characteristics of these species needs to be collected. Greater knowledge of the general biology would also improve the capture efficiency of small species. Due to higher levels of abundance associated with many forage species, the collection of adequate sample sizes would become very cost-effective.

- The NRBS studies demonstrated substantial variability in whole organism responses among reference populations of the small fish species studied, spoonhead sculpin and lake chub. More effort needs to be directed towards establishing the full range of variability associated with reference fish; both within a monitoring system and among a variety of similar aquatic systems. Interpretation of results of a small fish-based monitoring program will depend on a good understanding of the extent and causes of natural variability.

- More information is needed to further evaluate the mobility of smaller fish species. Although spoonhead sculpin does not appear to be as mobile as many of the large fish species, it is still necessary to increase our understanding of the degree and pattern of mobility, size of home range and habitat requirements associated with specific small species of interest. This is especially true for lake chub.

- To further evaluate the suitability of small fish species, it would be of interest to compare responses between small and large fish species of the same system. If possible, it would be advantageous to monitor a system possessing habitat or man-made barriers that would restrict the movement of the larger fish species. The comparison would examine the consistency of responses between species and investigate the relative sensitivity of each species.

- Laboratory evaluations of the potential of Athabasca effluents to disrupt steroids and induce MFO activity need to be repeated. Preliminary work was completed during the current project, but the steroid exposure protocol was still under development and has just been recently finalized. The exposures should be repeated using the final protocol. This information would be valuable to evaluate whether the effluents show the potential to induce the physiological changes.

6.0 CONCLUSIONS AND RECOMMENDATIONS

In summary, based on the basin-wide survey of physiological parameters in wild fish or from either of the newer approaches to specific monitoring, there was limited evidence of harmful effects of pulp mill effluents on biota in the basins. The sediment toxicity studies also supported this conclusion. There was suggestive evidence of physiological responses in fish downstream from the pulp mill examined in the preliminary studies, but by the end of the study, most of these responses were not evident. This apparent improvement was presumably due to process modifications that occurred at the mills during the study. Pulp mills in the basins introduced new treatment technologies resulting in a reduction of chlorinated organic compounds in the environment. Improvements involved the elimination or reduction in the use of elemental chlorine in the bleaching process, and upgrades to effluent secondary treatment. There was limited evidence that the pulp mills may still be causing depressed sex hormones in fish in a survey of small fish downstream from several pulp mills in the Athabasca River basin, but these depressed hormones apparently did not result in whole fish reproductive effects in that study. Although there was an indication in the basin-wide survey that the percentage of immature fish was higher downstream from the mills, the distribution of mature fish by sex was also anomalous suggesting that these distributions may be caused by biological rather than chemical effects.

An unexpected finding was that the highest EROD responses in the basin-wide survey were observed in areas of the lower Athabasca River far downstream from the pulp mills and in a tributary that did not receive pulp mill effluent. Tar sands and petroleum deposits are likely culprits. There were also unexplained responses in both the metallothionein and liver vitamin parts of the basin-wide survey. For the most part, these responses occurred mostly in the downstream areas and appeared to be unrelated to pulp mills or obvious discharge sources.

To further improve our understanding of the responses there are several scientific studies that could be conducted both in the laboratory and in the field:

- Long-term responses of fish in the lower Athabasca River to contaminants from the petroleum seeps, tar sands and other hydrocarbon-related pollution needs to be investigated. In particular, the connection, if any, with the distribution of immature burbot in the downstream areas needs to be addressed.

- More information needs to be available concerning the ecology of burbot to determine if the anomalous distributions observed in the NRBS studies were pollution-related or resulted from some previously aspect of burbot life history in these systems.

- The apparent anomalously high concentrations of free retinols and depressed tocopherol in burbot of the lower Peace River needs to be further investigated. If the observations can be replicated, they need to be explained.

- Not enough information on the growth rates, mobility, home range, habitat preferences, reproductive strategies or life histories of small fish is available to help plan monitoring programs based on these species. Baseline data on life histories of these species needs to be collected.

- In the NRBS studies, there was considerable variability in responses among reference populations of the small fish species studied, spoonhead sculpin and lake chub; this variability was particularly evident when populations in the Athabasca and North Saskatchewan Rivers were compared. More information is needed to explain this variability. It is recommended that the North Saskatchewan River not be used as a reference for the environmental effects studies in the Peace-Athabasca system.

- The responses of small fish and large fish in the same reach should be compared. This comparison would give an indication of inter-species variability and help identify sensitive species.

- Research into a short-term test for steroid depression needs to be conducted and effluents from various sources need to be tested for the presence of steroid-disrupting substances.

- Work on the use of SPMDs should be continued and a standardised protocol for their use in monitoring programs developed and validated.

7.0 REFERENCES

Adams, S.M., W.D. Crumby, M.S. Greeley, Jr., L.R. Shugart, and C.F. Saylor. 1992. Responses of fish populations and communities to pulp mill effluent: A holistic approach. Ecotoxicol. Environ. Saf. 24: 347-360.

Ankley, G.T., D. E. Tillitt, J. P. Giesy, P. D. Jones and D.A. Verbrugge. 1991, Bioassay-derived 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents in PCB-containing extracts from the flesh and eggs of Lake Michigan chinook salmon *(Oncorhynchus tshawytscha)* and possible implications for reproduction. Can. J. Fish. Aquat. Sci. 48:1685-1690.

Azais, V., M. Arand, P. Rauch, H. Schramm, P. Bellenand, J. F. Narbonne, F. Oesch, G. Pascal and L. W. Robertson. 1987. A time-course investigation of vitamin A levels and drug metabolising enzymes activities in rats following a single treatment with prototypic polychlorinated biphenyls and DDT. Toxicology 44: 341-354.

Balagus, P., A. De Vries and J. E. Green. 1993. Collection of fish from the traditional winter fishery on the Peace-Athabasca Delta, February, 1993. Northern River Basins Study Project Report No. 20.

Bank, P. A., K. L. Solyers and M. H. Zile. 1989. Effect of tetrachlorodibenzo-*p*-dioxin (TCDD) on the glucuronidation of retinoic acid in the rat. Biochem. Biophys. Acta. 993: 1-6.

Barton, B. A., C. P. Bjornson and K. L. Egan. 1993a. Special Fish Collections, Upper Athabasca River, May, 1992. Northern River Basins Study Project Report No. 8.

Barton, B. A., D. Patan and L. Seely. 1993b. Special Fish Collections, Upper Athabasca River, September and October, 1992. Northern River Basins Study Project Report No. 10.

Benson, W. H., K. N. Baer and C. F. Watson. 1990. Metallothionein as a biomarker of environmental metal contamination: Species-dependent effects. in *Biomarkers of Environmental Contamination*, J. F. McCarthy and L. R. Shugart (eds.), Lewis Publishers, Boca Raton, FL., pp. 255-265.

Benson, W. H., C. F. Watson, K. N. Baer and R. A. Stackhouse. 1988. Response of hematological and biochemical parameters to heavy metal exposure: Implications in environmental monitoring. Mar. Environ. Res. 24: 219-222.

Brouwer, A., and K. J. Van den Berg. 1986. Binding of a metabolite of 3,4,3',4'-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol. Appl. Pharmacol. 85: 301-312.

Brouwer, A., K. J. Van den Berg and A, Kuhler. 1985. Time course and dose response of the reduction in retinoid concentrations in C57BL/Rij and DBA/2 mice induced by 3,4,3',4'-tetrachlorobiphenyl. Toxicol. Appl. Pharmacol. 78: 180-189.

Brouwer, A., and K. J. Van den Berg. 1984. Early and differential decrease in retinoid levels in C57BL/Rij and DBA/2 mice by 3,4,3',4'-tetrachlorobiphenyl. Toxicol. Appl. Pharmacol. 73: 204-209.

Brown, J.H., U.T. Hammer and G.D. Koshinsky. 1970. Breeding biology of the lake chub, *Couesius plumbeus*, at Lac la Ronge, Saskatchewan. J. Fish. Res. Board Can. 27:1005-1015.

Brown, S. B., R. E. Evans, L. Vandenbyllaardt and A. Bordeleau. 1993. Analysis and interpretation of steroid hormones and gonad morphology in fish, Upper Athabasca River, 1992. Northern River Basins Study Project Report No. 13.

Brown, S. B., R. E. Evans and L. Vandenbyllaardt. 1996. Analysis for circulating gonadal steroids and gonad morphology in fish, Peace, Athabasca and Slave River Basins, September to December 1994. Northern River Basins Study Project Report No. 89.

Brown, S. B., and L. Vandenbyllaardt. 1996. Analysis of dehydroretinol, retinol, petinyl palmitate and tocopherol in fish, Peace, Athabasca and Slave River Basins, September to December 1994. Northern River Basins Study Project Report No. 90.

Chambers, P.A. 1996. Nutrient enrichment in the Peace, Athabsca and Slave Rivers: Assesment of present conditions and future trends. Northern River Basins Study Synthesis Report 4.

Chapman, P. M. 1990. The sediment quality triad approach to determining pollution-induced degradation. Sci. Tot. Environ. 97/98: 815-825.

Crosley, R. W. 1996. Environmental contaminants in water and sediments: PCDDs, PCDFs and resin acids, Athabasca River Basin, February-May, 1993. Northern River Basins Study Project Report No. 130.

Day, K., and T. Reynoldson. 1995. Ecotoxicology of depositional sediments, Athabasca River, May to December 1993. Northern River Basins Study Project Report No. 59.

Dobson, E., K. Day and T. Reynoldson. 1996. Ecotoxicology of depositional sediments, Athabasca, Smoky and Peace Rivers, June 1995. Northern River Basins Study Project Report No. 135.

Engel, D. W., and M. Brouwer. 1989. Metallothioneins and metallothionein-like proteins: Physiological importance. Adv. Comp. Environ. Physiol. 4: 53-75.

Freeman, H.C. and D.R. Idler. 1975. The effect of polychlorinated biphenyl on steroidogenesis and reproduction in the Brook Trout (*Salvelinus fontinalis*). Can. J. Biochem. 53:666-670.

Friesen, C., P.L. Wong, S.B. Brown and W.L. Lockhart. 1994. Changes in fish and aquatic habitat of the Winnipeg River downstream from the Pine Falls pulp mill. 2nd International Conference on Environmental Fate and Effects of Bleached Pulp Mill Effluent. Vancouver, British Columbia, November 6-10, 1994.

Gagnon, M.M., J.J. Dodson, and P.V. Hodson. 1994a. Ability of BKME (bleached kraft mill effluent) exposed white suckers (*Catostomus commersoni*) to synthesize steroid hormones. Comp. Biochem. Physiol. 107C: (2) 265-273.

Gagnon, M.M., J.J. Dodson, P.V. Hodson, G. Van Der Kraak and J. H. Carey. 1994b. Seasonal effects of bleached kraft mill effluent on reproductive parameters of white sucker (*Catostomus commersoni*) populations of the St. Maurice River, Quebec, Canada. Can. J. Fish. Aquat. Sci. 51: 337-347.

Gibbons, W.N., K.R. Munkittrick and W.D. Taylor. 1994. Peamouth chub (*Mylocheilus caurinus*) response to BKME exposure to effluent in the upper Fraser River, BC 2nd International Conference on Environmental Fate and Effects of Bleached Pulp Mill Effluent. Vancouver, British Columbia, November 6-10, 1994.

Gibbons, W.N., K.R. Munkittrick and W. Taylor. 1996. Suitability of small fish species for monitoring the effects of pulp mill effluent on fish populations, Athabasca River, 1994 and 1995. Northern River Basins Study Project report No. 100.

Giesy, J.P., J. P. Ludwig and D.E. Tillitt. 1994. Deformities in birds of the Great Lakes region - Assigning causality. Environ. Sci. Technol. 28:128A-135A

Hamer, D. H. 1986. Metallothionein. Ann. Rev. Biochem. 55: 913-951.

Hewitt, L.M., M.R. Servos, I.M. Scott, J.H. Carey and K.R. Munkittrick. 1995. Use of a MFO-directed toxicity identification evaluation to isolate and characterize bioactive contaminants from a lampricide formulation. Environ. Toxicol. Chem.

Hodson, P.V., M. McWhirther, K. Ralph, B. Gray, D. Thivierge, J. Carey, G. Van Der Kraak, D.M. Whittle, and M.C. Levesque. 1992. Effects of bleached kraft mill effluent on fish in the St. Maurice River, Quebec. Environ. Toxicol. Chem. 11: 1635-1651.

Huckins, J.N., M. W. Tubergen and G.K. Manuweera. 1990. Semipermeable membrane devices containing model lipid: A new approach to monitoring the bioavailability of lipophilic contaminants and estimating their bioconcentration potential. Chemosphere, 20:533-552.

Huckins, J.N., J. D. Petty, J.A. Lebo, C. E. Orazio, H. F. Prest, D. E. Tillitt, G. S. Ellis, B. T. Johnson and G.K. Manuweera. 1995. Semipermeable membrane devices (SPMDS) for the concentration and assessment of bioavailable organic contaminants in aquatic environments. In G. Ostrander, ed., *Techniques in Aquatic Toxicology.* Lewis publishers, *in press.*

Hvenegaard, P. J., and T. D. Boag. 1993. Burbot collections, Smoky, Wapiti and Peace Rivers, October and November, 1992. Northern River Basins Study Project Report No. 12.

Innami, S., A. Nakamura and S. Nagayama. 1974. Polychlorobiphenyl toxicity and nutrition. II. PCB toxicity and Vitamin A. J. Nutr. Sci. Vitaminol. 20: 363-370.

Jacobson, T. L. And T. D. Boag. 1995. Fish collections, Peace, Athabasca and Slave River Basins - September to December 1994. Northern River Basins Study Project Report No. 61.

Kidd, K., G. Van Der Kraak and K. Munkittrick. 1993. The effects of PCB126 on hepatic mixed function oxidase activity and steroidogenic capacity of the goldfish (Carassius auratus). Proc. 19th Aquatic Toxicity Workshop, Oct. 4-7, 1992, Edmonton, Alberta. E.G. Baddaloo, S. Ramamoorthy and J.W. Moore (eds.) Can. Tech. Rept. Fish. Aquat. Sci. 1942: 470-475.

Klaverkamp, J. F. And C. L. Baron. 1996. Concentrations of metallothionein in fish, Peace, Athabasca and Slave River Basins, September to December 1994. Northern River Basins Study Project report No. 93.

Kloepper-Sams, P.J., S.M. Swanson, T. Marchant, R. Schryer and J.W. Owens. 1994. Exposure of fish to biologically treated bleached-kraft effluent. 1. Biochemical, physiological and pathological assessment of rocky mountain whitefish (*Prosopium williamsoni*) and longnose sucker (*Catostomus catostomus*). Environ. Toxicol. Chem. 13: 1469-1482

Kloepper-Sams, P.J. and E. Benton. 1994. Exposure of fish to biologically treated bleached-kraft effluent. 2. Induction of hepatic cytochrome P4501A in mountain whitefish (*Prosopium williamsoni*) and other species. Environ. Toxicol. Chem. 13: 1483-1496.

Kovacs, T.G., J.S. Gibbons, L.A. Tremblay, B.I. O'Connor, P.H. Martel and R.H. Voss. 1995. The effects of a secondary-treated bleached kraft pulp mill effluent on aquatic organisms as assessed by short-term and long-term laboratory tests. Ecotox. Environ. Saf. 31: 7-22 .

Lindstrom-Seppa, P., S. Huuskonen, M. Pesonen, P. Muona and O. Hanninen. 1992. Unbleached pulp mill effluent affect cytochrome P-450 monooxygenase enzyme activities. Mar. Env. Res. 34: 157-161.

Lockhart, W. L. and D. A. Metner. 1996. Analysis for liver mixed-function oxygenase in fish, Peace, Athabasca and Slave River Drainages, September to December, 1994. Northern River Basins Study Project Report No. 104.

Lockhart, W. L., D. A. Metner, D. F. Rawn, R. J. Boychuk and J. R. Toews. 1996. Analysis for liver mixed-function oxygenase in fish, Upper Athabasca River, 1992. Northern River Basins Study Project Report No. 132.

Lowell, R.B., J.M. Culp and F.J. Wrona. 1995. Stimulation of increased short-term growth and development of mayflies by pulp mill effluent. Environmental Toxicology and Chemistry, 14(9): 1529-1541.

MacLatchy, D. and G. Van Der Kraak. 1995. The phytoestrogen β-sitosterol alters the reproductive status of goldfish. Toxicol. Appl. Pharmacol. 134: 305-312.

Martel, P.H., T.G. Kovacs and R.H. Voss. 1996. Effluent from Canadian pulp and paper mills: A recent investigation of their potential to induce mixed function oxygenase activity in fish. in *Environmental Fate and Effects of Pulp and Paper Mill Effluent*, (M.R. Servos, K.R. Munkittrick, J.H. Carey and G. Van Der Kraak, eds.), St. Lucie Press, Boca Raton, FL. pp 401-412.

McMaster, M.E., G.J. Van Der Kraak, C.B. Portt, K.R. Munkittrick, P.K. Sibley, I.R. Smith and D.G. Dixon. 1991. Changes in hepatic mixed function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft pulp mill effluent. Aquatic Toxicology 21: 199-218

McMaster, M. E., C. B. Portt, K. R. Munkittrick and D. G. Dixon. 1992b. Milt characteristics, reproductive performance and larval survival and development of white sucker exposed to bleached kraft mill effluent. Ecotoxicol. Environ. Saf. 23: 103-117.

McMaster, M.E. 1995. Mechanisms of reproductive dysfunction in fish exposed to various pulp mill effluents, and polycyclic aromatic hydrocarbon contamination. Ph.D. Thesis, University of Guelph, Guelph, Ontario. 278 p.

McMaster, M.E., K.R. Munkittrick, G.J. Van Der Kraak, P.A. Flett and M.R. Servos. 1995. Detection of steroid hormone disruptions associated with pulp mill effluent using artificial exposures to goldfish. In: *Environmental Fate and Effects of Pulp and Paper Mill Effluents*, (M.R. Servos, K.R. Munkittrick, J.H. Carey and G. Van Der Kraak, eds.), St. Lucie Press, Boca Raton, FL.

McMaster, M.E., K.R. Munkittrick, G.J. Van Der Kraak, P.A. Flett and M.R. Servos. 1996. Detection of steroid hormone disruptions associated with pulp mill effluent using artificial exposures to goldfish, in *Environmental Fate and Effects of Pulp and Paper Mill Effluent*, (M.R. Servos, K.R. Munkittrick, J.H. Carey and G. Van Der Kraak, eds.), St. Lucie Press, Boca Raton, FL., pp. 427-437.

Mercure, F. and G. Van Der Kraak. 1995. Inhibition of gonadotropin-stimulated ovarian steroid production by polyunsaturated fatty acids in teleost fish. Lipids 30: 547-554.

Muir, D.C.G., A.L. Yarechewski, D.A. Metner and W.L. Lockhart. 1990a. Dietary accumulation and sustained hepatic mixed function oxidase induction by 2,3,4,7,8-pentachlorodibenzofuran in rainbow trout. Environ. Toxicol. Chem. 9: 1463-1472.

Munkittrick, K.R. 1992. Recent North American studies of bleached kraft mill impacts on the reproduction and growth of fish populations. pp. 347-356 In (ed. A. Södergren) *Environmental Fate and Effects of Bleached Pulp Mill Effluents, Proceedings* November 19-21, 1991, Stockholm, Sweden. Swedish Environmental Protection Agency 4031.

Munkittrick, K.R., G.J. Van Der Kraak, M.E. McMaster and C.B. Portt. 1992a. Response of hepatic mixed function oxygenase (MFO) activity and plasma sex steroids to secondary treatment and mill shutdown. Environmental Toxicology and Chemistry 11: 1427-1439.

Munkittrick, K.R., M.E. McMaster, C.B. Portt, G.J. Van Der Kraak, I.R. Smith and D.G. Dixon. 1992b. Changes in maturity, plasma sex steroid levels, hepatic MFO activity and the presence of external lesions in lake whitefish exposed to bleached kraft mill effluent. Canadian Journal of Fisheries and Aquatic Sciences 49: 1560-1569.

Munkittrick, K.R., G.J. Van Der Kraak, M.E. McMaster, C.B. Portt, M.R. van den Heuvel and M.R. Servos. 1994a. Survey of receiving water environmental impacts associated with discharges from pulp mills. 2. Gonad size, liver size, hepatic EROD activity and plasma sex steroid levels in white sucker. Environ. Toxicol. Chem. 13: 1089-1101.

Munkittrick, K.R., M.R. Servos, J.L. Parrott, V. Martin, J.H. Carey, P.A. Flett and G.J. Van Der Kraak. 1994b. Identification of lampricide formulations as a potent inducer of MFO activity in fish. J. Great Lakes Res. 20: 355-365.

Nelson, J.S. and M.J. Paetz. 1992. The Fishes of Alberta. The University of Calgary Press, Calgary, AB. 437 pp.

Okey, A.B., D.S. Riddick and P.A. Harper. 1994. The Ah receptor: Mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Toxicol. Letters 70:1-22.

Owens, J.W., S.M. Swanson and D.A. Birkholtz. 1994a. Bioaccumulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran and extractable organic chlorine at a bleached-kraft mill site in a Northern Canadian river system. Environ. Toxicol. Chem. 13: 343-354.

Owens, J.W., S.M. Swanson and D.A. Birkholtz. 1994b. Environmental monitoring of bleached kraft pulp mill chlorophenolic compounds in a Northern Canadian river system. Chemosphere 29: 89-109.

Palace, V. P., and S. B. Brown. 1994. HPLC determination of tocopherol, retinol, dehydroretinol and retinyl palmitate in tissues of lake char (Salvelinus namacush) exposed to coplanar 3,3',4,4',5-pentachlorobiphenyl. Environ. Toxicol. Chem. 13: 473-476.

Parrott, J.L., P. V. Hodson, D. E. Tillitt, D.T. Bennie and M. E. Comba. 1996a. Accumulation of fish mixed function oxygenase inducers by semipermeable membrane devices in river water and effluents, Athabasca River, August and September, 1994. Northern River Basins Study Project Report No. 83.

Parrott, J. L., J. J. Whyte and M. E. Comba. 1996b. Accumulation of fish mixed function oxygenase (MFO) inducers by semipermeable membrane devices (SPMDs) in river water and effluents, Athabasca Peace and Wapiti Rivers, August and September, 1995. Northern Rivers Basins Study Project Report No. 127.

Persaud, P., Jaagumagi, R. and Hayton, A. 1992. Guidelines for the protection and management of sediment quality in Ontario. OMOEE Report, ISBN 0-7729-9248-7, Queen's Park Printer, Toronto, Ontario.

Pesonen, M. and T. Andersson.1992. Toxic effects of bleached and unbleached paper mill effluent in primary cultures of rainbow trout hepatocytes. Ecotox. Environ. Safety. 24: 63-71.

R. L. & L. Environmental Services Ltd. 1994a. A general fish and riverine habitat inventory, Athabasca River, April to May, 1992. Northern River Basins Study Project Report No. 32.

R. L. & L. Environmental Services Ltd. 1994b. A general fish and riverine habitat inventory, Athabasca River, October, 1993. Northern River Basins Study Project Report No. 40.

Roberts, W.E. 1988. The culpins of Alberta. Alberta Nat. 18:121-127.

Robinson, R.D., J.H. Carey, K.R. Solomon, I.R. Smith, M.R. Servos and K.R. Munkittrick. 1994. Survey of receiving water environmental impacts associated with discharges from pulp mills. I. Mill characteristics, receiving water chemical profiles and laboratory toxicity tests. Environ. Toxicol. Chem. 13:1075-1088.

Robinson, R.D. 1994. Evaluation and development of laboratory protocols for predicting the chronic toxicity of pulp mill effluents to fish. Univ. of Guelph, Ph.D. Thesis, 150 p.

Safe, S. 1990. Polychlorinated-biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). CRC Crit. Rev. Toxicol. 21: 51-88.

Sandström, O, E. Neuman and P. Karås. 1988. Effects of a bleached pulp mill effluent on growth and gonad function in Baltic coastal fish. Wat. Sci. Technol. 20: 107-118.

Sandström, O. 1994. Incomplete recovery in a coastal fish community exposed to effluent from a modernized Swedish coastal bleached kraft mill. Can. J. Fish. Aquat. Sci. 51: 2195-2202.

Sentar. 1994a. Pre-design study, environmental effects monitoring, Alberta Newsprint Company, Whitecourt, Alberta. Prepared for Alberta Newsprint Company, Whitecourt Alberta by Sentar Consultants Ltd., Calgary, Alberta. 107 pp. + appendices.

Sentar. 1994b. Pre-design study, environmental effects monitoring, Millar Western Pulp Ltd. Whitecourt, Alberta. Prepared for Millar Western Pulp Ltd., Whitecourt Alberta by Sentar Consultants Ltd., Calgary, Alberta. 117 pp. + appendices.

Servizi, J.A., R.W. Gordon, D.W. Martens, W.L. Lockhart, D.A. Metner, I.H. Rogers, J.R. McBride and R.J. Norstrom. 1993. Effects of biotreated bleached kraft mill effluent on fingerling chinook salmon (*Oncorhynchus tshawytcha*). Can. J. Fish. Aquat. Sci. 50: 846-857.

Servos, M., J. Carey, M. Ferguson, G. Van Der Kraak, H. Ferguson, J. Parrott, K. Gorman and R. Cowling. 1992. Impact of a modern bleached kraft mill with secondary treatment on white suckers. Water Pollut. Res. J. Can. 27: 423-437.

Servos, M.R., S. Huestis, D.M. Whittle, G.J. Van Der Kraak and K.R. Munkittrick. 1994. Survey of receiving water environmental impacts associated with discharges from pulp mills. 3. Polychlorinated dioxins and furans in muscle and liver of white sucker (*Catostomus commersoni*). Environ. Toxicol. Chem. 13: 1103-1115.

Servos, M.R., K.R. Munkittrick, E.A. Chisholm, M. Ferguson, and G. Van Der Kraak. 1995. Physiological responses in white sucker exposed to treated bleached kraft mill effluent in the Spanish River, Ontario, Canada. 2nd Internat. Conf. Environmental Fate and Effects of Bleached Pulp Mill Effluents, Nov. 6-10, 1994, Vancouver, BC

Singh, H. 1989. Interaction of xenobiotics with reproductive endocrine functions in a Protogynous Teleost, *Monopterus albus*. Mar. Environ. Res. 28:285-289.

Sivarajah, K., C.S. Franklin, and W.P. Williams. 1978. The effects of polychlorinated biphenyls on plasma steroid levels and hepatic microsomal enzymes in fish. J. Fish Biol. 13:401-409.

Södergren, A. 1989. Biological effects of bleached pulp mill effluents. National Swedish Environmental Protection Board Report 3558, Final Report. 139 p.

Spear, P.A., A.Y. Bilodeau and A. Branchard. 1992. Retinoids: from metabolism to environmental monitoring. Chemosphere. 25:1733-1738.

Spear, P. A., H. Garcin and J. F. Narbonne. 1988. Increased retinoic acid metabolism following 3,3',4,4',5,5'-hexabromobiphenyl injection. Can. J. Physiol. Pharmacol. 66: 1181-1186.

Spear, P. A., T. W. Moon and D. B. Peakall. 1986. Liver retinoid concentrations in natural populationa of herring gulls (Laurus argentatus) contaminated by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and in ring doves (Sterptopelia risoria) inhected with a dioxin analogue. Can. J. Zool. 64: 204-208.

Spear, P. A., P. Higuer and H. Garcin. 1994. Effects of fasting and 3,3',4,4',5,5'-hexabromobiphenyl on the plasma transport of thyroxin and retinol. J. Toxicol. Environ. Health 42: 173-183.

Spear. P. A., D. H. Bourbonnais, D. H. Peakall and T. W. Moon. 1989. Dove reproduction and retinoid (Vitamin A) dynamics in adult females and their eggs following exposure to 3,3',4,4'-tetrachlorobiphenyl. Can. J. Zool. 67: 908-913.

Swanson, S.M., R. Schyer, B. Shelast, K. Holley, I. Berbekar, P. Kloepper-Sams, J.W. Owens, L. Steeves, D. Birkholtz and T. Marchant. 1993. Wapiti/Smoky River Ecosystem Study. Weyerhaeuser Canada, Grande Prairie, Alberta

Swanson, S.M., R. Schryer, R. Shelast, P.J. Kloepper-Sams and J.W. Owens. 1994. Exposure of fish to biologically treated bleached-kraft effluent. 3. Fish habitat and population assessment. Environ. Toxicol. Chem. 13: 1497-1507.

Thomas, P. 1988. Reproductive endocrine function in female Atlantic Croaker exposed to pollutants. Mar. Environ. Res. 24:179-183.

Tillitt, D.E., Giesy, J.P. and G.T. Ankley. 1991. Characterization of the H4IIE rat hepatoma cell bioassay as a tool for assessing toxic potency of planar halogenated hydrocarbons in environmental samples. Environ. Sci. Technol. 25:87-92.

Truscott, B., J.M. Walsh, M.P. Burton, J.F. Payne and D.R. Idler. 1983. Effects of acute exposure to crude petroleum on some reproductive hormones in salmon and flounder. Comp. Biochem. Physiol. 75C: 121-130.

Wade, M.G., G. Van Der Kraak, M.F. Gerrits and J.S. Ballantyne. 1994. Release and steroidogenic actions of polyunsaturated fatty acids in the goldfish testis. Biol. Reprod. 51: 131-139.

White, R., S. Jobling, S.A. Hoare, J.P. Sumpter and M.G. Parker. 1994. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135: 175-182.

Williams, T.G., J. H. Carey, B. K. Burnison, D. G. Dixon and H.-B. Lee. 1996. Rainbow trout (*Oncorhynchus mykiss*) mixed function oxygenase responses caused by unbleached and bleached pulp mill effluent: A laboratory-based study. in *Environmental Fate and Effects of Pulp and Paper Mill Effluent*, (M.R. Servos, K.R. Munkittrick, J.H. Carey and G. Van Der Kraak, eds.), St. Lucie Press, Boca Raton, FL. pp 379-389.

Scientific Questions

1 a) How has the aquatic ecosystem, including fish and/or other aquatic organisms, been affected by exposure to organochlorines or other toxic compounds?

b) How can the ecosystem be protected from the effects of these compounds?

2 What is the current state of water quality in the Peace, Athabasca and Slave river basins, including the Peace-Athabasca Delta'.

3 Who are the stakeholders and what are the consumptive and non-consumptive uses of the water resources in the river basins?

4 a) What are the contents and nature of the contaminants entering the system and what is their distribution and toxicity in the aquatic ecosystem with particular reference to water, sediments and biota?

b) Are toxins such as dioxins, furans, mercury, etc. increasing or decreasing and what is their rate of change?

5 Are the substances added to the rivers by natural and man made discharge likely to cause deterioration of the water quality?

6 What is the distribution and movement of fish species in the watersheds of the Peace, Athabasca and Slave river? Where and when are they most likely to be exposed to changes in water quality and where are their important habitats?

7 What concentrations of dissolved oxygen are required seasonally to protect the various life stages of fish, and what factors control dissolved oxygen in rivers?

8 Recognizing that people drink water and eat fish from these river systems, what is the current concentration of contaminants in water and edible fish tissue and how are these levels changing through time and by location?

9 Are fish tainted in these waters and, if so, what is the source of the tainting compounds (i.e. compounds affecting how fish taste and smell to humans)?

10 How does and how could river flow regulation impact the aquatic ecosystem?

11 Have the riparian vegetation, riparian wildlife and domestic livestock in the river basin been affected by exposure to organochlorines or other toxic compounds?

12 What native traditional knowledge exists to enhance the physical science studies in all areas of enquiry?

13 a) What predictive tools are required to determine the cumulative effects of man made discharges on the water and aquatic environment'?

b) What are the cumulative effects of man made discharges on the water and aquatic environment?

14 What long term monitoring programs and predictive models are required to provide an ongoing assessment of the state of the aquatic ecosystems. These programs must ensure that all stakeholders have the opportunity for input.

Non-Scientific Questions

15 How can the Study results be communicated most effectively?

16 What form of interjurisdictional body can be established, ensuring stakeholder participation for the ongoing protection and use of the river basins?

The Northern River Basins Study was established to examine the relationship between industrial, municipal, agricultural and other development and the Peace, Athabasca and Slave river basins.

Synthesis Report

Over four and one half years, about 150 projects, or "mini studies" were contracted by the Study under eight component categories including contaminants, drinking water, nutrients, traditional knowledge, hydrology/hydraulics, synthesis and modelling, food chain and other river uses. The results of these projects, and other work and analyses conducted by the Study are provided in a series of synthesis reports.

This Synthesis Report documents the scientific findings and scientific recommendations of one of these component groups. This Synthesis Report is one of a series of documents which make up the Northern River Basins Study's final report. A separate document, the Final Report, provides further discussion on a number of scientific and river management issues, and outlines the Study Board's recommendations to the Ministers.

Project reports, synthesis reports, the Final Report and other NRBS documents are available to the public and to other interested parties.