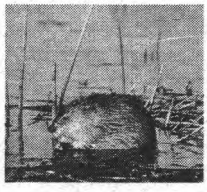
Canada Alberta Northwest Territories

# Northern River Basins Study





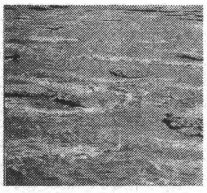


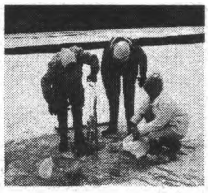





NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 104

# ANALYSIS FOR LIVER MIXED FUNCTION OXIDASE IN FISH, UPPER ATHABASCA RIVER, 1992






Prepared for the
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by

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NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 104

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**PREFACE:**

The Northern River Basins Study was initiated through the "Canada-Alberta-Northwest Territories Agreement Respecting the Peace-Athabasca-Slave River Basin Study, Phase II - Technical Studies" which was signed September 27, 1991. The purpose of the Study is to understand and characterize the cumulative effects of development on the water and aquatic environment of the Study Area by coordinating with existing programs and undertaking appropriate new technical studies.

This publication reports the method and findings of particular work conducted as part of the Northern River Basins Study. As such, the work was governed by a specific terms of reference and is expected to contribute information about the Study Area within the context of the overall study as described by the Study Final Report. This report has been reviewed by the Study Science Advisory Committee in regards to scientific content and has been approved by the Study Board of Directors for public release.

It is explicit in the objectives of the Study to report the results of technical work regularly to the public. This objective is served by distributing project reports to an extensive network of libraries, agencies, organizations and interested individuals and by granting universal permission to reproduce the material.

## NORTHERN RIVER BASINS STUDY
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Whereas the above publication is the result of a project conducted under the Northern River Basins Study and the terms of reference for that project are deemed to be fulfilled,
**IT IS THEREFORE REQUESTED BY THE STUDY OFFICE THAT;**
this publication be subjected to proper and responsible review and be considered for release to the public.

(Dr. Fred J. Wrona, Science Director) — (Date) 6 June 96

Whereas it is an explicit term of reference of the Science Advisory Committee "to review, for scientific content, material for publication by the Board",
**IT IS HERE ADVISED BY THE SCIENCE ADVISORY COMMITTEE THAT;**
this publication has been reviewed for scientific content and that the scientific practices represented in the report are acceptable given the specific purposes of the project and subject to the field conditions encountered.
**SUPPLEMENTAL COMMENTARY HAS BEEN ADDED TO THIS PUBLICATION: [ ] Yes [ ] No**

(Dr. P. A. Larkin, Ph.D., Chair) — (Date) 10 June/96

Whereas the Study Board is satisfied that this publication has been reviewed for scientific content and for immediate health implications,
**IT IS HERE APPROVED BY THE BOARD OF DIRECTORS THAT;**
this publication be released to the public, and that this publication be designated for: **[ ] STANDARD AVAILABILITY [ ] EXPANDED AVAILABILITY**

(Lucille Partington, Co-chair) — (Date) June 6/96

(Robert McLeod, Co-chair) — (Date) June 6/96

# ANALYSIS FOR LIVER MIXED FUNCTION OXYGENASE IN FISH, UPPER ATHABASCA RIVER, SPRING AND FALL, 1992

## STUDY PERSPECTIVE

The aquatic fauna of the northern rivers in Alberta are exposed to contaminants from pulp mill effluent, and other types of industrial and municipal effluents. Studies of sub-lethal effects on individuals can indicate an early response to environmental stress, and determine if further study is required to assess the ecological consequences for that population. For example, several recent studies in Canada have reported increased levels of mixed function oxygenase (MFO) activity, a sub-lethal effect, in the livers of fish collected near the effluents of pulp mills. MFOs are a family of detoxification enzymes found in the liver of many types of animals. Activity of these enzymes can be induced by the presence of a number of different foreign compounds, including those found in pulp mill effluents. MFO induction is an adaptive response, or defence mechanism, that has been associated with changes in sex steroid hormone levels, vitamin A metabolism, and impairment of reproductive and immune systems. Induction of MFOs is also one of the easiest and most sensitive responses to detect and has been adopted in a wide range of environmental monitoring programs. These enzyme activities can be used as an early warning to signal the need for further examination of other biological responses.

### *Related Study Questions*

| | |
|---|---|
| *1a)* | *How has the aquatic ecosystem, including fish and/or other aquatic organisms been affected by exposure to organochlorines or other toxic compounds?* |
| *4a)* | *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?* |
| *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?* |
| *13b)* | *What are the cumulative effects of man-made discharges on the water and aquatic environment?* |

The objective of this project was to examine MFO activity in fish collected from six sites on the upper Athabasca River, upstream and downstream of the bleached kraft pulp mill at Hinton. The fish species targeted for collection and analyses were mountain whitefish, longnose sucker, northern pike and white sucker. Two enzyme assays were applied to fish liver samples, ethoxyresorufin-O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH), and their activities are compared with contaminant residue levels and sex steroid hormones reported for the same fish in other NRBS project reports.

Results from 62 mountain whitefish and 26 northern pike collected in the spring, and 62 mountain whitefish, 23 northern pike, 57 longnose suckers and 26 white suckers collected in the fall were included into a portion or all of these analyses. The spring samples of mountain whitefish showed an elevated MFO response downstream of Hinton. The site by site pattern of enzyme activity was similar to the pattern shown by chlorinated dioxin and furan residues found in the muscle of these same fish. The fall collection of mountain whitefish did not show a consistent pattern of enzyme induction, but this is typical of fish during the spawning period. Female longnose suckers collected in the fall indicated a consistent pattern of elevated enzyme activities at the downstream sites. High enzyme activities in fall longnose suckers were associated with low levels of sex steroid hormones in the same individuals. Sample sizes of northern pike and white sucker were insufficient at some collection sites to support firm conclusions about site differences.

The results from this project are consistent with observations of fish downstream of pulp mills in other parts of Canada and the world. This project is one component of a study representing a concentrated effort to simultaneously evaluate contaminants levels, reproductive parameters and possible physiological effects of potential contaminant exposure in fish from the upper Athabasca River. This study will help determine the utility of these approaches in a larger scale analysis of fish physiology and contaminant levels in the Peace, Athabasca and Slave River drainages. In addition, results from this study will form important linkages with research on contaminant fate and food chain modelling, ecosystem health, cumulative effects assessment and human health consumption advisory assessments.

## REPORT SUMMARY

Since the late 1980s several studies of fish taken from habitat receiving effluents from bleached kraft pulp mills in Canada and northern Europe have shown a consistent pattern of increases in a series of liver enzymatic activities. These responses, called by a variety of names (monooxygenases, mixed-function oxidases or cytochrome P-450) have been found to respond to a number of chemical compounds including components of bleached kraft mill effluent. More recent study indicates that components of effluents from non-bleaching mills also cause this response. The responses arise from the synthesis of increased amounts of new enzyme by the exposed animals. These enzymes are often thought of as defense mechanisms used by the fish in an effort to detoxify chemical compounds and excrete them through bile or urine. There are a number of statistical relationships between these enzyme activities and other biological processes, notably those of reproduction and immune responses. Some evidence suggests that these processes are controlled independently, however, the statistical linkages exist, and so the enzyme activities can be used to signal the need for examination of other biological processes. The enzyme response is very sensitive, and so it offers an 'early warning' to signal the need for studies at higher levels of biological activity. However, if the enzyme activity fails to show a response, then other, more costly biological studies may not be required.

We applied two enzyme assays to the fish taken by Environmental Management Associates in the spring and fall of 1992 as part of the Representative Area Program of the Northern River Basins Study. The spring samples of mountain whitefish showed a response downstream from Hinton. The reach-by-reach pattern of enzyme activities was strikingly similar to the pattern shown by chlorinated dioxin and furan residues. Statistically, the enzyme activities were correlated with residue concentrations in the same fish (Pastershank and Muir, 1995). In comparison with other sites in western Canada, the response below Hinton was small and the activities found were all quite low. The fall collection (at spawning time) of mountain whitefish did not show a consistent pattern of induction. (Previous studies with other species have shown that enzyme activities are often low during spawning activity.) However, there were enough longnose sucker females taken in the fall to permit analysis for differences among reaches and these indicated a consistent pattern of elevated enzyme activities at the downstream sites, the same as that found in mountain whitefish in the spring. High enzyme activities in fall longnose suckers were associated with low levels of circulating steroid hormones (Brown et al., 1993) in the same individuals.

There were too few samples of northern pike or white suckers to support firm conclusions about site differences.

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## 1.0 INTRODUCTION

As agreed under the terms of contract 930380, fish liver samples were analyzed for activities of two liver microsomal catalytic activities, namely ethoxyresorufin-O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH). In addition, difference spectra were taken to allow calculation of the content of cytochrome P-450. Samples were collected by Environmental Management Associates and supplied to us by Alberta Environment. These analyses were selected because previous studies in Europe and Canada had indicated that they were sensitive to some component(s) in the effluents of bleached kraft pulp mills (Andersson *et al.*, 1988; Oikari, Lindström-Seppä and Kukkonen, 1988; Rogers *et al.*, 1989; Lindström-Seppä and Oikari, 1989; Lehtinen, 1990; Lehtinen et al., 1990; Oikari and Lindström-Seppä, 1990; McMaster *et al.*, 1991; Munkittrick *et al.*, 1992, Boyle *et al*, 1992; Servizi *et al.*, 1993). Recent studies have indicated that the enzyme induction response is elicited not only by effluents from kraft mills using chlorine bleaching, but also by effluents from mills using other processes (Martel *et al.*, 1992; Munkittrick et al., 1994; Friesen et al., 1994).

## 2.0 METHODS

### Fish Samples

Samples of liver of mountain whitefish (*Prosopium williamsoni*), northern pike (*Esox lucius*), white sucker (*Catostomus commersoni*) and Longnose sucker (*Catostomus catostomus*) were collected from several reaches of the Athabasca River by Environmental Management Associates, Calgary, Alberta, as described by Barton *et al.* (1992a,b). The same reaches were sampled in spring and fall; these were designated reaches A-F in the spring and G-M in the fall, as described below. The approximate extent of each reach is shown in Figure 1.

| Spring Reach | Fall Reach | Description |
|---|---|---|
| A | G | Near Entrance (near Highway 40 bridge) |
| B | H | Weldwood Haul Bridge at Hinton |
| C | J | Obed Mountain Coal Bridge |
| D | K | Emerson Lakes Bridge |
| E | L | Below Berland River confluence (bridge crossing) |
| F | M | Windfall Bridge (Windfall junction upstream of Whitecourt) |
| Reaches were the same in spring and fall but were designated with different letters | | |

Samples were sent, packed with dry ice, to the Freshwater Institute where they were stored at -80∘C until they were analyzed.

### Analysis of Livers

Recently the Department of Fisheries and Oceans published a technical report describing methods to perform monooxygenase assays (Hodson et al., 1991). These analyses can be done successfully in several ways; the methods described below are the procedures normally used in this laboratory and are consistent

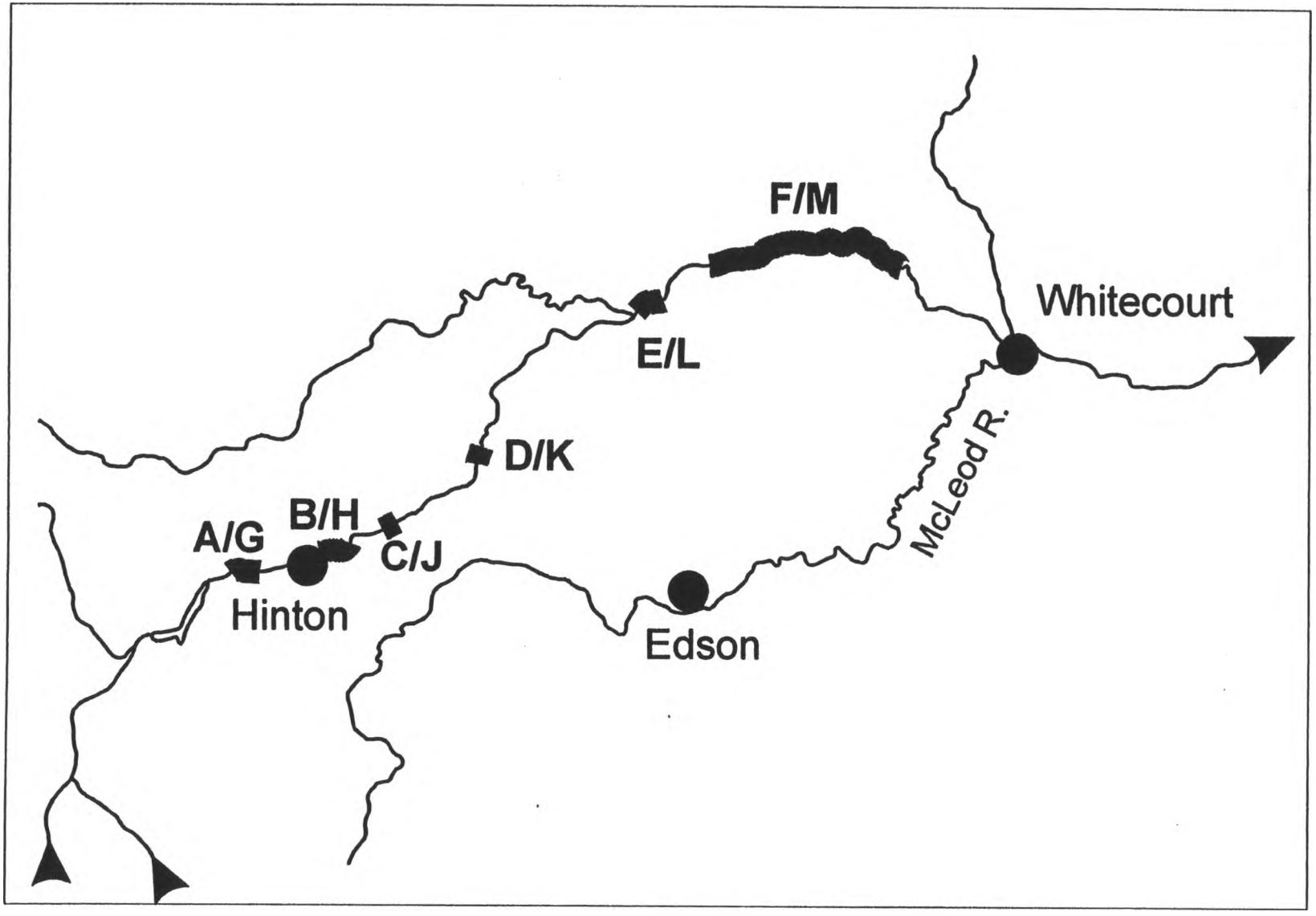


Figure 1. Approximate reaches of the Athabasca River where fish were collected in 1992. A-F, spring reach designation; G-M, fall reach designation.

with existing data on fish from other reaches of the Athabasca and Slave Rivers. The fluorometric assay for ethoxyresorufin O-deethylase (EROD) in Hodson et al. (1991) is identical to the procedure described below. The cytochrome P-450 assay below is similar to that described by Hodson et al. (1991), however, a different assay was used for aryl hydrocarbon hydroxylase (AHH) activity.

Reagents and chemicals were obtained from the following sources. Buffer salts were reagent grade and solvents were HPLC grade or distilled in glass. HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid), bovine serum albumin, dithiothreitol, ethylenediamine tetraacetic acid (EDTA), glycerol, nicotinimide adenine dinucleotide phosphate (NADP), nicotinimide adenine dinucleotide (reduced) (NADH), dl-isocitric acid trisodium salt (sodium isocitrate), and isocitric dehydrogenase were obtained from Sigma Chemicals. Potassium chloride (KCl), tris(hydroxy-methyl)methylamine (tris), sodium dihydrogen phosphate, disodium hydrogen phosphate, magnesium sulphate, magnesium choride, and potassium hydroxide (KOH) were obtained from BDH Chemicals. Resorufin and ethoxyresorufin were obtained from Molecular Probes. Methanol, hexane and dimethyl sulphoxide (DMSO) were obtained from Caledon Laboratories. Tritiated benzo(*a*)pyrene and Atomlight® liquid scintillation cocktail were obtained from DuPont Chemicals and unlabelled benzo(*a*)pyrene was obtained from Sigma-Aldrich. Sodium hydrosulphite (dithionite) was obtained from Fisher Scientific.

**Preparation of Liver Microsomes**

All tissue preparations were carried out in a controlled environment room at 4°C and care was taken to ensure that the temperature of samples or tissue preparations did not rise above 4°C during processing. The samples were received minced and frozen in 4 mL cryovials. The samples were removed from the freezer and allowed to thaw at 4°C until they could be removed from the cryovials. The samples were weighed and transferred to 15 mL cold glass tissue homogenizers, immersed in cold 0.02M HEPES/0.15M KCl buffer (pH 7.5) using 4 mL buffer per gram of tissue and homogenized using 5-7 passes with a motor-driven teflon pestle. The homogenate was then centrifuged at 12000 x g at 2°C for 20 min; the supernatant was further centrifuged at 105,000 x g at 2°C for 75 min to obtain a microsomal pellet. The pellet was washed and resuspended in tris/glycerol buffer to a target protein concentration between 5 and 10 mg $mL^{-1}$. The resuspension buffer consisted of 0.05 M tris, 1 mM dithiothreitol, 1 mM EDTA, and 20 % glycerol (v/v) adjusted to pH 7.4. Actual protein concentration in each microsomal suspension was measured by the method of Lowry et.al. (1951) as modified by Markwell et.al. (1981) using bovine serum albumin as a standard. Resuspended microsomes were frozen and stored in liquid nitrogen until analysis (Stegeman et al., 1981).

**Analysis of Microsomes for AHH Activity**

The AHH assay was based on that of Depierre et.al. (1975) as modified by Van Cantfort et.al. (1977) to measure the production of polar metabolites from non-polar tritiated benzo(*a*)pyrene. The microsomal suspension (150 µL) was incubated in tris-HCl buffer (800 µL, 0.1 M, pH 7.5) with magnesium chloride (10 µL, 500 mM) and an NADPH generating system (NADP, 10 µL, 78.7 mg $mL^{-1}$; sodium isocitrate 10 µL, 154.86 mg $mL^{-1}$; isocitrate dehydrogenase, q.s. 1 U per incubation tube (10 µL)) and tritiated benzo(*a*)pyrene (20 µL, 15-20 µCi per µmole in acetone, (specific activity accurately determined for each batch of substrate and corrected for time decay)) for 30 min at 25.0°C. The reaction was stopped by adding 2 ml 0.15 M KOH in 85 % DMSO: 15 % water. Unreacted benzo(*a*)pyrene was extracted with

two washes, each with 3 mL hexane. Radioactivity (benzo(*a*)pyrene metabolites) was counted in a 200-µL aliquot of the remaining aqueous layer using a Beckman LS-7500 liquid scintillation counter using Atomlight® liquid scintillation cocktail. Metabolites were assumed to have the same specific activity as substrate and to have been produced at 1:1 stoichiometry. Triplicate sample incubations were normally carried out for each microsomal suspension, and in each case triplicate blank incubations to which KOH-DMSO had been added prior to the addition of substrate were also run. Radioactivity remaining in the aqueous phase in the blanks was subtracted from that in the active preparations for calculation of activity as nanomoles of product per mg of microsomal protein per minute. The value reported was the mean of the replicate incubations.

Quench curves for the liquid scintillation counter were prepared using standard tritiated toluene obtained from DuPont. Counter performance was monitored using unquenched standards supplied by Beckman Instruments and analytical performance was monitored by reanalysing aliquots of two bulk preparations of charr microsomes (one of which had a high level of activity and one of which had a low level of activity) along with every second or third batch of samples.

**Analysis of Microsomes for EROD Activity**

Ethoxyresorufin-O-deethylase was measured by the deethylation of 7-ethoxyresorufin to yield resorufin which was detected by a fluorometric assay described by Pohl and Fouts (1980). The reaction mixture was 1100 µL of HEPES buffer (0.1 M, pH 7.8), 10 µL of magnesium sulfate (154 mg $mL^{-1}$), 10 µL NADP (98.4 mg $mL^{-1}$), 10 µL sodium isocitrate (193.58 mg $mL^{-1}$), isocitrate dehydrogenase (q.s. 1 U per incubation tube (10 µL)), and 50 µL bovine serum albumin (40 mg $mL^{-1}$), all mixed in a 16 x 100 mm disposable glass tubes where the mixture was incubated at room temperature for at least 10 min. The microsomal suspension (50 µL) was then added and the tubes and contents were equilibrated to 25.0°C in a water bath. The reaction initiated by the addition of 10 µL ethoxyresorufin (0.03 mg $mL^{-1}$ in DMSO) and tubes were incubated for an accurately timed period of two minutes at 25.0°C. The reaction was stopped by the addition of 2.5 mL methanol. The samples were centrifuged at 3700 x g for 15 min to remove precipitated protein and the resorufin in the supernatant measured fluorometrically at an excitation wavelength of 530 nm and an emission wavelength of 585 nm using either an Aminco-Bowman spectrofluorometer or a Perkin-Elmer LS50 spectrofluorometer. Standards which contained known amounts of resorufin were prepared in the same analytical matrix as samples and then used to determine a fluorescence response factor for the resorufin. Instrument response was monitored with each batch of samples by running samples consisting of resorufin in methanol. The LS50 was further monitored with solid fluorescence standards (Wilmad Glass) run with each batch of samples. Analytical quality was monitored by reanalysing aliquots of bulk arctic charr microsomes (one with a high level of activity and one with a low level of activity) with every second or third batch of samples. Triplicate incubation mixtures of each sample were normally run. Blanks consisting of samples to which the methanol had been added prior to the addition of substrate were run for each sample, and the final result corrected for any non-enzymatic production of resorufin. The value reported is the mean of the replicate assays.

**Analysis of Microsomes for Cytochrome P-450 Difference Spectra** .

The procedure is based on methods developed by Omura and Sato (1964a,b). The procedure used was similar to that described by Stegeman and Binder (1979). The microsome suspension was diluted to a

concentration of 1 mg $mL^{-1}$ (total volume 2 mL) in a phosphate buffer (0.1 M, pH 7.0) in a 12 x 75 mm disposable test tube. Twenty µL NADH solution (5.7 mg $mL^{-1}$) were added, mixed and then the suspension was transferred to a quartz cuvette. The suspension was bubbled for a minimum of 20 seconds with carbon monoxide gas to saturate the sample with CO. The absorbance of the carbon monoxide-saturated microsomes was then recorded from 400 to 500 nm using a Beckman DU-7 recording spectrophotometer and stored in the instrument memory as the background spectrum. Several milligrams of dithionite were added to the cuvette to reduce the cytochrome P-450 and the absorbance spectrum between 400 and 500 nm was recorded again, using the stored spectrum as the background thus creating the difference spectrum. The absorbance readings for the difference spectrum at 450 and 475 nm were used (Johannesen and DePierre, 1978) to calculate the amount of cytochrome P-450 present, using a millimolar extinction coefficient of 91 for a 1-cm light path (Omura and Sato, 1964a,b). Triplicate spectra were run for each sample if sufficient sample was available and the P-450 value reported was the mean of the replicates.

**Statistics**

All statistics given were calculated using SAS (Version 6.07) procedures (proc means, proc corr, proc glm) on a VAX 6000 computer at the Freshwater Institute. The level of probability taken to indicate a significant difference was 0.05. Unless otherwise stated, tabulated values were log transformed prior to comparisons among mean values. A few values were below our levels for quantitation and in those instances the different approaches described below were taken to include that information in a calculation. In the case of enzyme activities, we chose arbitrarily to express those values as the detection limit for calculations. That is, a value tabulated as <0.001 (Appendix 1) would be taken as 0.001 for calculations. In the case of chlorinated dioxin and furan residues, we treated residues reported as 'ND' or 'Trace' as zero, while those designated as NDR (x) were treated as x. (That is, NDR(0.55) was treated as 0.55.)

## 3.0 RESULTS AND DISCUSSION

The samples consisted of mountain whitefish and northern pike collected from several reaches of the Athabasca River (Figure 1) by Environmental Management Associates in the spring of 1992, and additional mountain whitefish, northern pike, longnose suckers and white suckers collected in the fall of 1992 (Barton *et al.*, 1992a,b). A list of all the samples analyzed is given in Appendix 1 showing physical data and liver biochemical measurements for each individual.

The cytochrome P-450 levels are tabulated, however, we consider them to be unreliable in view of the shapes of the difference spectra. Most samples exhibited a large peak near 420 nm, indicative of deterioration in sample quality, although the cause of this is not known. The two sample handling points most likely to provide opportunity for deterioration are in the field between sacrifice of the fish and freezing the sample, and in the laboratory during the recovery of the samples from the cryovials. Our initial thought was that perhaps the samples degraded during the interval between death and freezing of liver; however, we tested this interval for statistical relationships with cytochrome P450 or P420 for the spring mountain whitefish and none were found. The samples were difficult to extract from the cryovials, and had to be thawed somewhat more than usual prior to homogenization; this may have provided an occasion for some sample deterioration. Whatever its source, the deterioration indicated by the difference spectra may have compromised the EROD and AHH values.

**Mountain whitefish**

Group arithmetic mean enzymatic activities are listed for female mountain whitefish in Table 1 and for males in Table 2.

Spring samples

Considering the 62 (46 female, 16 male) mountain whitefish taken in the spring, there were patterns in both liver microsomal EROD and AHH activities consistent with a source of inducing compounds near reach B, the location nearest the Hinton mill (Figure 2). There is nothing in the records of sample handling to suggest selective deterioration of samples taken at the upstream reach (A), and we interpret the site differences in EROD and AHH activities as effects of mill effluent. Statistically there were real differences among the locations for EROD activity in spring when the sexes were pooled and when the females were considered alone; with spring males ($n=16$) site differences fell just short of the $p<0.05$ criterion, although the pattern was the same. With AHH means, activity in males and females combined differed among sites, however, neither sex individually met the statistical criterion. EROD and AHH activities were correlated with each other.

Brown et al. (1993) reported several measurements on the whitefish, and we have examined their data for statistical linkages between the enzymatic activities and their other measures associated with reproduction. In the whitefish females from the spring there was a weak but meaningful correlation between EROD activity and egg diameter ($r=0.32$, $p=0.032$); AHH activities were similarly related to egg diameter ($r=0.32$, $p=0.027$). There were no correlations of either enzymatic activity with the other measures available (length, weight, liver weight, gonad weight, estrogen, testosterone, egg weight, clutch, absolute fecundity, or relative fecundity). Taking males only, neither enzymatic activity correlated with the reproductive measurements. When (log10) egg diameter was incorporated into the covariance model with females, site differences remained significant for (log10) EROD ($p<0.0001$) and reached the criterion for (log10) AHH ($p=0.039$).

| Table 1. Summary statistics for female mountain whitefish | | | | | | |
|---|---|---|---|---|---|---|
| Reach (season) | Variable | N | Mean | Std Dev | Minimum | Maximum |
| A (spring) | EROD | 7 | 0.006 | 0.005 | 0.001 | 0.016 |
| B (spring) | EROD | 11 | 0.045 | 0.042 | 0.008 | 0.146 |
| C (spring) | EROD | 8 | 0.025 | 0.015 | 0.012 | 0.051 |
| D (spring) | EROD | 8 | 0.014 | 0.013 | 0.001 | 0.034 |
| E (spring) | EROD | 6 | 0.029 | 0.013 | 0.019 | 0.051 |
| F (spring) | EROD | 6 | 0.032 | 0.013 | 0.014 | 0.049 |
| G (fall) | EROD | 5 | 0.002 | 0.002 | 0.001 | 0.005 |
| H (fall) | EROD | 9 | 0.050 | 0.074 | 0.003 | 0.203 |
| J (fall) | EROD | 8 | 0.007 | 0.004 | 0.003 | 0.014 |
| K (fall) | EROD | 9 | 0.018 | 0.009 | 0.006 | 0.033 |
| L (fall) | EROD | 9 | 0.010 | 0.005 | 0.005 | 0.016 |
| M (fall) | EROD | 5 | 0.008 | 0.003 | 0.005 | 0.013 |
| A (spring) | AHH | 7 | 0.019 | 0.013 | 0.001 | 0.038 |
| B (spring) | AHH | 11 | 0.069 | 0.047 | 0.015 | 0.168 |
| C (spring) | AHH | 8 | 0.042 | 0.034 | 0.001 | 0.097 |
| D (spring) | AHH | 8 | 0.024 | 0.022 | 0.001 | 0.058 |
| E (spring) | AHH | 6 | 0.053 | 0.026 | 0.032 | 0.101 |
| F (spring) | AHH | 6 | 0.051 | 0.026 | 0.001 | 0.077 |
| G (fall) | AHH | 5 | 0.013 | 0.006 | 0.005 | 0.022 |
| H (fall) | AHH | 9 | 0.022 | 0.021 | 0.001 | 0.052 |
| J (fall) | AHH | 8 | 0.018 | 0.009 | 0.007 | 0.032 |
| K (fall) | AHH | 9 | 0.030 | 0.019 | 0.013 | 0.075 |
| L (fall) | AHH | 9 | 0.019 | 0.017 | 0.001 | 0.044 |
| M (fall) | AHH | 5 | 0.015 | 0.011 | 0.002 | 0.031 |
| A (spring) | P450 | 7 | 0.062 | 0.017 | 0.031 | 0.081 |
| B (spring) | P450 | 11 | 0.071 | 0.039 | 0.001 | 0.133 |
| C (spring) | P450 | 8 | 0.067 | 0.055 | 0.001 | 0.160 |
| D (spring) | P450 | 8 | 0.054 | 0.062 | 0.001 | 0.184 |
| E (spring) | P450 | 6 | 0.090 | 0.006 | 0.080 | 0.099 |
| F (spring) | P450 | 6 | 0.111 | 0.020 | 0.076 | 0.137 |
| G (fall) | P450 | 5 | 0.053 | 0.030 | 0.007 | 0.081 |
| H (fall) | P450 | 8 | 0.079 | 0.067 | 0.029 | 0.204 |
| J (fall) | P450 | 8 | 0.068 | 0.023 | 0.038 | 0.106 |
| K (fall) | P450 | 9 | 0.074 | 0.016 | 0.050 | 0.101 |
| L (fall) | P450 | 9 | 0.068 | 0.026 | 0.040 | 0.120 |
| M (fall) | P450 | 5 | 0.070 | 0.014 | 0.054 | 0.091 |

| Table 2. Summary statistics for male mountain whitefish | | | | | | |
|---|---|---|---|---|---|---|
| Reach (season) | Variable | N | Mean | Std dev. | Minimum | Maximum |
| A (spring) | EROD | 3 | 0.005 | 0.003 | 0.002 | 0.007 |
| B (spring) | EROD | 1 | 0.093 | . | 0.093 | 0.093 |
| C (spring) | EROD | 2 | 0.015 | 0.011 | 0.007 | 0.023 |
| D (spring) | EROD | 2 | 0.020 | 0.025 | 0.002 | 0.037 |
| E (spring) | EROD | 4 | 0.028 | 0.010 | 0.016 | 0.039 |
| F (spring) | EROD | 4 | 0.038 | 0.023 | 0.007 | 0.060 |
| G (fall) | EROD | 5 | 0.007 | 0.005 | 0.000 | 0.013 |
| H (fall) | EROD | 2 | 0.045 | 0.013 | 0.036 | 0.054 |
| J (fall) | EROD | 2 | 0.012 | 0.009 | 0.005 | 0.018 |
| K (fall) | EROD | 2 | 0.127 | 0.161 | 0.013 | 0.240 |
| L (fall) | EROD | 1 | 0.013 | . | 0.013 | 0.013 |
| M (fall) | EROD | 5 | 0.017 | 0.003 | 0.012 | 0.020 |
| A (spring) | AHH | 3 | 0.016 | 0.014 | 0.001 | 0.027 |
| B (spring) | AHH | 1 | 0.125 | . | 0.125 | 0.125 |
| C (spring) | AHH | 2 | 0.020 | 0.025 | 0.002 | 0.038 |
| D (spring) | AHH | 2 | 0.035 | 0.047 | 0.001 | 0.068 |
| E (spring) | AHH | 4 | 0.054 | 0.020 | 0.039 | 0.083 |
| F (spring) | AHH | 4 | 0.069 | 0.039 | 0.018 | 0.109 |
| G (fall) | AHH | 5 | 0.033 | 0.016 | 0.012 | 0.055 |
| H (fall) | AHH | 2 | 0.018 | 0.023 | 0.001 | 0.034 |
| J (fall) | AHH | 2 | 0.009 | 0.011 | 0.001 | 0.016 |
| K (fall) | AHH | 2 | 0.159 | 0.130 | 0.067 | 0.251 |
| L (fall) | AHH | 1 | 0.001 | . | 0.001 | 0.001 |
| M (fall) | AHH | 5 | 0.080 | 0.135 | 0.001 | 0.319 |
| A (spring) | P450 | 3 | 0.112 | 0.027 | 0.084 | 0.137 |
| B (spring) | P450 | 1 | 0.148 | . | 0.148 | 0.148 |
| C (spring) | P450 | 2 | 0.080 | 0.001 | 0.079 | 0.081 |
| D (spring) | P450 | 2 | 0.050 | 0.045 | 0.018 | 0.082 |
| E (spring) | P450 | 4 | 0.077 | 0.065 | 0.001 | 0.146 |
| F (spring) | P450 | 4 | 0.126 | 0.053 | 0.057 | 0.186 |
| G (fall) | P450 | 3 | 0.040 | 0.024 | 0.015 | 0.062 |
| H (fall) | P450 | 2 | 0.207 | 0.024 | 0.190 | 0.224 |
| M (fall) | P450 | 3 | 0.141 | 0.021 | 0.124 | 0.165 |

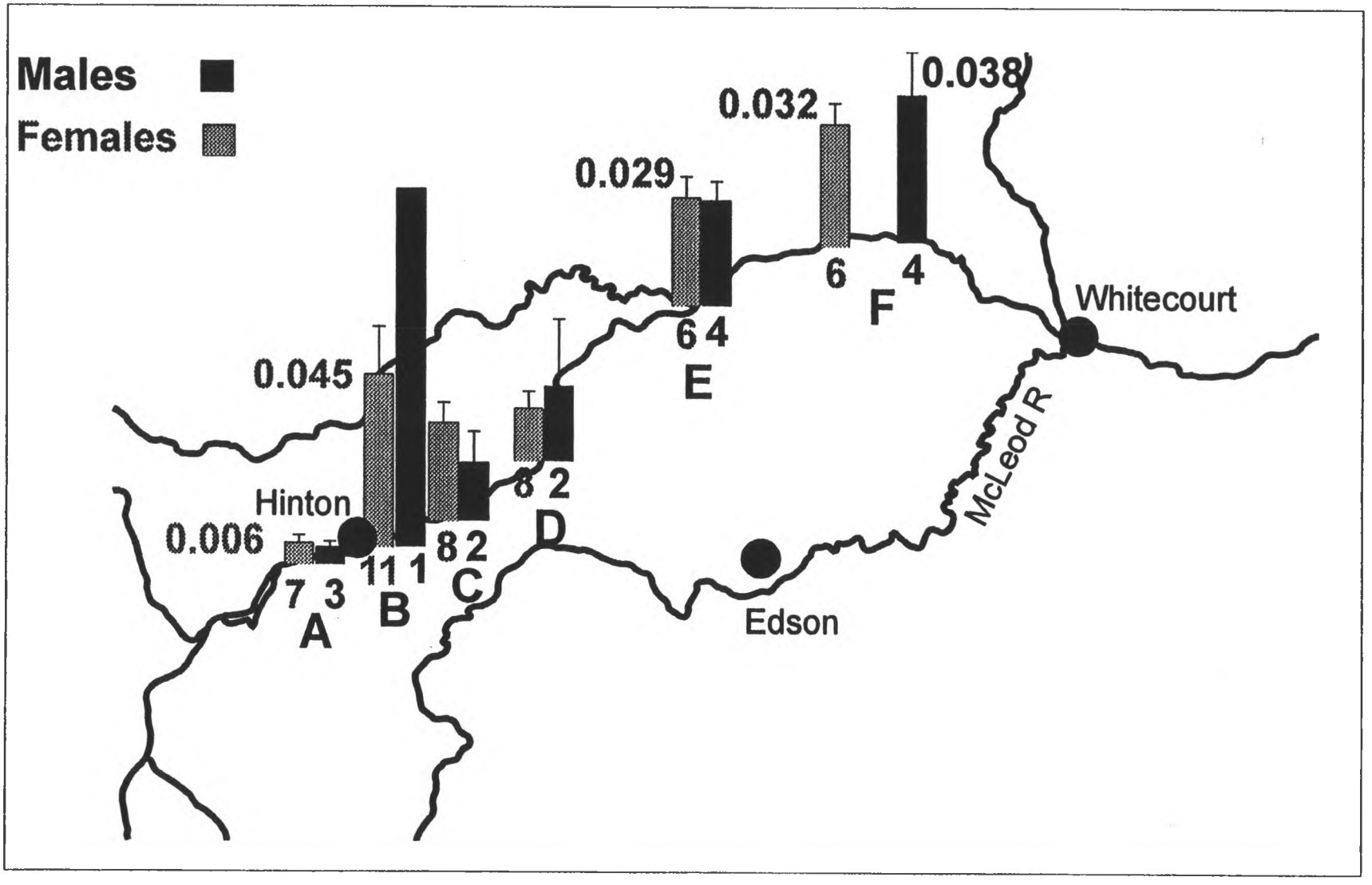


Figure 2. Liver microsomal EROD in mountain whitefish from several reaches of the Athabasca River in spring, 1992. Bars are means with standard errors. The number of fish represented by each bar is given beneath the bar.

Fall samples

Considering site means within the 62 mountain whitefish collected in the fall of 1992, (Tables 1 and 2) there were site-to-site differences in EROD activity in the combined sexes. AHH activities for the pooled sexes failed to differ among sites. Taking females alone (n=45) for EROD activities, there were site differences which could be interpreted as implicating a source at site B. The pattern of site means was quite similar with AHH activities. Males (n=17) failed to differ significantly among sites for either activity, and the pattern of activities gave no indication of a source; males from site D had the highest activities and the upstream males from site A had higher AHH activities than those from three of the other locations.

Fall-collected females were in various different states of sexual maturation, and there were significant statistical linkages with reproductive measurements reported by Brown et al., (1993). Specifically EROD correlated positively with egg diameter ($r=0.43$; $p=0.006$) and negatively with maturity index ($r=-0.55$; $p<0.0001$) and gonad weight ($r=-0.41$; $p=0.0057$). AHH correlated negatively with gonad weight ($r=-0.31$; $p=0.037$) and maturity index ($r=-0.48$; $p=0.0009$). When these factors (log10 length, log10 egg diameter, log10 gonad weight and maturity index) were incorporated into the covariance model to test for site differences in (log10) EROD, the site differences were significant ($F=7.84$ $p<0.0001$). Site A differed from all the other sites; the only other difference was between sites C and D. With (log10) AHH, however, the same model failed to detect any site differences. Males collected in the fall failed to show correlations between the enzymatic activities and measures associated with reproduction (gonad weight, testosterone, 11-ketotestosterone, maturity index) and no site differences were indicated by the analysis of covariance. A strong correlation between EROD and AHH activities often results when a sample contains both induced and uninduced fish; with the fall whitefish, there was no correlation between these activities, and so we interpret the fall data to mean that very little or no induction response was present.

Mountain whitefish spawn in the fall. There have been previous instances where fish showed the induction response in one season but not in another. Munkittrick et al. (1991) found exactly this with white suckers when they compared a site receiving effluent (Jackfish Bay, Lake Superior) with a reference site (Mountain Bay, Lake Superior). The fish from those two sites differed in the summer (non-spawning time) but not in the spring (spawning time for suckers). We have essentially the same phenomenon in the Athabasca samples, a difference in enzymatic activities during the non-spawning (spring) period but little or no difference during the spawning (fall) period.

**Correlations with chemical residues in spring mountain whitefish**

Values for chemical residues of chlorinated dibenzodioxins and dibenzofurans in muscle of the spring-sampled mountain whitefish were obtained from Ms. G. Pastershank (Pastershank & Muir, 1995). The values were simplified for calculations by making three arbitrary assumptions. Values reported as "ND" or "Trace" were taken as zero; values reported as "NDR (x)" were reported as x. That is, a value of NDR (0.095) was taken as 0.095. The residue values used for calculation are listed in Table 3. The value calculated for toxic equivalents (TEQ) was taken to be the TCDD concentration plus one-tenth of the TCDF concentration. The chemical residues showed a pattern of increasing amounts in fish taken downstream from the mill site (Figure 3), especially at sites B, C, and D. There were significant positive correlations between the liver enzyme activities and the chemical residues in females (tabulated below

and plotted in Figure 4), however, these were driven largely by two points with TCDD values over 20 pg/g and TCDF values over 30 pg/g (fish numbers B-I-9 and D-I-6). The correlations were recalculated excluding those points and significant correlations were absent without them.

Correlation coefficients between chlorinated dioxin and furan residues and liver microsomal enzymes in female mountain whitefish taken from sites in the Athabasca River in spring, 1992.

| Pair compared | Correlation coefficient, r, when all points are used (n=46) | | Correlation coefficient, r, with B-I-9 and D-I-6 excluded (n=44) | |
|---|---|---|---|---|
| | r | p | r | p |
| EROD vs TCDD | 0.45 | 0.002 | 0.24 | ns |
| AHH vs TCDD | 0.34 | 0.021 | 0.13 | ns |
| EROD vs TCDF | 0.55 | 0.0001 | 0.24 | ns |
| AHH vs TCDF | 0.43 | 0.003 | 0.17 | ns |
| EROD vs TEQ | 0.47 | 0.0009 | 0.25 | ns |
| AHH vs TEQ | 0.36 | 0.015 | 0.14 | ns |

**Comparisons with other published results for mountain whitefish**

We have tabulated those other published mountain whitefish assays known to us (Table 4). Means for "control" samples from the N. Saskatchewan River and from the Kootenay River ranged from 0.021 to 0.029 nmol mg protein$^{-1}$ min$^{-1}$, values substantially above those for the "control" (site A) reported here. This does not appear to be a case of analytical differences among different laboratories since the Smokey/Wapiti samples were analyzed in the Procter and Gamble laboratory in Cincinatti and the Columbia River samples were done in our laboratory, and the two sets of data agree well. Rather the difference suggests that the Athabasca samples are low and the reason(s) for that are unknown. Low values are predicted for these fish based on their cytochrome P-450 difference spectra. Perhaps some biological or habitat variable can explain why the activities should be low in the Athabasca system, but it seems more likely that the explanation lies with the sample quality as suggested by the cytochrome P-450 difference spectra. However, the low values cannot explain the site differences in the absence of some systematic artifact applied only to the upstream reach. Lacking any such known artifact, we conclude that the site differences relate to the source of effluent and not to procedural artifacts.

Table 3. Chlorinated dioxin and furan residues in muscle of mountain whitefish, Athabasca River, spring, 1992. TEQ=TCDD + (0.1 x TCDF) (Pastershank & Muir, 1995)

| Fish No. | Location | Sex | EROD | AHH | TCDD | TCDF | TEQ |
|---|---|---|---|---|---|---|---|
| A-I-1 | A | F | 0.003 | 0.007 | 0 | 0 | 0 |
| A-I-2 | A | M | 0.007 | 0.027 | 0.51 | 0.97 | 0.607 |
| A-I-3 | A | F | 0.016 | 0.038 | 1.4 | 4 | 1.8 |
| A-I-4 | A | F | 0.008 | 0.023 | 0 | 0 | 0 |
| A-I-5 | A | F | 0.002 | 0.021 | 0 | 0 | 0 |
| A-I-6 | A | F | 0.003 | 0.001 | 0 | 0.92 | 0.092 |
| A-I-7 | A | M | 0.002 | 0.001 | 0.92 | 1.3 | 1.05 |
| A-I-8 | A | F | 0.001 | 0.029 | 0 | 0 | 0 |
| A-I-9 | A | M | 0.005 | 0.021 | 0.16 | 0.37 | 0.197 |
| A-I-10 | A | F | 0.007 | 0.016 | 0 | 0 | 0 |
| B-I-1 | B | F | 0.008 | 0.015 | 6.7 | 12 | 7.9 |
| B-I-2 | B | F | 0.026 | 0.047 | 3.2 | 9.9 | 4.19 |
| B-I-3 | B | F | 0.092 | 0.13 | 13 | 21 | 15.1 |
| B-I-4 | B | F | 0.061 | 0.105 | 6.1 | 11 | 7.2 |
| B-I-5 | B | F | 0.01 | 0.031 | 6.7 | 16 | 8.3 |
| B-I-6 | B | F | 0.04 | 0.059 | 5 | 6.4 | 5.64 |
| B-I-7 | B | F | 0.017 | 0.045 | 10 | 14 | 11.4 |
| B-I-8 | B | M | 0.093 | 0.125 | 4.1 | 2.6 | 4.36 |
| B-I-9 | B | F | 0.146 | 0.168 | 23 | 47 | 27.7 |
| B-I-10 | B | F | 0.024 | 0.039 | 5.2 | 3.8 | 5.58 |
| B-I-11 | B | F | 0.044 | 0.073 | 6.3 | 8.2 | 7.12 |
| B-I-12 | B | F | 0.025 | 0.045 | 2.5 | 5.5 | 3.05 |
| C-I-1 | C | F | 0.023 | 0.033 | 14 | 21 | 16.1 |
| C-I-2 | C | F | 0.014 | 0.024 | 9.3 | 13 | 10.6 |
| C-I-3 | C | F | 0.051 | 0.097 | 11 | 17 | 12.7 |
| C-I-4 | C | M | 0.023 | 0.038 | 18 | 30 | 21 |
| C-I-5 | C | F | 0.032 | 0.069 | 1.8 | 6.7 | 2.47 |
| C-I-6 | C | F | 0.042 | 0.078 | 6.2 | 7.4 | 6.94 |
| C-I-7 | C | F | 0.013 | 0.001 | 3.2 | 8 | 4 |
| C-I-8 | C | F | 0.012 | 0.023 | 3.8 | 3.6 | 4.16 |
| C-I-9 | C | M | 0.007 | 0.002 | 2.1 | 2.9 | 2.39 |
| C-I-10 | C | F | 0.016 | 0.014 | 11 | 14 | 12.4 |

Table 3. (continued) Chlorinated dioxin and furan residues in muscle of mountain whitefish, Athabasca River, spring, 1992.
TEQ=TCDD + (0.1 x TCDF) (Pastershank & Muir, 1995)

| Fish No. | Location | Sex | EROD | AHH | TCDD | TCDF | TEQ |
|---|---|---|---|---|---|---|---|
| D-I-1 | D | M | 0.037 | 0.068 | 3.1 | 4.9 | 3.59 |
| D-I-2 | D | F | 0.01 | 0.024 | 7.3 | 25 | 9.8 |
| D-I-3 | D | F | 0.005 | 0.02 | 2.1 | 3 | 2.4 |
| D-I-4 | D | M | 0.002 | 0.001 | 1 | 2.5 | 1.25 |
| D-I-5 | D | F | 0.007 | 0.005 | 16 | 8.2 | 16.82 |
| D-I-6 | D | F | 0.033 | 0.055 | 29 | 39 | 32.9 |
| D-I-7 | D | F | 0.001 | 0.001 | 3.7 | 6.9 | 4.39 |
| D-I-8 | D | F | 0.012 | 0.008 | 8.7 | 15 | 10.2 |
| D-I-9 | D | F | 0.034 | 0.058 | 9.1 | 18 | 10.9 |
| D-I-10 | D | F | 0.008 | 0.018 | 4.8 | 14 | 6.2 |
| E-I-1 | E | M | 0.016 | 0.039 | 0.7 | 13 | 2 |
| E-I-2 | E | F | 0.022 | 0.032 | 1.1 | 3 | 1.4 |
| E-I-3 | E | F | 0.019 | 0.035 | 2.7 | 1.2 | 2.82 |
| E-I-4 | E | F | 0.037 | 0.063 | 3.1 | 3.3 | 3.43 |
| E-I-5 | E | F | 0.051 | 0.101 | 0.4 | 0.4 | 0.44 |
| E-I-6 | E | M | 0.026 | 0.045 | 5.5 | 4.4 | 5.94 |
| E-I-7 | E | M | 0.039 | 0.083 | 3.1 | 1.1 | 3.21 |
| E-I-8 | E | F | 0.019 | 0.037 | 1.3 | 2.8 | 1.58 |
| E-I-9 | E | F | 0.023 | 0.05 | 0.9 | 4.3 | 1.33 |
| E-I-10 | E | M | 0.03 | 0.047 | 11 | 15 | 12.5 |
| F-I-1 | F | M | 0.06 | 0.109 | 8.7 | 16 | 10.3 |
| F-I-2 | F | F | 0.046 | 0.077 | 2.2 | 6.3 | 2.83 |
| F-I-3 | F | M | 0.007 | 0.018 | 9.5 | 14 | 10.9 |
| F-I-4 | F | M | 0.048 | 0.085 | 3.6 | 8.2 | 4.42 |
| F-I-5 | F | F | 0.014 | 0.001 | 0.7 | 0.91 | 0.791 |
| F-I-6 | F | F | 0.028 | 0.055 | 0 | 0.96 | 0.096 |
| F-I-7 | F | F | 0.029 | 0.059 | 1.3 | 1.2 | 1.42 |
| F-I-8 | F | F | 0.049 | 0.057 | 1.8 | 2.9 | 2.09 |
| F-I-9 | F | M | 0.038 | 0.063 | 10 | 35 | 13.5 |
| F-I-10 | F | F | 0.026 | 0.056 | 0.32 | 0.83 | 0.403 |

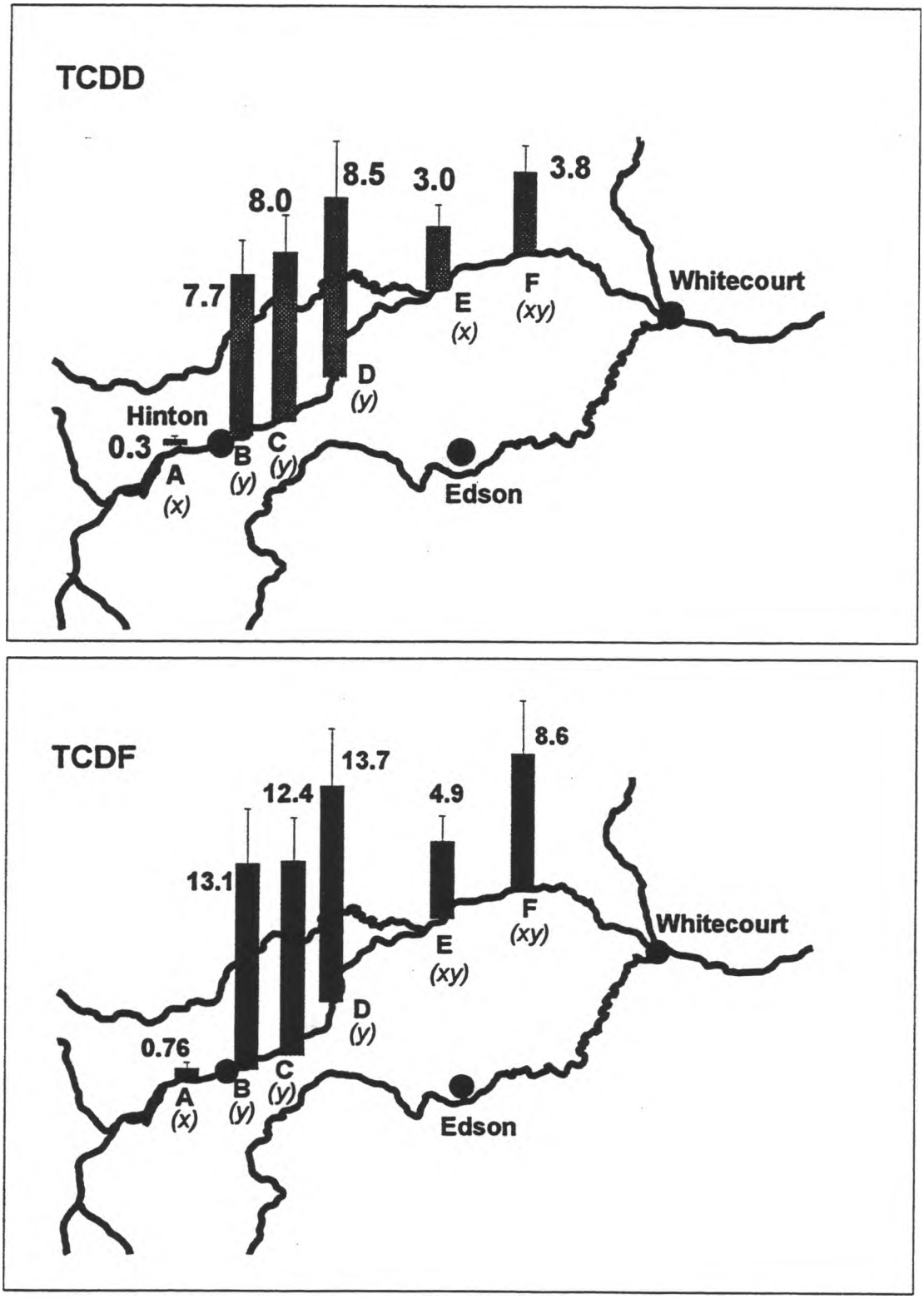


Figure 3. TCDD (above) and TCDF (below) residues in mountain whitefish from reaches of the Athabasca River in spring, 1992. Bars are means of concentrations (pg/g wet weight) of muscle, with standard errors. Reach locations are identified as capital letters near bases of bars. Italicized lower case letters below reach letters are Duncan's statistical groups established from an analysis of covariance incorporating fish length and residue concentration.

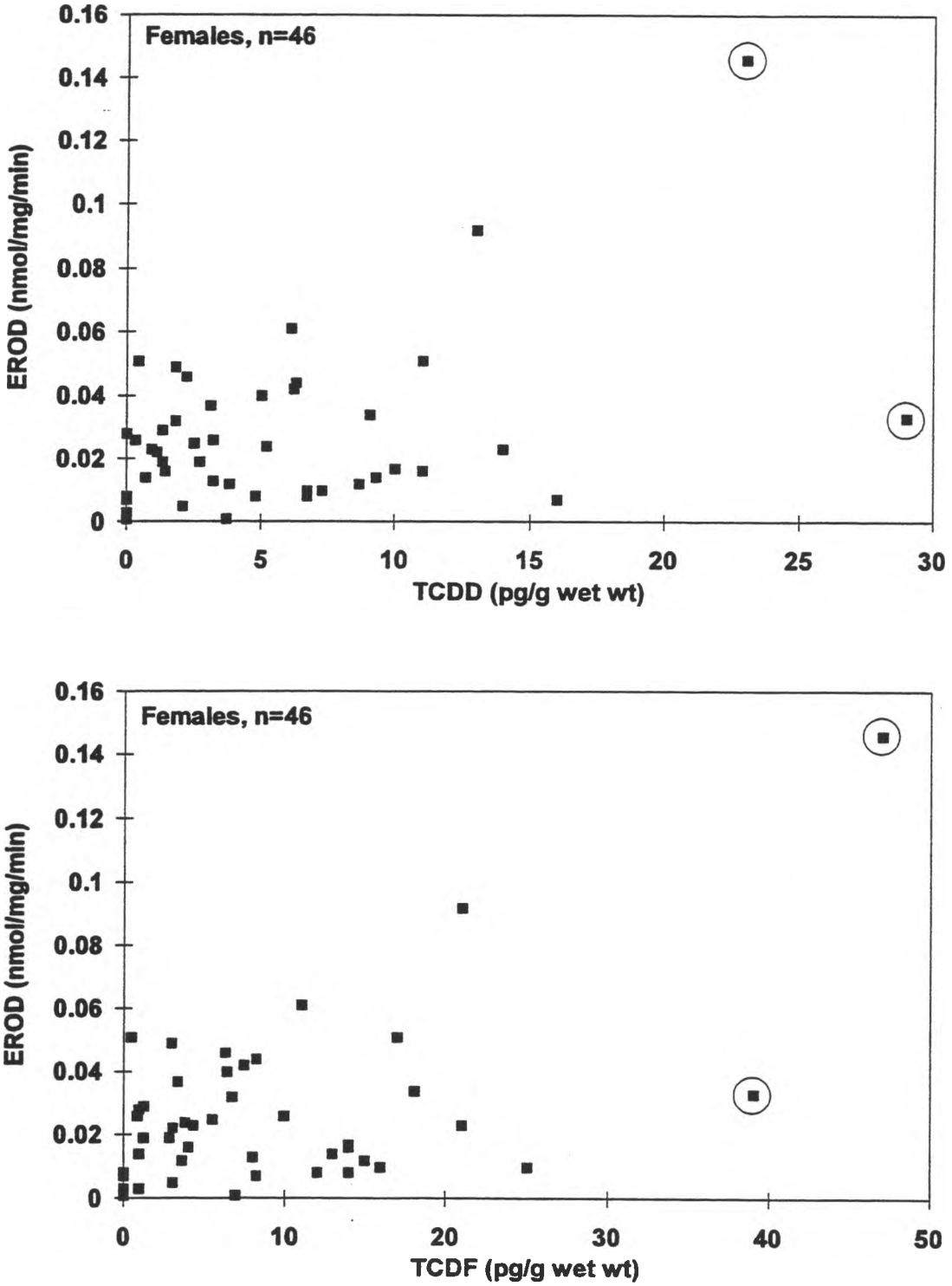


Figure 4. EROD activities in female mountain whitefish as a function of TCDD residue levels (above) and TCDF residue levels (below). Correlations are not significant if the circled points are excluded from the analysis.

| Table 4. Other published mountain whitefish microsomal EROD activities | | | | |
|---|---|---|---|---|
| Source | Time | n | EROD (Std. Dev.) | Reference |
| N. Saskatchewan R. Reference site | Spring/summer 1991 | 24 | 0.021 (0.014) | Kloepper-Sams & Benton., 1994 |
| Wapiti/Smokey, site 2A | Spring/summer 1991 | 18 | 0.557 (0.420) | " |
| Wapiti/Smokey, site 2B | Spring/summer 1991 | 10 | 0.627 (0.506) | " |
| Wapiti/Smokey, site 5 | Spring/summer 1991 | 6 | 0.231 (0.444) | " |
| N. Saskatchewan Reference | Fall, 1991 | 6 | 0.029 (0.013) | " |
| Wapiti/Smokey Site 2A | Fall, 1991 | 10 | 0.227 (0.112) | " |
| Wapiti/Smokey Site 2A | Spring, 1992 | 19 | 0.218 (0.125) | " |
| Wapiti/Smokey Site 2A | Spring, 1992 | 19 | 0.279 (0.135) | " |
| Brilliant Reservoir, B.C. (control site) females | January, 1991 | 12 | 0.022 (0.014) | Boyle et al., 1992 |
| Brilliant Reservoir B.C. (control site) males | January, 1991 | 13 | 0.021 (0.008) | " |
| Columbia River Genelle, B.C. (mill site) females | January, 1991 | 10 | 0.358 (0.212) | " |
| Columbia River Genelle, B.C. (mill site) males | January, 1991 | 11 | 0.547 (0.223) | " |

**Northern Pike**

Twenty-nine northern pike are reported from the spring collection, close to spawning; they were in either pre-spawning or spent condition. Generally, with cytochrome P450-linked responses, it is preferred to obtain samples at non-spawning times. Mean EROD and AHH activities are listed in Table 5 for females and Table 6 for males. With the northern pike taken in the spring, there was no geographic pattern to suggest a source of inducing materials near site B. The only sites where adequate numbers of pike were obtained were E and F, and males taken there had the highest values obtained. In view of the spawning condition of this species, it is not known whether the individuals may have moved recently, and hence whether they represented the reaches where they were collected. There was, however, a strong correlation between the two catalytic activities ($r = 0.98$) and this may indicate the presence of some mildly induced individuals. In the fall, no samples were available from site G, the upstream control, and so any treatment effects could not be detected.

In some species where systematic studies of seasonal variation has been studied, enzymatic activities are low at times near spawning (Luxon *et al.*, 1987; Boychuk, 1994).

There are few published reports of mixed function oxidase activities in pike. We obtained two previous collections of them, one of 20 fish from the Slave River at Fort Smith in September/October, 1990, and the other of 12 fish from Leland Lake in October, 1990. Mean EROD activities for females and males respectively were 0.032 and 0.048 nmol mg $prot^{-1}$ $min^{-1}$ from the Slave River, and comparable values from Leland Lake were 0.010 and 0.027. For AHH, the corresponding activities were 0.048 nmol mg $prot^{-1}$ $min^{-1}$ for females and 0.052 nmol mg $prot^{-1}$ $min^{-1}$ for males from the Slave river, and 0.015 and 0.040 from Leland Lake. Balk *et al.* (1980) reported AHH activity of 0.012 nmol mg $prot^{-1}$ $min^{-1}$ (at 32°C) for sexually immature northern pike from near Stockholm, Sweden. Clearly the males from sites E and F exceed other pike values; we cannot tell whether this represents a response to chemical inputs in view of the small numbers of pike from the sites A and B. We have not previously examined pike at spawning time.

**Longnose suckers**

Samples from longnose suckers taken in the fall were in frozen storage over a year between collection and analysis. Comparison of males and females, ignoring location, indicated significantly higher activities of EROD and AHH in males than in females (Tables 7 and 8). Analysis of covariance of (log 10) EROD activities (taking log of length as covariate) in females indicated significant differences among sites ($p=0.0012$), with reach G (upstream) having lower activities than the other sites (Figure 5). Again, there were numerous correlations linking the enzymatic activities and the measures of reproduction reported by Brown et al. (1993). Figure 6 illustrates two of the correlations between EROD activity and the endocrine measurements; the upper panel shows the relationship between testosterone and EROD and the lower one shows the relationship between estradiol and EROD. The fall-captured fish were scored microscopically for "maturation index" by Brown et al, and the enzymatic values correlated with that measurement also, leading one to suspect that the enzymatic activities may have been a reflection of the

| Table 5. Summary statistics for female northern pike | | | | | | |
|---|---|---|---|---|---|---|
| Reach (season) | Variable | N | Mean | Std. dev | Minimum | Maximum |
| A (spring) | EROD | 3 | 0.029 | 0.010 | 0.020 | 0.039 |
| B (spring) | EROD | 1 | 0.014 | . | 0.014 | 0.014 |
| C (spring) | EROD | 1 | 0.012 | . | 0.012 | 0.012 |
| E (spring) | EROD | 5 | 0.011 | 0.006 | 0.005 | 0.020 |
| F (spring) | EROD | 4 | 0.019 | 0.008 | 0.011 | 0.026 |
| H (fall) | EROD | 1 | 0.035 | . | 0.035 | 0.035 |
| K (fall) | EROD | 1 | 0.007 | . | 0.007 | 0.007 |
| L (fall) | EROD | 4 | 0.014 | 0.015 | 0.005 | 0.036 |
| M (fall) | EROD | 6 | 0.016 | 0.016 | 0.004 | 0.048 |
| A (spring) | AHH | 3 | 0.032 | 0.019 | 0.013 | 0.050 |
| B (spring) | AHH | 1 | 0.025 | . | 0.025 | 0.025 |
| C (spring) | AHH | 1 | 0.012 | . | 0.012 | 0.012 |
| E (spring) | AHH | 5 | 0.016 | 0.008 | 0.006 | 0.026 |
| F (spring) | AHH | 4 | 0.025 | 0.013 | 0.010 | 0.039 |
| H (fall) | AHH | 1 | 0.035 | . | 0.035 | 0.035 |
| K (fall) | AHH | 1 | 0.007 | . | 0.007 | 0.007 |
| L (fall) | AHH | 4 | 0.016 | 0.015 | 0.006 | 0.038 |
| M (fall) | AHH | 6 | 0.016 | 0.016 | 0.001 | 0.045 |
| A (spring) | P450 | 3 | 0.218 | 0.025 | 0.190 | 0.238 |
| B (spring) | P450 | 1 | 0.084 | . | 0.084 | 0.084 |
| C (spring) | P450 | 1 | 0.136 | . | 0.136 | 0.136 |
| E (spring) | P450 | 5 | 0.093 | 0.033 | 0.045 | 0.122 |
| F (spring) | P450 | 4 | 0.143 | 0.043 | 0.092 | 0.182 |
| H (fall) | P450 | 1 | 0.468 | . | 0.468 | 0.468 |
| K (fall) | P450 | 1 | 0.278 | . | 0.278 | 0.278 |
| L (fall) | P450 | 4 | 0.308 | 0.052 | 0.255 | 0.379 |
| M (fall) | P450 | 6 | 0.247 | 0.049 | 0.199 | 0.308 |

| Table 6. Summary statistics for male northern pike | | | | | | |
|---|---|---|---|---|---|---|
| Reach (season) | Variable | N | Mean | Std dev | Minimum | Maximum |
| A (spring) | EROD | 2 | 0.017 | 0.011 | 0.009 | 0.024 |
| B (spring) | EROD | 1 | 0.001 | . | 0.001 | 0.001 |
| E (spring) | EROD | 6 | 0.059 | 0.031 | 0.003 | 0.090 |
| F (spring) | EROD | 6 | 0.077 | 0.043 | 0.012 | 0.144 |
| K (fall) | EROD | 1 | 0.026 | . | 0.026 | 0.026 |
| L (fall) | EROD | 6 | 0.040 | 0.009 | 0.026 | 0.050 |
| M (fall) | EROD | 4 | 0.080 | 0.028 | 0.055 | 0.121 |
| A (spring) | AHH | 2 | 0.025 | 0.023 | 0.009 | 0.041 |
| B (spring) | AHH | 1 | 0.001 | . | 0.001 | 0.001 |
| E (spring) | AHH | 6 | 0.060 | 0.029 | 0.010 | 0.087 |
| F (spring) | AHH | 6 | 0.086 | 0.044 | 0.013 | 0.149 |
| K (fall) | AHH | 1 | 0.045 | . | 0.045 | 0.045 |
| L (fall) | AHH | 6 | 0.047 | 0.008 | 0.037 | 0.058 |
| M (fall) | AHH | 4 | 0.077 | 0.031 | 0.053 | 0.123 |
| A (spring) | P450 | 2 | 0.095 | 0.018 | 0.082 | 0.108 |
| B (spring) | P450 | 1 | 0.001 | . | 0.001 | 0.001 |
| E (spring) | P450 | 6 | 0.119 | 0.018 | 0.096 | 0.146 |
| F (spring) | P450 | 6 | 0.163 | 0.030 | 0.119 | 0.199 |
| K (fall) | P450 | 1 | 0.318 | . | 0.318 | 0.318 |
| L (fall) | P450 | 6 | 0.329 | 0.045 | 0.287 | 0.402 |
| M (fall) | P450 | 4 | 0.350 | 0.041 | 0.300 | 0.400 |

| Table 7. Summary statistics for female longnose suckers. | | | | | | |
|---|---|---|---|---|---|---|
| Reach (season) | Variable | N | Mean | Std Dev | Minimum | Maximum |
| G (fall) | EROD | 7 | 0.005 | 0.001 | 0.004 | 0.006 |
| H (fall) | EROD | 4 | 0.018 | 0.016 | 0.006 | 0.042 |
| J (fall) | EROD | 5 | 0.028 | 0.029 | 0.013 | 0.08 |
| K (fall) | EROD | 3 | 0.046 | 0.025 | 0.019 | 0.068 |
| L (fall) | EROD | 8 | 0.017 | 0.009 | 0.005 | 0.031 |
| M (fall) | EROD | 6 | 0.015 | 0.009 | 0.006 | 0.028 |
| G (fall) | AHH | 7 | 0.019 | 0.006 | 0.007 | 0.025 |
| H (fall) | AHH | 4 | 0.043 | 0.038 | 0.018 | 0.1 |
| J (fall) | AHH | 5 | 0.062 | 0.039 | 0.037 | 0.131 |
| K (fall) | AHH | 3 | 0.094 | 0.035 | 0.054 | 0.119 |
| L (fall) | AHH | 8 | 0.043 | 0.02 | 0.022 | 0.077 |
| M (fall) | AHH | 6 | 0.062 | 0.064 | 0.014 | 0.184 |
| G (fall) | P450 | 7 | 0.219 | 0.0208 | 0.187 | 0.249 |
| H (fall) | P450 | 4 | 0.202 | 0.06 | 0.149 | 0.283 |
| J (fall) | P450 | 5 | 0.217 | 0.058 | 0.122 | 0.274 |
| K (fall) | P450 | 3 | 0.202 | 0.063 | 0.13 | 0.247 |
| L (fall) | P450 | 8 | 0.235 | 0.044 | 0.189 | 0.321 |
| M (fall) | P450 | 6 | 0.236 | 0.043 | 0.184 | 0.291 |

| Table 8. Summary statistics for male longnose suckers | | | | | | |
|---|---|---|---|---|---|---|
| Reach (season) | Variable | N | Mean | Std Dev | Minimum | Maximum |
| G (fall) | EROD | 3 | 0.025 | 0.012 | 0.018 | 0.038 |
| H (fall) | EROD | 6 | 0.039 | 0.015 | 0.012 | 0.053 |
| J (fall) | EROD | 5 | 0.083 | 0.028 | 0.061 | 0.131 |
| K (fall) | EROD | 7 | 0.057 | 0.012 | 0.043 | 0.076 |
| L (fall) | EROD | 2 | 0.038 | 0.014 | 0.028 | 0.048 |
| M (fall) | EROD | 1 | 0.021 | . | 0.021 | 0.021 |
| G(fall) | AHH | 3 | 0.066 | 0.030 | 0.047 | 0.100 |
| H (fall) | AHH | 6 | 0.088 | 0.030 | 0.038 | 0.121 |
| J (fall) | AHH | 5 | 0.157 | 0.043 | 0.120 | 0.231 |
| K (fall) | AHH | 7 | 0.116 | 0.027 | 0.080 | 0.152 |
| L (fall) | AHH | 2 | 0.092 | 0.035 | 0.067 | 0.116 |
| M (fall) | AHH | 1 | 0.119 | . | 0.119 | 0.119 |
| G(fall) | P450 | 3 | 0.296 | 0.105 | 0.186 | 0.395 |
| H (fall) | P450 | 6 | 0.254 | 0.066 | 0.169 | 0.329 |
| J (fall) | P450 | 5 | 0.305 | 0.013 | 0.283 | 0.316 |
| K (fall) | P450 | 7 | 0.205 | 0.077 | 0.130 | 0.362 |
| L (fall) | P450 | 2 | 0.281 | 0.069 | 0.232 | 0.330 |
| M (fall) | P450 | 1 | 0.287 | . | 0.287 | 0.287 |

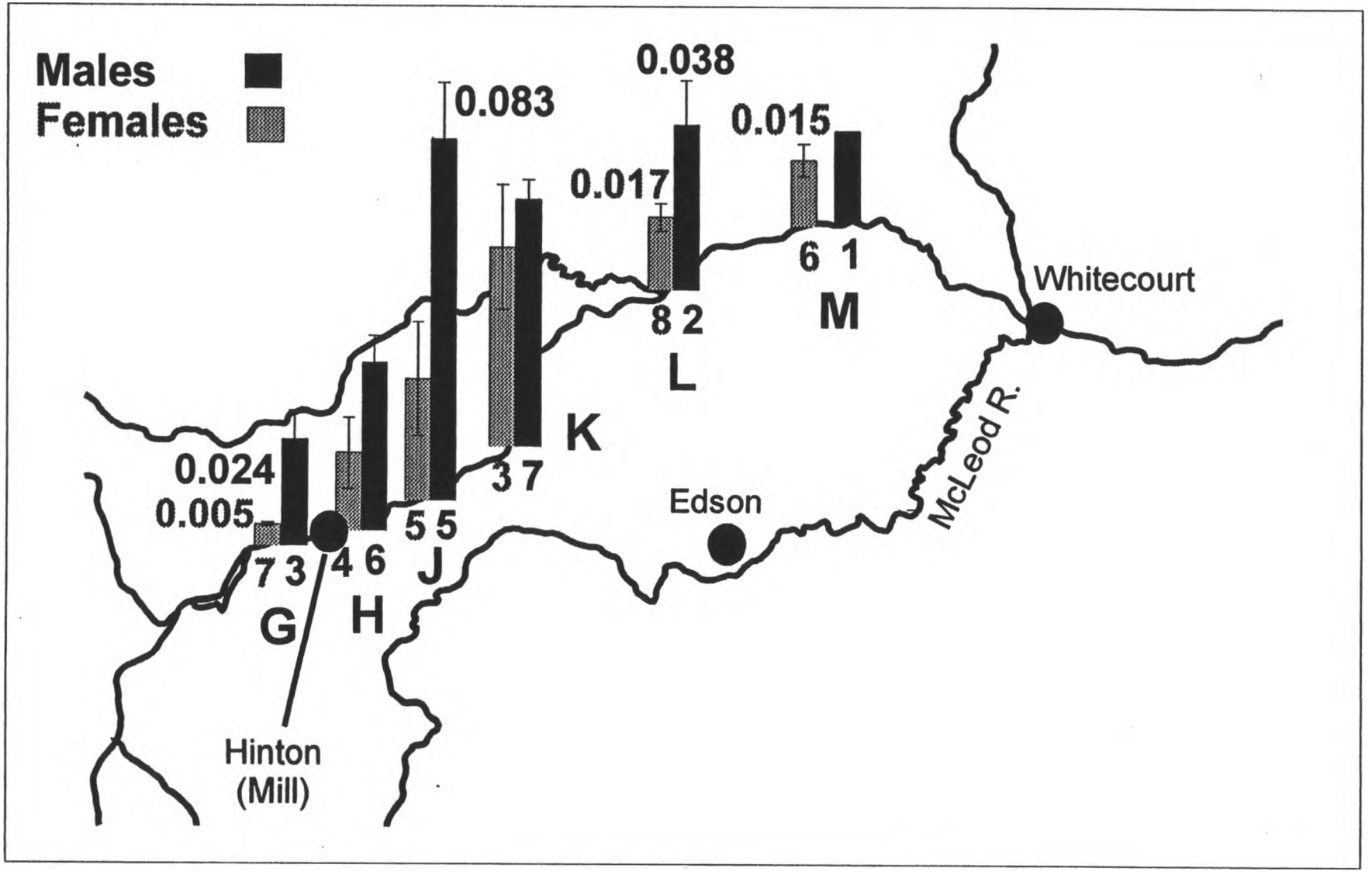


Figure 5. Liver microsomal EROD activities in longnose suckers from reaches G-M of the Athabasca River in fall, 1992. Bars are means with standard errors. The number of fish represented by each bar is given beneath the bar.

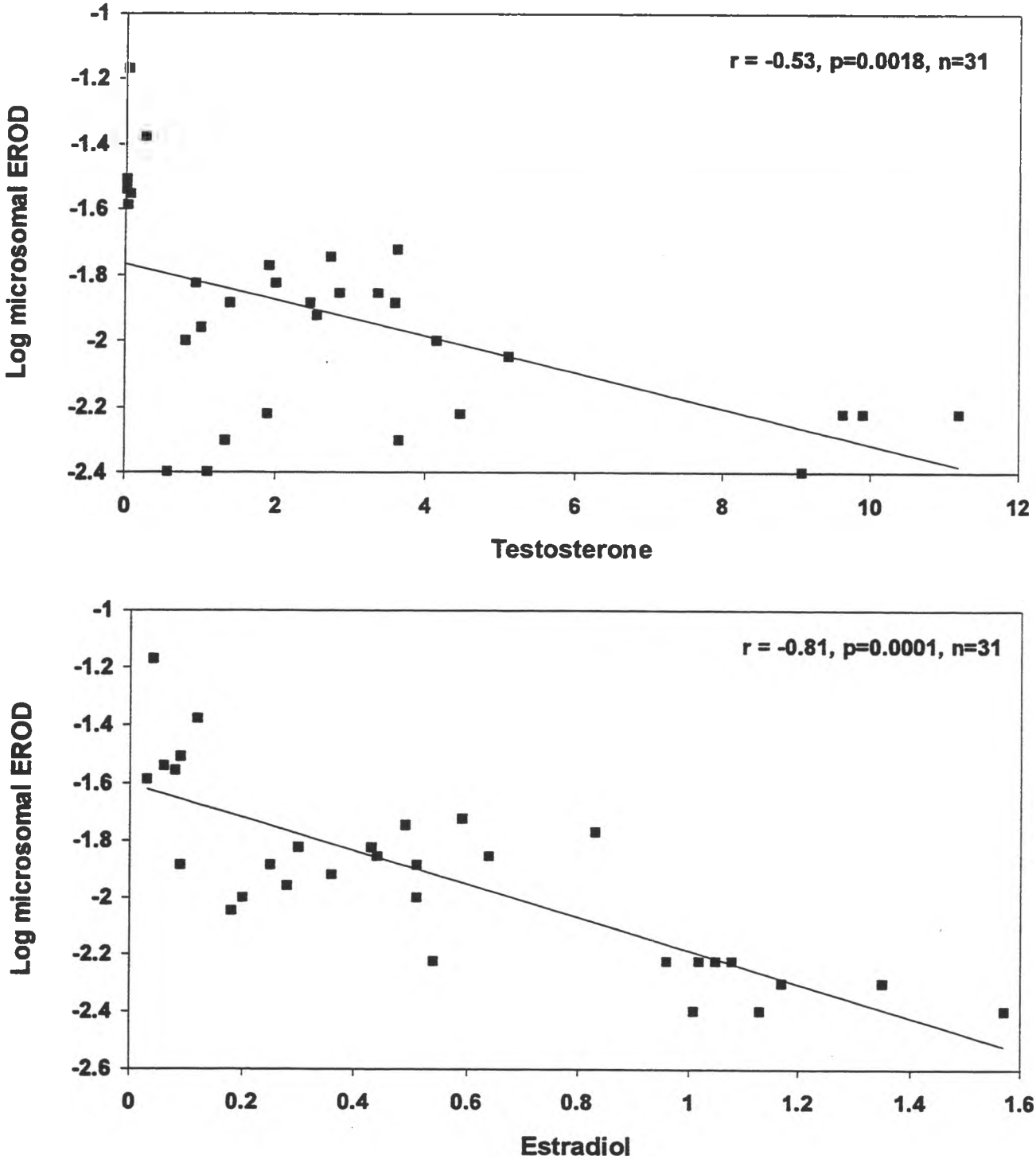


Figure 6. Relationships between EROD and blood steroid hormone measurements (testosterone, above and estradiol, below) from female longnose suckers taken from the Athabasca River in the fall, 1992. Data on hormones from Brown et al., 1993.

state of sexual maturity and not related to site differences. However, statistically, the site differences remained when the maturity index was incorporated into the analysis of covariance, suggesting that both the endocrine and the enzymatic measures were responding to some other influence, presumably effluent.

Kloepper-Sams and Benton (1994) reported EROD activities for longnose suckers from the Saskatchewan River and Wapiti/Smokey River in 1990 and 1991 (Table 9) and the results were quite similar to those from this study. Their mean values for Saskatchewan River suckers were 0.019 in summer, 1990, and 0.030 in summer, 1991. The EROD values from the upstream site (G) in this study were somewhat lower in females (mean 0.005, Table 7) than in the Saskatchewan River (Table 9), but were about the same in males (0.025, Table 8). In this study, values from most downstream sites were higher than those from site G, ranging up to 0.046 for females (Table 7) and 0.083 for males (Table 8). Kloepper-Sams and Benton found very similar ranges in their downstream fish (Table 9) except for one unusually high value from a single male in spring, 1991. We have analyzed two longnose suckers from Alberta previously, both from Mildred Lake (collected by Janice Smith, Environment Canada, Burlington, studies at Syncrude, August, 1989) and these had EROD values of 0.099 and 0.110 and AHH values of 0.085 and 0.074. These values are a little higher than those from the upper Athabasca River reported here, but it is not surprising in view of their source.

Table 9. Published EROD activities (means and standard deviations) in longnose suckers (from Kloepper-Sams & Benton, 1994)

| Source | Time | EROD (nmol/mg prot/min) |
|---|---|---|
| Saskatchewan River | Summer, 1990 | 0.019 (0.010) |
| Wapiti/Smokey, Site 2 | Summer, 1990 | 0.069 (0.068) |
| Wapiti/Smokey, Site 3 | Summer, 1990 | 0.038 (0.027) |
| Wapiti/Smokey, Site 4 | Summer, 1990 | 0.063 (0.045) |
| Wapiti/Smokey, Site 1B | Spring, 1991 | 0.050 (0.032) (males)<br>0.019 (0.016) (females) |
| Wapiti/Smokey, Site 2A | Spring, 1991 | 0.075 (0.035) |
| Wapiti/Smokey, Site 2B | Spring, 1991 | 0.277 (n=1) (male)<br>0.024 (0.008) (females) |
| Wapiti/Smokey, Site 6 | Spring, 1991 | 0.099 (0.069) |
| Saskatchewan River | Summer, 1991 | 0.030 (0.021) |
| Smokey/Wapiti, Site 2A | Summer, 1991 | 0.060 (0.035) |
| Smokey/Wapiti, Site 2B | Summer, 1991 | 0.087 (0.047) |
| Smokey/Wapiti, Site 5 | Summer, 1991 | 0.066 (0.017) (males)<br>0.037 (0.02) (females) |

**White suckers**

Summary statistics for white suckers taken in the fall are listed in Tables 10 and 11. Mean EROD activities fell in the narrow range of 0.008 to 0.011 nmol/mg protein/min for females (Table 10) and from 0.018 to 0.023 for males (Table 11). White suckers could not be obtained from the upstream reach, and so no treatment effects could be detected. Boychuk (1994) studied the seasonal variation in enzymatic activities in white suckers at the Experimental Lakes Area (ELA) of northwestern Ontario. For comparison, Boychuk's values for EROD and AHH activities are given below:

Mean EROD and AHH values from white suckers from the Experimental Lakes Area, July, 1990 and August, 1991 (from Boychuk, 1994)

| Activity | July, 1990 | August, 1991 |
|---|---|---|
| EROD (females) | 0.016 ± 0.005 | 0.010 ± 0.004 |
| EROD (males) | 0.025 ± 0.011 | 0.015 ± 0.009 |
| AHH (females) | 0.052 ± 0.007 | 0.081 ± 0.017 |
| AHH (males) | 0.061 ± 0.028 | 0.088 ± 0.021 |

The August values for female suckers from ELA were about the same as the fall values white suckers for the Athabasca River (Table 10) for EROD, but were about twice as high for AHH. While this comparison is less satisfactory than comparison with upstream samples from the Athabasca River, it is the best we have available. The ELA values suggest that the Athabasca River white suckers had experienced little or no induction. Studies of white suckers from the lower Athabasca area by Brian Brownlee and staff from Environment Canada in Burlington have resulted in the analysis of several white suckers from several locations in the oil sands area. These values are summarized below. The activities from suckers from the oil sands area were considerably higher than those from

EROD and AHH values from white suckers taken from the oil sands area of Alberta, August, 1989 (unpublished data from studies by Dr. B. Brownlee, Environment Canada, Burlington, Ontario).

| Location | N | EROD Mean ± S.D. | AHH Mean ± S.D. |
|---|---|---|---|
| Mildred Lake | 1 | 0.065 | 0.121 |
| Syncrude | 10, 9 | 0.157 ± 0.098 | 0.178 ± 0.096 |
| Beaver Creek Reservoir | 11 | 0.084 ± 0.026 | 0.131 ± 0.043 |

the present samples for both EROD and AHH. Given the similarities between the present samples and those from ELA, it seems likely that the enzyme actitivies in fish from the oil sands area are high rather than the present values being low because of some sampling or analytical artifact.

| Table 10. Summary statistics for female white suckers | | | | | | |
|---|---|---|---|---|---|---|
| Reach (season) | Variable | N | Mean | Std Dev | Minimum | Maximum |
| H (fall) | EROD | 1 | 0.009 | . | 0.009 | 0.009 |
| K (fall) | EROD | 3 | 0.009 | 0.003 | 0.007 | 0.012 |
| L (fall) | EROD | 7 | 0.011 | 0.005 | 0.006 | 0.019 |
| M (fall) | EROD | 8 | 0.008 | 0.002 | 0.005 | 0.011 |
| H (fall) | AHH | 1 | 0.042 | . | 0.042 | 0.042 |
| K (fall) | AHH | 3 | 0.040 | 0.013 | 0.030 | 0.055 |
| L (fall) | AHH | 7 | 0.038 | 0.021 | 0.013 | 0.066 |
| M (fall) | AHH | 8 | 0.034 | 0.023 | 0.014 | 0.088 |
| H (fall) | P450 | 1 | 0.195 | . | 0.195 | 0.195 |
| K (fall) | P450 | 3 | 0.162 | 0.010 | 0.151 | 0.171 |
| L (fall) | P450 | 7 | 0.175 | 0.028 | 0.146 | 0.218 |
| M (fall) | P450 | 8 | 0.155 | 0.024 | 0.121 | 0.189 |

| Table 11. Summary statistics for male white suckers | | | | | | |
|---|---|---|---|---|---|---|
| Reach (season) | Variable | N | Mean | Std dev | Minimum | Maximum |
| H (fall) | EROD | 1 | 0.023 | . | 0.023 | 0.023 |
| L (fall) | EROD | 3 | 0.018 | 0.011 | 0.005 | 0.026 |
| M (fall) | EROD | 2 | 0.019 | 0.009 | 0.012 | 0.025 |
| H (fall) | AHH | 1 | 0.072 | . | 0.072 | 0.072 |
| L (fall) | AHH | 3 | 0.067 | 0.036 | 0.029 | 0.100 |
| M (fall) | AHH | 2 | 0.056 | 0.002 | 0.054 | 0.057 |
| H (fall) | P450 | 1 | 0.247 | . | 0.247 | 0.247 |
| L (fall) | P450 | 3 | 0.207 | 0.079 | 0.116 | 0.260 |
| M (fall) | P450 | 2 | 0.239 | 0.001 | 0.238 | 0.239 |

## Conclusions

1. Liver enzymatic activities of both mountain whitefish in the spring and and longnose suckers in the fall showed geographic patterns of activities consistent with a source of inducing compounds between sites A and B. This is similar to a number of studies at other mill sites in Canada and Europe.

2. Liver enzymatic activities for mountain whitefish were low compared with other studies by our own and other laboratories. The cytochrome P-450 difference spectra indicated deterioration of the samples but the cause for this is speculative. We suspect the problem may have been with extraction of the samples from the cryovials. Use of small, plastic bags is recommended for future sampling. However, there is no reason to believe that a procedural artifact of unknown nature could produce the geographic patterns observed, and so it is concluded that while the values are low, their relative standings are correct.

3. The pattern of enzymatic activities and the reproductive measurements indicate that the differences among sites were not reflections of differing reproductive states, but rather were responses to exogenous inducer(s).

4. Weak, positive correlations between the enzymatic activities and the chlorinated dioxin and furan residues in mountain whitefish taken in the spring suggest that the response observed in the fish may have been related to sources of those compounds. The data do not establish that the chlorinated compounds were responsible for the induction; rather, they may have been partially responsible. Experimental studies are recommended below to help resolve the question of the nature of the inducers.

## Recommendations

Recommendations are made to try to begin discrimination of the types of compounds that might be responsible for the induction observed in the fish.

1. Experimental studies of mill effluent would help to determine whether the observed induction can be produced by relatively short-term exposures, and especially to determine whether induction decays rapidly after exposure is terminated. We have observed both rapid induction of enzyme activities and rapid decay of induction following cessation of exposure to the effluent of the mill at Pine Falls, Manitoba, where chlorine bleaching is not used. Induction by higher chlorinated dioxins and furans is very stable and does not decay readily after exposure is terminated, and so experimental induction/decay experiments would offer insight into the nature of the inducers. Parallel experimental designs could be conducted at sites in the Athabasca River by holding fish in cages for short periods to determine whether inducers have similar properties as those in the effluent.

3. Residue measurements should be made on the longnose suckers taken in the fall in order that we can test for relationships between body residues and biochemical responses.

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## 5.0 APPENDICES

| Appendix 1. List of all samples analyzed for mixed-function oxygenase activities for the Northern River Basins Study | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Field number | Species | Loc | Sex | Len (mm) | Wt (g) | EROD | AHH | P450 |
| 9203101 | A-I-1 | mountain whitefish | A | F | 314 | 325 | 0.003 | 0.007 | 0.057 |
| 9203102 | A-I-2 | mountain whitefish | A | M | 330 | 381 | 0.007 | 0.027 | 0.137 |
| 9203103 | A-I-3 | mountain whitefish | A | F | 446 | 841 | 0.016 | 0.038 | 0.065 |
| 9203104 | A-I-4 | mountain whitefish | A | F | 292 | 308 | 0.008 | 0.023 | 0.08 |
| 9203105 | A-I-5 | mountain whitefish | A | F | 298 | 290 | 0.002 | 0.021 | 0.031 |
| 9203106 | A-I-6 | mountain whitefish | A | F | 366 | 531 | 0.003 | <0.001 | 0.081 |
| 9203107 | A-I-7 | mountain whitefish | A | M | 363 | 492 | 0.002 | <0.001 | 0.084 |
| 9203108 | A-I-8 | mountain whitefish | A | F | 413 | 795 | <0.001 | 0.029 | 0.057 |
| 9203109 | A-I-9 | mountain whitefish | A | M | 333 | 409 | 0.005 | 0.021 | 0.116 |
| 9203110 | A-I-10 | mountain whitefish | A | F | 314 | 360 | 0.007 | 0.016 | 0.065 |
| 9203111 | B-I-1 | mountain whitefish | B | F | 292 | 309 | 0.008 | 0.015 | 0.033 |
| 9203112 | B-I-2 | mountain whitefish | B | F | 285 | 310 | 0.026 | 0.047 | 0.091 |
| 9203113 | B-I-3 | mountain whitefish | B | F | 459 | 1199 | 0.092 | 0.13 | 0.133 |
| 9203114 | B-I-4 | mountain whitefish | B | F | 402 | 819 | 0.061 | 0.105 | 0.093 |
| 9203115 | B-I-5 | mountain whitefish | B | F | 283 | 291 | 0.01 | 0.031 | <0.001 |
| 9203116 | B-I-6 | mountain whitefish | B | F | 372 | 674 | 0.04 | 0.059 | 0.096 |
| 9203117 | B-I-7 | mountain whitefish | B | F | 328 | 472 | 0.017 | 0.045 | 0.026 |
| 9203118 | B-I-8 | mountain whitefish | B | M | 314 | 357 | 0.093 | 0.125 | 0.148 |
| 9203119 | B-I-9 | mountain whitefish | B | F | 320 | 400 | 0.146 | 0.168 | 0.1 |
| 9203120 | B-I-10 | mountain whitefish | B | F | 375 | 560 | 0.024 | 0.039 | 0.047 |
| 9203121 | B-I-11 | mountain whitefish | B | F | 357 | 526 | 0.044 | 0.073 | 0.082 |
| 9203122 | B-I-12 | mountain whitefish | B | F | 418 | 961 | 0.025 | 0.045 | 0.081 |
| 9203131 | C-I-1 | mountain whitefish | C | F | 401 | 1029 | 0.023 | 0.033 | 0.16 |
| 9203132 | C-I-2 | mountain whitefish | C | F | 420 | 1030 | 0.014 | 0.024 | <0.001 |
| 9203133 | C-I-3 | mountain whitefish | C | F | 421 | 1030 | 0.051 | 0.097 | 0.103 |
| 9203134 | C-I-4 | mountain whitefish | C | M | 393 | 742 | 0.023 | 0.038 | 0.079 |
| 9203135 | C-I-5 | mountain whitefish | C | F | 385 | 550 | 0.032 | 0.069 | 0.081 |
| 9203136 | C-I-6 | mountain whitefish | C | F | 313 | 435 | 0.042 | 0.078 | 0.033 |
| 9203137 | C-I-7 | mountain whitefish | C | F | 297 | 341 | 0.013 | <0.001 | 0.097 |
| 9203138 | C-I-8 | mountain whitefish | C | F | 385 | 599 | 0.012 | 0.023 | 0.058 |
| 9203139 | C-I-9 | mountain whitefish | C | M | 406 | 692 | 0.007 | 0.002 | 0.081 |
| 9203140 | C-I-10 | mountain whitefish | C | F | 414 | 812 | 0.016 | 0.014 | <0.001 |
| 9203141 | D-I-1 | mountain whitefish | D | M | 373 | 631 | 0.037 | 0.068 | 0.082 |
| 9203142 | D-I-2 | mountain whitefish | D | F | 305 | 361 | 0.01 | 0.024 | <0.001 |

| Appendix 1 (continued). List of all samples analyzed for mixed-function oxygenase activities for the Northern River Basins Study | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Field number | Species | Loc | Sex | Len (mm) | Wt (g) | EROD | AHH | P450 |
| 9203143 | D-I-3 | mountain whitefish | D | F | 329 | 403 | 0.005 | 0.02 | <0.001 |
| 9203144 | D-I-4 | mountain whitefish | D | M | 326 | 422 | 0.002 | <0.001 | 0.018 |
| 9203145 | D-I-5 | mountain whitefish | D | F | 433 | 920 | 0.007 | 0.005 | 0.033 |
| 9203146 | D-I-6 | mountain whitefish | D | F | 433 | 1038 | 0.033 | 0.055 | 0.087 |
| 9203147 | D-I-7 | mountain whitefish | D | F | 320 | 415 | 0.001 | <0.001 | <0.001 |
| 9203148 | D-I-8 | mountain whitefish | D | F | 395 | 812 | 0.012 | 0.008 | 0.067 |
| 9203149 | D-I-9 | mountain whitefish | D | F | 350 | 602 | 0.034 | 0.058 | 0.057 |
| 9203150 | D-I-10 | mountain whitefish | D | F | 356 | 570 | 0.008 | 0.018 | 0.184 |
| 9203151 | E-I-1 | mountain whitefish | E | M | 335 | 412 | 0.016 | 0.039 | <0.001 |
| 9203152 | E-I-2 | mountain whitefish | E | F | 453 | 1173 | 0.022 | 0.032 | 0.088 |
| 9203153 | E-I-3 | mountain whitefish | E | F | 342 | 468 | 0.019 | 0.035 | 0.099 |
| 9203154 | E-I-4 | mountain whitefish | E | F | 392 | 715 | 0.037 | 0.063 | 0.093 |
| 9203155 | E-I-5 | mountain whitefish | E | F | 354 | 491 | 0.051 | 0.101 | 0.088 |
| 9203156 | E-I-6 | mountain whitefish | E | M | 369 | 648 | 0.026 | 0.045 | 0.113 |
| 9203157 | E-I-7 | mountain whitefish | E | M | 389 | 748 | 0.039 | 0.083 | 0.146 |
| 9203158 | E-I-8 | mountain whitefish | E | F | 412 | 932 | 0.019 | 0.037 | 0.08 |
| 9203159 | E-I-9 | mountain whitefish | E | F | 459 | 952 | 0.023 | 0.05 | 0.092 |
| 9203160 | E-I-10 | mountain whitefish | E | M | 370 | 611 | 0.03 | 0.047 | 0.048 |
| 9203161 | F-I-1 | mountain whitefish | F | M | 418 | 891 | 0.06 | 0.109 | 0.186 |
| 9203162 | F-I-2 | mountain whitefish | F | F | 316 | 428 | 0.046 | 0.077 | 0.137 |
| 9203163 | F-I-3 | mountain whitefish | F | M | 343 | 502 | 0.007 | 0.018 | 0.057 |
| 9203164 | F-I-4 | mountain whitefish | F | M | 327 | 395 | 0.048 | 0.085 | 0.137 |
| 9203165 | F-I-5 | mountain whitefish | F | F | 333 | 472 | 0.014 | <0.001 | 0.076 |
| 9203166 | F-I-6 | mountain whitefish | F | F | 347 | 465 | 0.028 | 0.055 | 0.105 |
| 9203167 | F-I-7 | mountain whitefish | F | F | 338 | 497 | 0.029 | 0.059 | 0.116 |
| 9203168 | F-I-8 | mountain whitefish | F | F | 302 | 371 | 0.049 | 0.057 | 0.109 |
| 9203169 | F-I-9 | mountain whitefish | F | M | 414 | 914 | 0.038 | 0.063 | 0.125 |
| 9203170 | F-I-10 | mountain whitefish | F | F | 384 | 722 | 0.026 | 0.056 | 0.12 |
| 9203171 | A-II-1 | northern pike | A | M | 470 | 785 | 0.024 | 0.041 | 0.108 |
| 9203172 | A-II-2 | northern pike | A | I | 467 | 712 | 0.005 | 0.01 | 0.048 |
| 9203173 | A-II-3 | northern pike | A | F | 659 | 1792 | 0.02 | 0.013 | 0.226 |
| 9203174 | A-II-4 | northern pike | A | M | 590 | 1399 | 0.009 | 0.009 | 0.082 |
| 9203175 | A-II-5 | northern pike | A | F | 440 | 632 | 0.028 | 0.032 | 0.19 |
| 9203176 | A-II-6 | northern pike | A | F | 483 | 910 | 0.039 | 0.05 | 0.238 |
| 9203181 | B-II-1 | northern pike | B | F | 477 | 781 | 0.014 | 0.025 | 0.084 |
| 9203182 | B-II-2 | northern pike | B | M | 395 | 415 | 0.001 | <0.001 | <0.001 |

| Appendix 1 (continued). List of all samples analyzed for mixed-function oxygenase activities for the Northern River Basins Study | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Field number | Species | Loc | Sex | Len (mm) | Wt (g) | EROD | AHH | P450 |
| 9203201 | D-II-1 | northern pike | D | F | 698 | 2596 | 0.012 | 0.012 | 0.136 |
| 9203211 | E-II-1 | northern pike | E | F | 699 | 2152 | 0.02 | 0.026 | 0.122 |
| 9203212 | E-II-2 | northern pike | E | M | 405 | 543 | 0.053 | 0.053 | 0.096 |
| 9203212 | E-II-2 | northern pike | E | M | 405 | 543 | 0.053 | 0.053 | 0.124 |
| 9203213 | E-II-3 | northern pike | E | M | 612 | 1693 | 0.085 | 0.074 | 0.11 |
| 9203214 | E-II-4 | northern pike | E | F | 835 | 5409 | 0.013 | 0.013 | 0.119 |
| 9203215 | E-II-5 | northern pike | E | F | 665 | 2413 | 0.013 | 0.022 | 0.107 |
| 9203216 | E-II-6 | northern pike | E | F | 667 | 2212 | 0.005 | 0.006 | 0.045 |
| 9203217 | E-II-7 | northern pike | E | M | 650 | 1680 | 0.069 | 0.084 | 0.107 |
| 9203218 | E-II-8 | northern pike | E | M | 548 | 1110 | 0.003 | 0.01 | 0.13 |
| 9203219 | E-II-9 | northern pike | E | M | 507 | 1093 | 0.09 | 0.087 | 0.146 |
| 9203220 | E-II-10 | northern pike | E | F | 631 | 720 | 0.005 | 0.013 | 0.074 |
| 9203221 | F-II-1 | northern pike | F | F | 455 | 632 | 0.013 | 0.019 | 0.175 |
| 9203222 | F-II-2 | northern pike | F | M | 465 | 674 | 0.012 | 0.013 | 0.138 |
| 9203223 | F-II-3 | northern pike | F | M | 540 | 1129 | 0.073 | 0.085 | 0.199 |
| 9203224 | F-II-4 | northern pike | F | M | 537 | 1100 | 0.095 | 0.097 | 0.179 |
| 9203225 | F-II-5 | northern pike | F | M | 538 | 1084 | 0.144 | 0.149 | 0.184 |
| 9203226 | F-II-6 | northern pike | F | M | 530 | 970 | 0.067 | 0.093 | 0.119 |
| 9203227 | F-II-7 | northern pike | F | M | 546 | 1118 | 0.072 | 0.081 | 0.159 |
| 9203228 | F-II-8 | northern pike | F | F | 792 | 3735 | 0.026 | 0.039 | 0.124 |
| 9203229 | F-II-9 | northern pike | F | F | 521 | 1045 | 0.026 | 0.03 | 0.182 |
| 9203230 | F-II-10 | northern pike | F | F | 637 | 2045 | 0.011 | 0.01 | 0.092 |
| 9203561 | G-I-1 | mountain whitefish | G | M | 323 | 380 | 0.005 | 0.031 | 0.044 |
| 9203562 | G-1-2 | mountain whitefish | G | M | 322 | 380 | 0.009 | 0.025 | . |
| 9203563 | G-I-3 | mountain whitefish | G | F | 383 | 740 | 0.002 | 0.009 | 0.04 |
| 9203564 | G-I-4 | mountain whitefish | G | M | 350 | 435 | 0.013 | 0.055 | 0.062 |
| 9203565 | G-I-5 | mountain whitefish | G | F | 360 | 510 | 0.002 | 0.014 | 0.075 |
| 9203566 | G-I-6 | mountain whitefish | G | F | 366 | 580 | 0.002 | 0.005 | 0.081 |
| 9203567 | G-I-7 | mountain whitefish | G | M | 326 | 390 | 0.01 | 0.041 | . |
| 9203568 | G-I-8 | mountain whitefish | G | F | 325 | 390 | 0.005 | 0.013 | 0.007 |
| 9203569 | G-I-9 | mountain whitefish | G | F | 405 | 695 | 0.001 | 0.022 | 0.062 |
| 9203570 | G-I-10 | mountain whitefish | G | M | 370 | 575 | 0 | 0.012 | 0.015 |
| 9203571 | H-I-1 | mountain whitefish | H | F | 382 | 720 | 0.003 | 0.011 | 0.037 |
| 9203572 | H-I-2 | mountain whitefish | H | F | 401 | 790 | 0.012 | 0.002 | 0.03 |
| 9203573 | H-I-3 | mountain whitefish | H | F | 440 | 1100 | 0.011 | 0.022 | 0.046 |
| 9203574 | H-I-4 | mountain whitefish | H | F | 413 | 820 | 0.012 | 0.006 | 0.037 |
| 9203575 | H-I-5 | mountain whitefish | H | F | 402 | 870 | 0.018 | 0.052 | 0.09 |

| Appendix 1 (continued). List of all samples analyzed for mixed-function oxygenase activities for the Northern River Basins Study | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Field number | Species | Loc | Sex | Len (mm) | Wt (g) | EROD | AHH | P450 |
| 9203576 | H-I-6 | mountain whitefish | H | F | 314 | 360 | 0.003 | 0.011 | 0.156 |
| 9203577 | H-I-7 | mountain whitefish | H | F | 321 | 375 | 0.038 | 0.044 | 0.029 |
| 9203578 | H-I-8 | mountain whitefish | H | F | 315 | 360 | 0.15 | <0.001 | . |
| 9203579 | H-I-9 | mountain whitefish | H | M | 341 | 485 | 0.054 | <0.001 | 0.224 |
| 9203580 | H-I-10 | mountain whitefish | H | F | 469 | 1220 | 0.203 | 0.052 | 0.204 |
| 9203581 | H-I-11 | mountain whitefish | H | M | 334 | 420 | 0.036 | 0.034 | 0.19 |
| 9203582 | J-I-1 | mountain whitefish | J | M | 316 | 385 | 0.005 | 0.016 | . |
| 9203583 | J-I-2 | mountain whitefish | J | F | 377 | 675 | 0.007 | 0.02 | 0.048 |
| 9203584 | J-I-3 | mountain whitefish | J | M | 306 | 345 | 0.018 | <0.001 | . |
| 9203585 | J-I-4 | mountain whitefish | J | F | 385 | 745 | 0.004 | 0.012 | 0.048 |
| 9203586 | J-I-5 | mountain whitefish | J | F | 358 | 570 | 0.013 | 0.026 | 0.091 |
| 9203587 | J-I-6 | mountain whitefish | J | F | 353 | 580 | 0.014 | 0.032 | 0.106 |
| 9203588 | J-I-7 | mountain whitefish | J | F | 344 | 480 | 0.007 | 0.021 | 0.071 |
| 9203589 | J-I-8 | mountain whitefish | J | F | 416 | 900 | 0.003 | 0.007 | 0.071 |
| 9203590 | J-I-9 | mountain whitefish | J | F | 315 | 365 | 0.007 | 0.015 | 0.067 |
| 9203591 | J-I-10 | mountain whitefish | J | F | 340 | 510 | 0.003 | 0.007 | 0.038 |
| 9203592 | K-I-1 | mountain whitefish | K | F | 381 | 740 | 0.012 | 0.025 | 0.073 |
| 9203593 | K-I-2 | mountain whitefish | K | F | 329 | 430 | 0.019 | 0.031 | 0.059 |
| 9203594 | K-I-3 | mountain whitefish | K | F | 324 | 380 | 0.033 | 0.042 | 0.101 |
| 9203595 | K-I-4 | mountain whitefish | K | F | 379 | 640 | 0.024 | 0.026 | 0.079 |
| 9203596 | K-I-5 | mountain whitefish | K | F | 322 | 425 | 0.01 | 0.02 | 0.085 |
| 9203597 | K-I-6 | mountain whitefish | K | F | 444 | 1160 | 0.006 | 0.013 | 0.058 |
| 9203598 | K-I-7 | mountain whitefish | K | F | 358 | 530 | 0.029 | 0.075 | 0.075 |
| 9203599 | K-I-8 | mountain whitefish | K | M | 337 | 425 | 0.013 | 0.067 | . |
| 9203600 | K-I-9 | mountain whitefish | K | F | 346 | 540 | 0.012 | 0.026 | 0.05 |
| 9203601 | K-I-10 | mountain whitefish | K | M | 332 | 345 | 0.24 | 0.251 | . |
| 9203602 | K-I-11 | mountain whitefish | K | F | 352 | 600 | 0.019 | 0.014 | 0.088 |
| 9203603 | L-I-1 | mountain whitefish | L | M | 332 | 390 | 0.013 | <0.001 | . |
| 9203604 | L-I-2 | mountain whitefish | L | F | 319 | 450 | 0.016 | 0.04 | 0.04 |
| 9203605 | L-I-3 | mountain whitefish | L | F | 382 | 710 | 0.015 | 0.007 | 0.12 |
| 9203606 | L-I-4 | mountain whitefish | L | F | 423 | 860 | 0.005 | 0.044 | 0.064 |
| 9203607 | L-I-5 | mountain whitefish | L | F | 379 | 720 | 0.007 | 0.016 | 0.065 |
| 9203608 | L-I-6 | mountain whitefish | L | F | 415 | 805 | 0.006 | 0.001 | 0.058 |
| 9203609 | L-I-7 | mountain whitefish | L | F | 426 | 1010 | 0.006 | 0.013 | 0.041 |
| 9203610 | L-I-8 | mountain whitefish | L | F | 407 | 790 | 0.006 | 0.007 | 0.083 |
| 9203611 | L-I-9 | mountain whitefish | L | F | 409 | 860 | 0.014 | 0.007 | 0.09 |
| 9203612 | L-I-10 | mountain whitefish | L | F | 338 | 485 | 0.013 | 0.04 | 0.051 |

| Appendix 1 (continued). List of all samples analyzed for mixed-function oxygenase activities for the Northern River Basins Study | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Field number | Species | Loc | Sex | Len (mm) | Wt (g) | EROD | AHH | P450 |
| 9203613 | M-I-1 | mountain whitefish | M | M | 431 | 960 | 0.018 | 0.049 | 0.124 |
| 9203614 | M-I-2 | mountain whitefish | M | F | 378 | 560 | 0.013 | 0.008 | 0.077 |
| 9203615 | M-I-3 | mountain whitefish | M | M | 359 | 435 | 0.017 | <0.001 | |
| 9203616 | M-I-4 | mountain whitefish | M | F | 399 | 680 | 0.007 | 0.002 | 0.054 |
| 9203617 | M-I-5 | mountain whitefish | M | M | 363 | 585 | 0.02 | 0.019 | 0.134 |
| 9203618 | M-I-6 | mountain whitefish | M | M | 389 | 720 | 0.012 | 0.01 | 0.165 |
| 9203619 | M-I-7 | mountain whitefish | M | F | 400 | 840 | 0.008 | 0.031 | 0.091 |
| 9203620 | M-I-8 | mountain whitefish | M | F | 450 | 1280 | 0.005 | 0.012 | 0.068 |
| 9203621 | M-I-9 | mountain whitefish | M | F | 362 | 625 | 0.008 | 0.021 | 0.062 |
| 9203622 | M-I-10 | mountain whitefish | M | M | 347 | 510 | 0.02 | 0.319 | |
| 9203623 | G-IV-1 | longnose sucker | G | F | 375 | 690 | 0.004 | 0.007 | 0.216 |
| 9203624 | G-IV-2 | longnose sucker | G | F | 416 | 820 | 0.005 | 0.02 | 0.187 |
| 9203625 | G-IV-3 | longnose sucker | G | M | 362 | 570 | 0.018 | 0.047 | 0.395 |
| 9203626 | G-IV-4 | longnose sucker | G | M | 375 | 470 | 0.018 | 0.051 | 0.186 |
| 9203627 | G-IV-5 | longnose sucker | G | F | 412 | 870 | 0.004 | 0.022 | 0.209 |
| 9203628 | G-IV-6 | longnose sucker | G | F | 426 | 940 | 0.004 | 0.021 | 0.241 |
| 9203629 | G-IV-7 | longnose sucker | G | M | 378 | 670 | 0.038 | 0.1 | 0.306 |
| 9203630 | G-IV-8 | longnose sucker | G | F | 388 | 760 | 0.006 | 0.025 | 0.211 |
| 9203631 | G-IV-9 | longnose sucker | G | F | 400 | 870 | 0.006 | 0.018 | 0.223 |
| 9203632 | G-IV-10 | longnose sucker | G | F | 378 | 630 | 0.006 | 0.023 | 0.249 |
| 9203633 | H-II-1 | northern pike | H | F | 485 | 825 | 0.035 | 0.035 | 0.468 |
| 9203634 | H-III-1 | white sucker | H | M | 370 | 730 | 0.023 | 0.072 | 0.247 |
| 9203635 | H-III-2 | white sucker | H | F | 388 | 960 | 0.009 | 0.042 | 0.195 |
| 9203636 | H-IV-1 | longnose sucker | H | F | 414 | 880 | 0.042 | 0.1 | 0.283 |
| 9203637 | H-IV-2 | longnose sucker | H | M | 393 | 760 | 0.053 | 0.121 | 0.274 |
| 9203638 | H-IV-3 | longnose sucker | H | M | 411 | 820 | 0.012 | 0.038 | 0.169 |
| 9203639 | H-IV-4 | longnose sucker | H | M | 374 | 670 | 0.043 | 0.105 | 0.274 |
| 9203640 | H-IV-5 | longnose sucker | H | F | 412 | 960 | 0.006 | 0.018 | 0.21 |
| 9203641 | H-IV-6 | longnose sucker | H | M | 400 | 800 | 0.031 | 0.07 | 0.177 |
| 9203642 | H-IV-7 | longnose sucker | H | F | 400 | 710 | 0.013 | 0.029 | 0.166 |
| 9203643 | H-IV-8 | longnose sucker | H | F | 400 | 830 | 0.011 | 0.025 | 0.149 |
| 9203644 | H-IV-9 | longnose sucker | H | M | 402 | 850 | 0.053 | 0.107 | 0.329 |
| 9203645 | H-IV-10 | longnose sucker | H | M | 386 | 700 | 0.039 | 0.087 | 0.299 |
| 9203646 | J-IV-1 | longnose sucker | J | F | 376 | 680 | 0.015 | 0.053 | 0.245 |
| 9203647 | J-IV-2 | longnose sucker | J | F | 406 | 835 | 0.014 | 0.037 | 0.23 |
| 9203648 | J-IV-3 | longnose sucker | J | M | 352 | 580 | 0.061 | 0.12 | 0.307 |
| 9203649 | J-IV-4 | longnose sucker | J | M | 361 | 650 | 0.066 | 0.133 | 0.31 |

| Appendix 1 (continued). List of all samples analyzed for mixed-function oxygenase activities for the Northern River Basins Study | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Field number | Species | Loc | Sex | Len (mm) | Wt (g) | EROD | AHH | P450 |
| 9203650 | J-IV-5 | longnose sucker | J | M | 381 | 780 | 0.085 | 0.148 | 0.316 |
| 9203651 | J-IV-6 | longnose sucker | J | F | 386 | 750 | 0.017 | 0.051 | 0.122 |
| 9203652 | J-IV-7 | longnose sucker | J | F | 398 | 870 | 0.08 | 0.131 | 0.274 |
| 9203653 | J-IV-8 | longnose sucker | J | M | 397 | 715 | 0.131 | 0.231 | 0.308 |
| 9203654 | J-IV-9 | longnose sucker | J | M | 397 | 720 | 0.072 | 0.155 | 0.283 |
| 9203655 | J-IV-10 | longnose sucker | J | F | 408 | 810 | 0.013 | 0.039 | 0.212 |
| 9203656 | K-II-1 | northern pike | K | M | 491 | 925 | 0.026 | 0.045 | 0.318 |
| 9203657 | K-II-2 | northern pike | K | F | 565 | 1260 | 0.007 | 0.007 | 0.278 |
| 9203658 | K-III-1 | white sucker | K | F | 393 | 825 | 0.007 | 0.03 | 0.151 |
| 9203659 | K-III-2 | white sucker | K | F | 340 | 550 | 0.012 | 0.055 | 0.171 |
| 9203660 | K-III-3 | white sucker | K | U | 333 | 470 | . | . | . |
| 9203661 | K-III-4 | white sucker | K | F | 370 | 650 | 0.007 | 0.035 | 0.164 |
| 9203662 | K-IV-1 | longnose sucker | K | F | 377 | 600 | 0.051 | 0.109 | 0.247 |
| 9203663 | K-IV-2 | longnose sucker | K | M | 348 | 560 | 0.043 | 0.08 | 0.13 |
| 9203664 | K-IV-3 | longnose sucker | K | M | 411 | 825 | 0.044 | 0.112 | 0.166 |
| 9203665 | K-IV-4 | longnose sucker | K | M | 356 | 555 | 0.048 | 0.084 | 0.155 |
| 9203666 | K-IV-5 | longnose sucker | K | M | 384 | 660 | 0.076 | 0.137 | 0.194 |
| 9203667 | K-IV-6 | longnose sucker | K | M | 357 | 590 | 0.061 | 0.116 | 0.193 |
| 9203668 | K-IV-7 | longnose sucker | K | F | 372 | 650 | 0.019 | 0.054 | 0.13 |
| 9203669 | K-IV-8 | longnose sucker | K | F | 374 | 640 | 0.068 | 0.119 | 0.23 |
| 9203670 | K-IV-9 | longnose sucker | K | M | 376 | 670 | 0.065 | 0.152 | 0.362 |
| 9203671 | K-IV-10 | longnose sucker | K | M | 356 | 590 | 0.061 | 0.128 | 0.234 |
| 9203672 | L-II-1 | northern pike | L | F | 438 | 620 | 0.036 | 0.038 | 0.379 |
| 9203673 | L-II-2 | northern pike | L | M | 441 | 600 | 0.046 | 0.048 | 0.289 |
| 9203674 | L-II-3 | northern pike | L | F | 876 | 5135 | 0.008 | 0.012 | 0.29 |
| 9203675 | L-II-4 | northern pike | L | M | 538 | 1050 | 0.04 | 0.037 | 0.287 |
| 9203676 | L-II-5 | northern pike | L | M | 643 | 2150 | 0.034 | 0.043 | 0.307 |
| 9203677 | L-II-6 | northern pike | L | M | 499 | 930 | 0.026 | 0.041 | 0.332 |
| 9203678 | L-II-7 | northern pike | L | F | 636 | 2090 | 0.005 | 0.006 | 0.255 |
| 9203679 | L-II-8 | northern pike | L | F | 562 | 1370 | 0.007 | 0.006 | 0.307 |
| 9203680 | L-II-9 | northern pike | L | M | 573 | 1340 | 0.05 | 0.058 | 0.402 |
| 9203681 | L-II-10 | northern pike | L | M | 549 | 1450 | 0.044 | 0.055 | 0.358 |
| 9203682 | L-III-1 | white sucker | L | M | 352 | 636 | 0.026 | 0.1 | 0.26 |
| 9203683 | L-III-2 | white sucker | L | F | 397 | 820 | 0.016 | 0.057 | 0.205 |
| 9203684 | L-III-3 | white sucker | L | F | 389 | 820 | 0.012 | 0.03 | 0.183 |
| 9203685 | L-III-4 | white sucker | L | F | 425 | 1105 | 0.006 | 0.013 | 0.166 |
| 9203686 | L-III-5 | white sucker | L | M | 360 | 570 | 0.005 | 0.029 | 0.116 |

| Appendix 1 (continued). List of all samples analyzed for mixed-function oxygenase activities for the Northern River Basins Study | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Field number | Species | Loc | Sex | Len (mm) | Wt (g) | EROD | AHH | P450 |
| 9203687 | L-III-6 | white sucker | L | F | 410 | 950 | 0.01 | 0.055 | 0.218 |
| 9203688 | L-III-7 | white sucker | L | M | 377 | 720 | 0.023 | 0.071 | 0.244 |
| 9203689 | L-III-8 | white sucker | L | F | 395 | 800 | 0.008 | 0.015 | 0.149 |
| 9203690 | L-III-9 | white sucker | L | F | 390 | 820 | 0.019 | 0.066 | 0.146 |
| 9203691 | L-III-10 | white sucker | L | F | 435 | 1090 | 0.007 | 0.033 | 0.161 |
| 9203692 | L-IV-1 | longnose sucker | L | F | 384 | 690 | 0.014 | 0.039 | 0.204 |
| 9203693 | L-IV-2 | longnose sucker | L | F | 442 | 1060 | 0.012 | 0.035 | 0.2 |
| 9203694 | L-IV-3 | longnose sucker | L | F | 380 | 620 | 0.031 | 0.077 | 0.321 |
| 9203695 | L-IV-4 | longnose sucker | L | F | 423 | 910 | 0.005 | 0.022 | 0.189 |
| 9203696 | L-IV-5 | longnose sucker | L | M | 358 | 540 | 0.048 | 0.116 | 0.33 |
| 9203697 | L-IV-6 | longnose sucker | L | M | 392 | 765 | 0.028 | 0.067 | 0.232 |
| 9203698 | L-IV-7 | longnose sucker | L | F | 385 | 680 | 0.029 | 0.069 | 0.274 |
| 9203699 | L-IV-8 | longnose sucker | L | F | 399 | 760 | 0.018 | 0.034 | 0.221 |
| 9203700 | L-IV-9 | longnose sucker | L | F | 412 | 800 | 0.01 | 0.025 | 0.22 |
| 9203701 | L-IV-10 | longnose sucker | L | F | 414 | 810 | 0.015 | 0.045 | 0.248 |
| 9203702 | M-II-1 | northern pike | M | F | 558 | 1280 | 0.007 | 0.009 | 0.296 |
| 9203703 | M-II-2 | northern pike | M | F | 766 | 3450 | 0.048 | 0.045 | 0.219 |
| 9203704 | M-II-3 | northern pike | M | M | 471 | 765 | 0.055 | 0.053 | 0.347 |
| 9203705 | M-II-4 | northern pike | M | F | 603 | 1610 | 0.015 | 0.018 | 0.308 |
| 9203706 | M-II-5 | northern pike | M | M | 677 | 2540 | 0.121 | 0.123 | 0.4 |
| 9203707 | M-II-6 | northern pike | M | F | 700 | 2650 | 0.009 | 0.007 | 0.199 |
| 9203708 | M-II-7 | northern pike | M | F | 730 | 2860 | 0.011 | 0.014 | 0.199 |
| 9203709 | M-II-8 | northern pike | M | F | 706 | 2780 | 0.004 | <0.001 | 0.263 |
| 9203710 | M-II-9 | northern pike | M | M | 667 | 2340 | 0.072 | 0.063 | 0.353 |
| 9203711 | M-II-10 | northern pike | M | M | 770 | 3715 | 0.073 | 0.07 | 0.3 |
| 9203712 | M-III-1 | white sucker | M | F | 430 | 1110 | 0.007 | 0.021 | 0.141 |
| 9203713 | M-III-2 | white sucker | M | F | 408 | 920 | 0.007 | 0.022 | 0.121 |
| 9203714 | M-III-3 | white sucker | M | F | 399 | 835 | 0.008 | 0.043 | 0.144 |
| 9203715 | M-III-4 | white sucker | M | F | 380 | 710 | 0.011 | 0.088 | 0.175 |
| 9203716 | M-III-5 | white sucker | M | F | 406 | 980 | 0.005 | 0.014 | 0.14 |
| 9203717 | M-III-6 | white sucker | M | M | 350 | 570 | 0.012 | 0.054 | 0.238 |
| 9203718 | M-III-7 | white sucker | M | M | 389 | 750 | 0.025 | 0.057 | 0.239 |
| 9203719 | M-III-8 | white sucker | M | F | 431 | 1020 | 0.009 | 0.032 | 0.15 |
| 9203720 | M-III-9 | white sucker | M | F | 406 | 960 | 0.007 | 0.027 | 0.181 |
| 9203721 | M-III-10 | white sucker | M | F | 446 | 1170 | 0.01 | 0.027 | 0.189 |
| 9203722 | M-IV-1 | longnose sucker | M | F | 407 | 830 | 0.028 | 0.084 | 0.263 |
| 9203723 | M-IV-2 | longnose sucker | M | M | 383 | 690 | 0.021 | 0.119 | 0.287 |

| Appendix 1 (continued). List of all samples analyzed for mixed-function oxygenase activities for the Northern River Basins Study | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Field number | Species | Loc | Sex | Len (mm) | Wt (g) | EROD | AHH | P450 |
| 9203724 | M-IV-3 | longnose sucker | M | F | 409 | 880 | 0.026 | 0.184 | 0.291 |
| 9203725 | M-IV-4 | longnose sucker | M | F | 403 | 940 | 0.009 | 0.014 | 0.184 |
| 9203726 | M-IV-5 | longnose sucker | M | F | 417 | 840 | 0.013 | 0.032 | 0.188 |
| 9203727 | M-IV-6 | longnose sucker | M | F | 376 | 730 | 0.006 | 0.024 | 0.259 |
| 9203728 | M-IV-7 | longnose sucker | M | F | 427 | 975 | 0.01 | 0.034 | 0.233 |
| 9403011 | 20,21 | mountain whitefish | * | . | . | . | 0.008 | <0.001 | . |
| 9403012 | 15,23-30 | mountain whitefish | * | . | . | . | 0.019 | 0.01 | . |
| 9403013 | 1 | mountain whitefish | . | . | . | . | 0.063 | 0.003 | . |
| 9403014 | 2-9 | mountain whitefish | * | . | . | . | 0.04 | 0.036 | . |
| 9403015 | 12 | mountain whitefish | . | . | . | . | 0.009 | <0.001 | <0.001 |
| 9403016 | 14 | mountain whitefish | . | . | . | . | 0.014 | 0.005 | 0.07 |
| 9403017 | 45 | mountain whitefish | . | . | . | . | 0.023 | 0.005 | 0.086 |
| 9403018 | 46 | mountain whitefish | . | . | . | . | 0.011 | 0.01 | 0.027 |

* pooled samples

# NORTHERN RIVER BASINS STUDY

# APPENDIX 2 - TERMS OF REFERENCE

## PROJECT: 2351-B1: BIOCHEMICAL ANALYSIS OF FISH LIVER FOR MIXED-FUNCTION OXIDASE ACTIVITIES

## I. PROJECT DESCRIPTION

The Northern River Basins Study requires the contract laboratory to analyze 120 fish liver samples for two liver microsomal mixed-function oxidase enzyme activities, namely ethoxyresorufin-O-deethylase (EROS) using 7-ethoxyresorufin as substrate and aryl hydrocarbon hydroxylase (AHH) using benzo(a)pyrene as substrate, microsomal protein content, and to supply a cytochrome-P-450 difference spectrum for each liver analyzed.

## II. TERMS OF REFERENCE

1. The laboratory will prepare a microsomal suspension from each liver, and assay the suspension for protein content, EROD activity, AHH activity, and it must record cytochrome P-450 difference spectra from 400 to 500 nanometres. Recently the Department of Fisheries and Oceans has published a technical report describing acceptable methods to perform the assays required here [3]. These analyses can be done in several ways, but in order to remain consistent with existing data, the methods described in detail below are those which have been applied to previous fish samples from the Athabasca and Slave rivers.

**Preparation of Microsomes**

Pieces are to be cut from the frozen liver and allowed to thaw partially at 2-4°C until they could be cut with scissors. The small pieces are suspended in cold 0.02M HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid)/0.15M KC1(potassium chloride) buffer (Ph 7.5) using 4 mL buffer per g of tissue in cold glass tissue homogenizers, and homogenized using 5-7 passes with a motor-driven teflon pestle. The homogenate is then centrifuged at 12000 x g at 2°C for 20 min.;the supernatant is further centrifuged at 105,000 x g at 2°C for 75-90 min. to obtain a microsomal pellet. The pellet is washed and resuspended in HEPES/KC1 buffer to a protein concentration between 5 and 15 mg $L^{-1}$. Protein in microsomal suspensions is measured by the method of Lowry *et.al.* [5] as modified by Markwell *et.al.* [6] with bovine serum albumin as a standard. The microsomes are resuspended on in a buffer consisting of 0.05 M tris(hydroxymethyl)methylamine (tris), 1 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid (EDTA), and 20 (v/v) pH 7.4 and then frozen and stored in liquid nitrogen analysis [10].

## Analysis of Microsomes for AHH Activity

The AHH assay is based on that of DePierre *et.al.* [1] as modified by Van Cantfort *et.al.* [11] to measure the production of polar metabolites from non-polar tritiated benzo(*a*)pyrene used as substrate. The microsomal suspension (150 $\mu$L) is incubated in tris-HC1 buffer (800 $\mu$L, 0.1 M, pH 7.5) with magnesium chloride (10 $\mu$L, 500 mM) and an NADPH generating system (NADP, 10 $\mu$L, 78.7 mg mL-$^{1}$; sodium isocitrate 10 $\mu$L, 154.86 mg mL-$^{1}$; isocitrate dehydrogenase, q.s. i U per incubation tube) and tritiated benzo(*a*)pyrene (20 $\mu$L, 15-20 $\mu$Ci $\mu$M-$^{1}$ in acetone, 1.01 mg mL-$^{1}$) for 30 min. at 25 °C. The reaction is stopped by adding 2 ml 0.15 M potassium hydroxide (KOH) in 85% dimethyl sulfoxide (DMSO). Unreacted benzo(*a*)pyrene is extracted with two washes, each with 3 mL hexane. Radioactivity (benzo(*a*)pyrene metabolites) is counted in duplicate 200-$\mu$L aliquots of the remaining aqueous layer using a liquid scintillation counter. Metabolites are assumed to have the same specific activity as substrate and to have been produced at 1;1 stoichiometry. Three separate incubations are carried out for each microsomal suspension, and in each case triplicate blanks were run to which KOH-DMSO had been added prior to the addition of substrate. Radioactivity remaining in the aqueous phase in the blanks was subtracted from that in the active preparations for calculation of activity as nanomoles of product per mg of microsomal protein per minutes.

## Analysis of Microsomes for EROD Activity

Ethoxyresorufin-O-deethylase is measured by the deethylation of 7-ethoxyresorufin to yield resorufin which is detected by the fluorometric procedure described by Pohl and Fouts [9]. The reaction mixture is 1100 $\mu$L of HEPES buffer (0.1 M, pH 7.8), 10 $\mu$L of magnesium sulfate (154 mg mL-$^{1}$), 10 $\mu$L NADP (98.4 mg mL-$^{1}$), isocitrate dehydrogenase (q.s. 1 U per incubation tube), and 50 $\mu$L bovine serum albumin (40 mg mL-$^{1}$), all mixed in a Corex centrifuge tube where the mixture is incubated at room temperature for at least 10 min. The microsomal suspension (50 $\mu$L) is added and the reaction is initiated by the addition of 10 $\mu$L ethoxyresorufin (0.03 mg mL-$^{1}$ in DMSO) and the tubes are incubated for an accurately timed period of two min. at 25 °C. Then the reaction is stopped by the addition of 2.5 mL methanol. The samples are centrifuged at 18000 x g for 5 min. to remove precipitated protein and the resorufin in the supernatant is measured with a spectrofluorometer as an excitation wavelength of 530 nm and an emission wavelength of 585 nm. Standards consist of killed reaction mixture (methanol added) to which a known amount of resorufin has been added and which have been carried through the centrifugation step. Blanks consisting of samples to which the methanol has been added prior to the addition of substrate are rune for each sample, and the final result corrected for any non-enzymatic production of resorufin. Triplicate incubation mixtures of each sample are run, along with triplicate blanks for each sample. Results are reported as nanomoles of product per mg of microsomal protein per minute.

**Analysis of Microsomes for Cytochrome P-450 Difference Spectra**

The procedure for measuring cytochrome P-450 is based on methods described by Omura and Sato [7, 8]. Estabrooke *et.al.* [2], and Johannesen and DePierre [4] have described some of the problems with this assay. The microsome suspension is diluted to a concentration of 1 mg $mL^{-1}$ in a phosphate buffer (0.1 M, pH 7.4) in a 12 x 75 mm disposable test tube and then transferred to a quartz cuvette. The suspension is bubbled for a minimum of 20 seconds with carbon monoxide gas to saturate the sample with CO. The absorbance of the carbon monoxide-saturated microsomes is then recorded from 400 to 500 nm using a recording spectrophotometer. Several milligrams of dithionite were added to the cuvette to reduce the cytochrome P-450 and the absorbance spectrum was recorded again, using the carbon monoxide spectrum as the reference. The difference absorbance readings at 450 and 490 nm were used to calculate the amount of cytochrome P-450 present, taking the difference between the absorbance at 450 and that at 490 nm, and using a millimolar extinction coefficient of 91 for a 1-cm light path [7] and the appropriate protein concentration. Analyses were done in triplicate. For recording the difference spectra, alternate procedures may be used depending on the model of spectrophotometer available. For example, if a double-beam instrument is used, then one cuvette can be used as the reference with the dithionite tube taken as the sample and difference spectrum can be recorded in a single scan.

## III. REPORTING REQUIREMENTS

1. The laboratory will record all information supplied with each liver sample and code laboratory record numbers with Northern River Basins Study sample numbers so that the enzyme analyses can be compared with other data generated on the same fish.

2. The laboratory will report the mean activity of its replicate analyses for AHH and EROD (as nanomoles of produce per mg microsomal protein per minute) for each liver analyzed. Cytochrome P-450 content will be reported as nanomoles per mg microsomal protein and copies of each difference spectrum will be supplied. Digital spectral data will be retained and made available on request by the Northern River Basins Study.

3. Details of all calculations will be retained by the laboratory, but will be made available to the Northern River Basins Study upon request.

4. The report to the Northern River Basins Study will be by printed paper, with electronic submission of data to follow in formats to be determined by the Northern River Basins Study.

## IV. INTELLECTUAL PROPERTY

Upon completion or termination of this project, all data, documents and materials which are acquired or produced under this project shall become the sole property of the Northern River Basins Study.

## V. REFERENCES

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