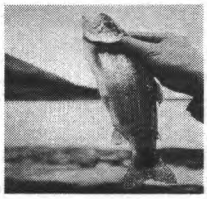
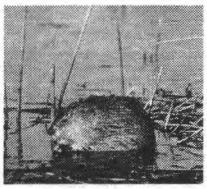
Canada Alberta Northwest Territories

Northern River Basins Study



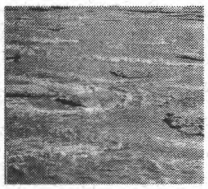


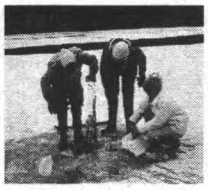





NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 132

ANALYSIS FOR LIVER MIXED FUNCTION OXYGENASE IN FISH, PEACE, ATHABASCA AND SLAVE RIVER BASINS, SEPTEMBER TO DECEMBER, 1994






Prepared for the
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by

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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.

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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) 22 Apr 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) April 23/96

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) April 23/96

(Robert McLeod, Co-chair) — (Date) 30/04/96

ANALYSIS FOR LIVER MIXED FUNCTION OXYGENASE IN FISH, PEACE, ATHABASCA AND SLAVE RIVER BASINS, SEPTEMBER TO DECEMBER, 1994

STUDY PERSPECTIVE

The aquatic fauna of the northern rivers in Alberta are exposed to 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. A previous Northern River Basins Study (NRBS) project also found elevated levels of MFO activity in mountain whitefish and longnose suckers collected from the Athabasca River downstream of a bleached kraft pulp mill located at Hinton. 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 and impairment of important biochemical and reproductive functions. 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. Activity of these enzymes 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?*

This project report describes MFO analyses done on fish collected from 23 sites on the Peace, Athabasca and Slave rivers and their major tributaries in the fall of 1994. The fish species targeted for collection and analyses were burbot (primary target species), northern pike, longnose sucker and flathead chub. Three enzyme assays were applied to fish liver samples; ethoxyresorufin-O-deethylase (EROD), aryl hydrocarbon hydroxylase (AHH), and cytochrome P-450.

Results from a total of 187 burbot, 37 northern pike, 82 longnose sucker and 21 flathead chub were included into a portion or all of these analyses. Results from the collection sites were organized into reference (upstream locations and tributaries receiving no inputs from pulp mills), near-field (<100 km downstream of a pulp mill source) and far-field (>100 km downstream of a pulp mill source). Small regional differences in EROD activities were found in burbot, particularly for adult male and immature fish, but there was no clear relationship between enzyme activities and the locations of pulp mills. Mature male and immature burbot from the Wabasca River and lower Athabasca River at Fort McKay had higher MFO activities than those from other sites. The presence of MFO inducing compounds in the lower Athabasca River can likely be attributed to natural erosion of the oil sands along the river, but this explanation is speculative for the Wabasca River. Mean EROD values were higher in northern pike collected from far-field regions, but regional differences in EROD were not detected for longnose sucker and flathead chub.

The results from this project indicate that average MFO responses are generally low in these river systems, except for fish collected from the lower Athabasca River and the Wabasca River. This project is one component of a study representing a large-scale effort to simultaneously evaluate contaminant levels, reproductive parameters and possible physiological effects of potential contaminant exposure in fish. Data from these fish will also provide comparative information in relation to previous contaminant and biochemical analyses conducted on these species by NRBS and other agencies. 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

Collections of fish were made at locations throughout the Peace, Athabasca and Slave River drainage basins in 1994 with a view to determining whether the effects of pulp mills could be detected in the fish. Previous studies had indicated that biochemical effects could be detected in fish from the Wapiti River near Grande Prairie and from the Athabasca River near Hinton. Four species of fish were collected, namely burbot (*Lota lota*), flathead chub (*Platygobio gracilis*), longnose sucker (*Catostomus catostomus*) and northern pike (*Esox lucius*). Collections were found to contain mature males, mature females and immature fish and it was generally necessary for statistical reasons to consider these gender/maturity classes separately, although numbers of fish in each class were often small. The fish were analyzed for mixed-function oxygenase (MFO) activities in microsomes prepared from livers. These enzymes have been found to respond to pulp mill effluents in populations of fish sampled near other pulp mill locations in Canada and Europe.

Collections were considered first at a regional scale in which collection sites were arbitrarily assigned to one of three types of regional scale. Control regions were located upstream from any known source of pulp mill effluent; near-field regions were <100 km downstream of a pulp mill source; and far-field regions were >100 km downstream of a pulp mill source. Using this regional grouping of collection sites, the result anticipated if mills were contributing MFO inducing compounds would be higher MFO activities in fish from the near-field region than in those from control or far-field region.

Analysis to compare the enzyme activities among regions revealed no clear regional patterns that might be attributable to pulp mills in burbot, flathead chub or northern pike, although sample numbers were small and several sites were missing with the latter two species. With longnose suckers, the pattern of enzymatic activities may have suggested the anticipated regional response, but the differences did not meet the criterion of statistical significance. With northern pike, the highest activities were found in fish from the far-field region.

In instances where there were four or more fish from a given gender/maturity group at several sites, we also examined the results to determine whether fish from different reaches of the rivers differed in a manner consistent with locations of mills. This type of analysis revealed elevated enzymatic activities in immature and male burbot (but not in females) not from reaches near pulp mills, but instead from the lower Athabasca River near the oil sands and also from the Wabasca River. There were too few flathead chub for meaningful reach-by-reach comparisons. Relatively high enzyme activities were found in longnose suckers from two upper Peace River reaches but the geographic pattern implicated unknown sources instead of, or in addition to, pulp mills. Very few longnose suckers were obtained from the upper Athabasca River in 1994 and so it was not possible to determine rigorously whether the enzyme induction reported downstream from Hinton in 1992 was still present in 1994; the few samples available suggest that it was still present. Numbers of northern pike were too small to make statistically meaningful comparisons among reaches but the highest activities were in pike from the Jackfish Village sites in the Athabasca Delta.

Taken together the results suggest that the enzyme activities of burbot were not affected by the pulp mills but were affected (in male and immature fish) by unidentified materials in the lower Athabasca River and Wabasca River, possibly associated with the presence of oil sands since petroleum-related compounds are good inducers of this enzyme system. Enzyme activities in longnose suckers (immature fish) were highest in those from two reaches of the upper Peace River and it seems unlikely that this pattern can be explained fully by the presence of pulp mills. Very few samples of longnose suckers were obtained from the upper Athabasca River in 1994, but the results were consistent with enzyme induction detected downstream from Hinton in 1992 still being present in 1994.

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1.0 INTRODUCTION

Fish were collected from several areas throughout the Peace, Athabasca and Slave River drainages during 1994. Among the analyses applied to these fish were the liver microsomal mixed-function oxygenase (MFO) enzyme activities. The rationale for making these collections was essentially the same as the that for more localized collections made from the reach of the Athabasca River near Hinton in 1992. That earlier study showed responses in mountain whitefish and longnose suckers consistent with a source of inducing compounds near Hinton (Lockhart et al., 1996). The MFO activities of fish have been shown to respond to the effluents of bleached kraft pulp mills in a number of studies (Anderson et al., 1988; Rogers et al., 1989; Munkittrick et al., 1991, 1994; Hodson et al., 1992; Swanson et al., 1993). When exposed to compounds with the required structural properties, the fish synthesize supplementary amounts of several enzymes collectively called mixed-function oxygenases (MFO). Two of these MFO activities (ethoxyresorufin-O-deethylase, EROD) and aryl hydrocarbon hydroxylase (AHH) have been measured in the fish collected for this study. The biochemical roles of these enzymes seem likely to remain under study for many years since they can catalyze a number of cellular biochemical changes. There seems little doubt that the presence of unusually high activities of these enzymes indicates that fish have been exposed to inducing compounds. However, it is not clear what role(s), if any, the induced activities have in toxicological responses at higher levels of biological organization.

1.1 Collections of Fish

Collections of fish were made by EnviResource Consulting Ltd. and have been described in their report (EnviResource Consulting Ltd., 1995). Fish were removed from the water and taken to live holding tanks on shore from which they were removed for measurements, descriptions and tissue sampling. Liver samples were taken within two minutes after the death of each fish sealed in Whirlpak bags and frozen immediately on dry ice. Livers were maintained frozen either on dry ice or in a freezer at -80∘C until analyzed at the Freshwater Institute. The site descriptions in Table 1 have been taken from the EnviResource report and are also shown on the map in Figure 1.

Table 1. Sites where fish were collected during the fall and winter of 1994 (from Table 1 of EnviResource Consulting Ltd., 1995) with approximate latitudes and longitudes.

River	Site	General Location	Latitude	Longitude
Peace	PR1	Includes Many Islands Prov. Pk.	56.29	119.12
	PR2	Near the Notikewin River	57.28	117.10
	PR3	Upstream from Fort Vermillion	58.41	116.13
Smoky	SR1	Near Watino at Highway 49	55.71	117.62
	SR2	Near Grande Cache		
	SR3[1]	Canfor main haul road bridge		
Wapati	WR1	Near Pipestone Ck. Prov. Pk.	55.04	119.21
	WR2	Near O'Brian Prov. Pk.		
Little Smoky	LSR1	Near Highway 744 crossing	55.62	117.39
	LSR2[2]	3 km downstream from LSR1		
Wabasca	WAB	Near Highway 67 crossing	57.87	115.39
Athabasca	A1	Near Highway 947 crossing	54.01	116.84
		Near the Berland River		
	A2	Upstream from Hinton	53.38	117.71
	A3	Near Fort Assiniboine	54.32	114.79
	A4	Near the Calling River	55.10	112.89
	A5	Near Fort Mackay	57.10	111.57
McLeod	MR	Near Eagle campground	54.10	115.81
	MCR2[1]	At Big Eddy upstream from Edson		
Pembina	P	Near the town of Jarvie	54.46	113.98
Lesser Slave	LSV	Downstream from Slave Lake Pulp and Paper	55.27	114.34
Clearwater	CW	Upstream from Fort McMurray	56.70	111.29
Peace Athabasca Delta	JV	At Jackfish Village	58.45	111.06
Slave River Delta	SR	Upstream from Fort Resolution	61.27	113.41

[1] Collections made in mid-December, 1994. SR3 samples (n=1 burbot) was denoted SR2 to correspond with sites listed in the Terms of Reference.

[2] Collections made in mid-September and mid-December, 1994

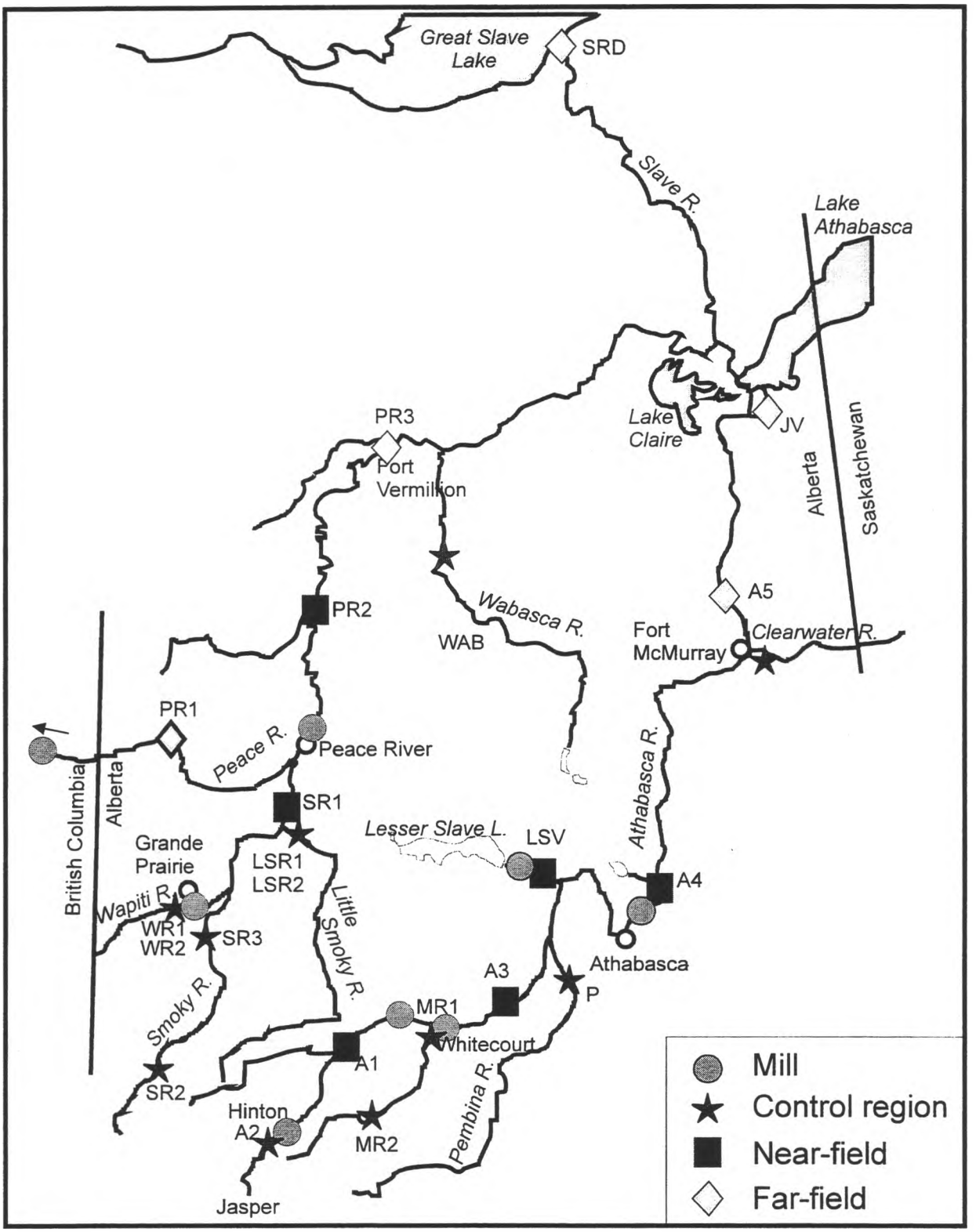

Figure 1. Approximate locations of pulp mills (solid circles) and locations where fish were collected for the Northern River Basins Study in 1994. Shapes of collection site markers reflect the regions defined with respect to locations of pulp mill

File nrbmapb.cdr

2.0 BIOCHEMICAL METHODS

Analyses for MFO catalytic activities were described in our report of activities in fish collected near Hinton in 1992 (Lockhart et al., 1996); they are repeated here. In 1991, 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 with existing data on fish from other collections from the Athabasca and Slave Rivers. The fluorometric assay for ethoxyresorufin O-deethylase (EROD) described by Hodson et al. (1991) is 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.

2.1 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 frozen in Whirlpak bags on dry ice and were held at -80◦C until analyzed. The samples were removed from the freezer, subsampled while still frozen and the remaining portion was returned to the freezer. The analytical samples were weighed and allowed to thaw at 4°C until they could be cut up with scissors. The samples were 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).

2.2 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.

2.3 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 of 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 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.

2.4 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.

2.5 Statistical Comparisons

Means (arithmetic) were calculated using PROC MEANS of SAS. Comparisons among means were made with PROC GLM of SAS using log(10) transformed values of biochemical measurements as inputs to the program. Individual means were compared with each other using the 'lsmeans' and 'Duncan' options of PROC GLM. Correlations were calculated using PROC CORR of SAS.

3.0 RESULTS AND DISCUSSION

3.1 Burbot

The three biochemical measurements described in this report are listed for all the fish individually in Appendix 1.

3.1.1 Enzyme activities of burbot at a regional scale

Seven pulp mills operate within the Peace and Athabasca drainages of Alberta (Table 2 and Figure 1) and there are additional mills upstream on the Peace River drainage in British Columbia. Some of the mills have operated for decades and these older mills have been upgraded extensively to meet new pollution control standards. A number of studies have indicated that effluents from bleached kraft pulp mills contain material that induces the microsomal MFO system of fish. Within Alberta, studies on the

Smoky/Wapiti system near Grande Prairie (Kloepper-Sams and Benton, 1994; Kloepper-Sams et al., 1994) and our study of the Athabasca River between Hinton and Whitecourt for the NRBS Representative Area Program in 1992 (Lockhart et al., 1996) have implicated two Alberta mills as sources of these materials. Recently studies in Finland (Lindstrom-Seppa et al, 1992) Ontario (Munkittrick et al., 1994) and Manitoba (Friesen et al., 1994) have indicated that effluents from non-chlorine-bleaching mills can also affect these activities in some fish, and so all the mills in Alberta must be considered potential sources of inducing compounds.

Table 2. Existing pulp mills of the Peace and Athabasca drainages in Alberta (also shown in Figure 1)

Mill	Location and Drainage
Weldwood Canada	Hinton, upper Athabasca R.
Alberta Newsprint Co.	Near Whitecourt, upper Athabasca R.
Millar Western Pulp	Near Whitecourt, upper Athabasca R.
Slave Lake Pulp	Lesser Slave River (Central Athabasca drainage)
Alberta Pacific Forest Industries	Near Athabasca, Central Athabasca R.
Weyerhauser Canada	Grande Prairie, Wapiti River (upper Peace drainage)
Daishowa-Marubeni International	Near Peace River (upper Peace R.)

In addition to these mills in Alberta, there are three mills within the Peace River drainage on the British Columbia side of the border.

The initial objective was to determine whether differences could be detected in the MFO activites of fish collected at sites grouped into regions. For this purpose, the sites were grouped into three regional classes: control, upstream from any known source; near-field, within approximately 100 km of a known source; and far-field, more than approximately 100 km from a known source (Table 3). Most sites fell readily into one of the three regions but a few did not fit neatly into any group. For example, the McLeod River site MR1 was quite close to the confluence of the McLeod and Athabasca rivers at Whitecourt and so fish taken there may have been represented either or both rivers.

Table 3. Regional grouping of sampling sites into control, near-field and far-field sites based on locations of pulp mills and sampling sites	
Region	Sites assigned to regional groupings (sites described above in Table 1, from Enviresource Consulting, 1995)
Control (upstream from any known sources)	A2, SR2, WR1, WR2, MR1, MR2, P, CW, WAB1, LSR1, LSR2
Near field (less than 100 km downstream from a known source)	A1 (a & b), A3, LSV, SR1, A4, PR2
Far field (more than 100 km downstream from a known source)	PR1, PR3, A5, JV, SRD

Initially, all the fish collected within each of the regions were pooled into a single regional group and the regional means were compared using an analysis of variance (using PROC GLM of SAS). EROD and AHH values were transformed to logarithms before making the comparisons, although arithmetic means are listed in Table 4. The statistical analysis indicated very little difference in EROD activities. The far-field group had significantly higher ($p=0.02$) EROD values than the controls, but the near-field values differed from neither the controls nor the far-field group. AHH activities failed to differ among the regions. Small regional differences existed in EROD activities, but there was no clear relationship between the enzyme activities and the locations of the mills. If the effects of the mills were present at a regional scale, then we should have expected the near-field region to exceed the controls. The cytochrome P-450 levels differed among the regions with the near-field group higher than controls and the far-field group declining to intermediate levels (Table 4). It has generally been our experience that the cytochrome P-450 assay is less sensitive than the individual catalytic activities (AHH, EROD) and so the pattern of P-450 in the absence of a similar pattern in EROD and AHH activites is not interpreted as a regional effect of the mills.

Table 4. Mean (arithmetic) MFO activities (nmol/mg protein/min) and cytochrome P-450 content (nmol/mg protein) in burbot (males, females, immatures together) from collections sites pooled into three regions, control, near-field and far-field, 1994.

	Control sites			Far Field sites			Near sites		
Variable	N	Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev
EROD	51	0.0242	0.0199	49	0.0380	0.0393	77	0.0223	0.0104
AHH	51	0.0148	0.0153	49	0.0301	0.0421	87	0.0117	0.0082
P450	51	0.2044	0.0545	49	0.2333	0.0662	87	0.2574	0.0590

Enzymatic activities in burbot are often higher in males than in females (Lockhart & Metner, 1992); we have not previously reported activities in immature burbot, but in view of the relatively large proportion of immature fish in these collections, the regions were compared using males only, then using females only and finally using immature fish only (Table 5).

Table 5. Biochemical values from burbot from the three regions, segregated by gender and maturity.

	Males only		Females only		Immatures only	
	N	EROD	N	EROD	N	EROD
Control	14	0.036	30	0.012	5	0.058
Near	33	0.024	12	0.011	32	0.025
Far	22	0.045	14	0.010	13	0.056
	N	AHH	N	AHH	N	AHH
Control	14	0.015	30	0.009	5	0.043
Near	36	0.012	16	0.006	35	0.014
Far	22	0.035	14	0.006	13	0.049
	N	P450	N	P450	N	P450
Control	14	0.239	30	0.175	5	0.274
Near	36	0.276	16	0.202	35	0.263
Far	22	0.242	14	0.185	13	0.270

Higher EROD and AHH values were found in the males and immature fish from the control and far-field regions than in the near-field region; the means for the regional groups are shown in Figure 2. Females were relatively uniform throughout the region. The cytochrome P-450 values were more uniform than the catalytic activities, with near-field fish having slightly higher values than either far-field or control fish. Again, with gender and maturity classes considered separately, the pattern of activities did not suggest a relationship to the mills.

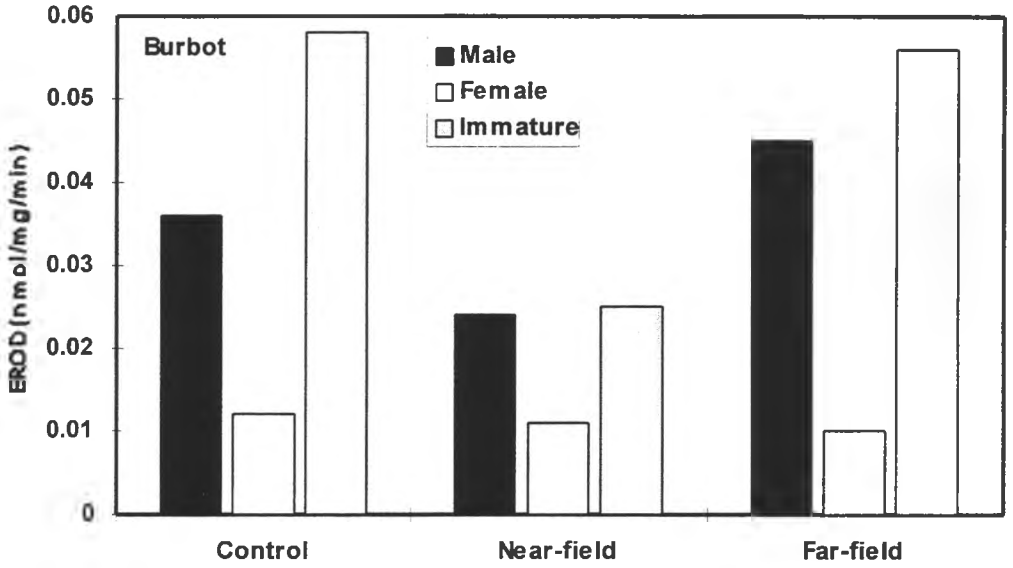

Figure 2. EROD activities in male, female and immature burbot from the three regions.

The relative abundance of immature fish was higher for the near-field and far field regions than for controls. The overall mean age for the 50 fish from the control region was 7.29 years as compared with 7.59 years for the near-field (n=86) and 7.27 years for the far-field (n=49) region. The 5 immature control fish averaged 5.0 years in age while the 35 immature fish from the near-field region averaged 7.34 years and those from the far-field region averaged 6.38 years in age. The immature fish from the near-field region (7.34 years) and the far field region (6.38 years) were almost the same ages as the mature fish from the control region (7.14 years for males and 7.82 years for females). This may suggest that burbot from both the near-field and far-field regions take longer to mature than those from the control region. If this is indeed the case, then the reasons for a longer maturation period in near-field and far-field regions are speculative but Munkittrick et al. (1991, 1992) reported delayed maturation in white suckers and lake whitefish from Jackfish Bay in Lake Superior, a site receiving effluent from a pulp mill there.

Taking the sexually mature burbot as two groups (males and females) without regard for region or site of collection, the EROD activities and gonad sizes were correlated but positively in males and weakly but negatively in females. (Correlations were calculated using PROC CORR of SAS on log10 EROD and log10 GSI.) The gonad size, (gonado-somatic index, GSI) was calculated as:

$$GSI = (\text{gonad wt}/(\text{total wt} - \text{gonad wt}))*100.$$

With males, the correlation coefficient, r, between EROD activity and GSI was + 0.467 (p=0.0001, n=69) while with females r = - 0.418 (p=0.0015, n=55). The GSI was highest in both males (12.1 %) and females (5.48 %) from the control region; it was lower from the far-field (males = 9.51 %, females = 4.73 %) and lower yet in the near-field (males = 4.31 %, females = 2.88 %) regions. This regional pattern in male GSI is similar to that for EROD activities in males (Figure 2); taken together they suggest that

factors were acting to increase EROD activity and increase the rate of transfer of material to the testes of males. Kloepper-Sams et al. (1994) studied mountain whitefish and longnose suckers from the Smoky/Wapiti system and reported no reductions in gonad weight in those fish with elevated EROD activities. Munkittrick et al. (1994) found reduced or unchanged gonad sizes in male white suckers taken from several mill sites in Ontario. We are not aware of previous studies in which a positive correlation between EROD and GSI has been reported. The effect was different in females; the correlation was negative and EROD activities were relatively uniform and low (Figure 2). The Ontario mill survey by Munkittrick et al. (1994) found low gonad weights in female white suckers from all sites except one (Espanola). These authors also noted the EROD effect in male but not in female white suckers at some sites.

3.1.2 Effects on Burbot at a Reach Scale

Since MFO enzyme activities in males often exceed those in females, the results for each collection have been presented for gender groups separately in Tables 6-8. We have not previously examined data from immature fish separately from mature fish, but in these burbot, the immature fish differed from the females, and so they have been presented separately. Mean EROD activities are shown in Table 6 for females, males and immature fish; similarly AHH activities and cytochrome P-450 levels are listed in Tables 7 and 8 respectively.

Taking the data site by site reduced the number of individuals available from some sites and so the rigor of statistical treatments was reduced. Inspection of the MFO activities for mature females revealed generally low values with no obvious geographic trend that might be related to a known source of inducing material. The highest mean EROD activity tabulated for a female (0.031 nmol/mg protein/min) was obtained from the Little Smoky River near its confluence with the Smoky River and the second highest value was from a group of four females from the Pembina River. Both of these sites are in the 'control' region.

Table 6. Mean liver microsomal EROD activities (nmol/mg protein/min) in mature female, mature male, and immature burbot from the Northern River Basin Study, 1994.

Locations	Female		Male		Immature	
	N	EROD	N	EROD	N	EROD
Athabasca 1	2	0.0090	2	0.0230	0	.
Athabasca 2	5	0.0112	1	0.0330	0	.
Athabasca 3	3	0.0150	14	0.0272	5	0.0248
Athabasca 4	2	0.0125	1	0.0140	9	0.0316
Athabasca 5	3	0.0157	10	0.0685	4	0.1295
Clearwater	5	0.0122	0	.	0	.
Little Smoky R. 2	1	0.0310	0	.	0	.
Little Smoky R. 3	1	0.0090	0	.	0	.
Lesser Slave	2	0.0090	6	0.0270	6	0.0298
McLeod R. 2	4	0.0038	2	0.0260	1	0.0150
Pembina	4	0.0220	1	0.0270	0	.
Peace River 1	1	0.0110	2	0.0235	4	0.0205
Peace River 2	0	.	3	0.0307	4	0.0163
Peace River 3	0	.	1	0.0200	5	0.0244
Smoky R. 1	3	0.0073	7	0.0160	8	0.0166
Smoky R. 2	1	0.0090	0	.	0	.
Slave River Delta	10	0.0083	9	0.0273	0	.
Wabasca R. 1	2	0.0170	1	0.0680	4	0.0690
Wapati R. 1	2	0.0120	2	0.0240	0	.
Wapati R. 2	5	0.0086	7	0.0391	0	.

Mature male and immature burbot from site A5 on the Athabasca River near Fort MacKay and from the site on the Wabasca River had higher activities than those from other sites. The relative scale of these activities are shown graphically in Figure 3 for immature fish and in Figure 4 for mature males.

Table 7. Mean liver microsomal AHH activities (nmol/mg protein/min) in mature female, mature male, and immature burbot from the Northern River Basin Study, 1994.

Locations	Female		Male		Immature	
	N	AHH	N	AHH	N	AHH
Athabasca 1	5	0.0038	3	0.0073	0	.
Athabasca 2	5	0.0062	1	0.0190	0	.
Athabasca 3	3	0.0103	14	0.0128	5	0.0102
Athabasca 4	2	0.0050	1	0.0070	9	0.0190
Athabasca 5	3	0.0160	10	0.0641	4	0.1350
Clearwater	5	0.0126	0	.	0	.
Little Smoky R. 2	1	0.023	0	.	0	.
Little Smoky R. 3	1	0.005	0	.	0	.
Lesser Slave	2	0.005	8	0.0129	8	0.0153
McLeod R. 2	4	0.0013	2	0.0170	1	0.0120
Pembina	4	0.0170	1	0.008	0	.
Peace River 1	1	0.0020	2	0.0070	4	0.0093
Peace River 2	1	0.0040	3	0.0107	5	0.0080
Peace River 3	0	.	1	0.0210	5	0.0108
Smoky R. 1	1	0.0050	7	0.0111	8	0.0154
Smoky R. 2	1	0.0020	0	.	0	.
Slave River Delta	10	0.0035	9	0.0094	0	.
Wabasca R. 1	2	0.0130	1	0.0340	4	0.0508
Wapati R. 1	2	0.0115	2	0.0185	0	.
Wapati R. 2	5	0.0028	7	0.0113	0	.

Earlier, as part of the regional scale analyses, the A5 site was assigned to the far-field region and the Wabasca River site to the control region; unexpectedly high values from these two sites tend to confound the regional analysis. Given the small numbers of males taken at most locations, an analysis of variance was carried out by restricting the comparisons to those 6 sites (A3, A5, WR2, SR1, LSV and SR) where at least four males were available. The results (using PROC GLM of SAS with least square means and Duncan's test to rank means) indicated that the A5 site with a mean of 0.069 nmol/mg/min differed from the other sites as indicated below.

	A5,	WR2,	SR, LSV,	A3,	SR1
Duncan group (burbot males)	a	b	ccccccccccc		d

Site WR2 had the second highest mean but its value was only 0.039 nmol/mg/min. Elevated values from these two sites cannot be explained by their spatial relationship to pulp mills, and so some other explanation must be sought. One possibility for the A5 site is that it is located near sites of current

production of oil from oil sands. Petroleum oils and specific polycyclic aromatic hydrocarbons are able to induce microsomal MFO enzyme systems in burbot (Lockhart et al., 1989) and so the Fort McMurray area may supply natural or effluent sources. There is a regulated effluent discharged from the Suncor plant to the Athabasca River near site A5 and it contains petroleum hydrocarbons. However, oil sands themselves contain a number of polycyclic aromatic hydrocarbons (Lockhart & Billeck, unpublished data), and there is some natural erosion of these formations; consequently it is difficult to associate induction at site A5 with either potential source without further work. Previous analyses of goldeye from this reach of the river also indicated high values (B. Brownlee et al., Environment Canada, unpublished data). The Wabasca River site was not included in this analysis because only one mature male was obtained from there, however, it had a high EROD activity of 0.068 nmol/mg protein/min. The WR2 site (0.039 nmol/mg/min) was different in a statistical sense but it was much closer to the remaining sites than it was to the A5 site.

Considering the immature fish, nine sites offered four or more individuals for comparison; burbot from the A5 and WAB1 sites had higher activities than any of the others as shown below:

	A5,	WAB1,	A4,	LSV,	A3,	PR3,	PR1,	PR2,	SR1
Duncan group	a	a	a	a	a	a	a		
			b	b	b	b	b	b	b
(immature, EROD)								c	c

Essentially the same pattern was obtained with AHH values; sites A5 and WAB1 had higher activities than all the other sites. Reasons for high MFO activities in burbot from the Wabasca River site are more speculative than at the A5 site; a map of oil sands deposits (Figure 5) shows that a deposit of oil sand does occur near the Wabasca River, and its presence there may may offer an explanation of the high values at the WAB site. However, the map also reveals deposits in the area of the Peace River in the vicinity of site PR2, and so we might expect to see elevated enzyme activities there too. However, this was not the case with burbot, although it was the case with immature suckers (see below). In all three of these sites, some additional analyses of suspended and bottom sediments for PAHs would help to test the argument that proximity to oil sand deposits may have been responsible for the apparent induction of fish enzymatic activities.

Table 8. Mean liver microsomal cytochrome P-450 (nmol/mg protein) in mature female, mature male, and immature burbot from the Northern River Basin Study, 1994.

Locations	Female		Male		Immature	
	N	P-450	N	P-450	N	P-450
Athabasca 1	5	0.2322	3	0.279	0	.
Athabasca 2	5	0.1904	1	0.231	0	.
Athabasca 3	3	0.225	14	0.30279	5	0.2826
Athabasca 4	2	0.1745	1	0.128	9	0.26389
Athabasca 5	3	0.145	10	0.2343	4	0.315
Clearwater	5	0.1574	0	.	0	.
Little Smoky R. 2	1	0.276	0	.	0	.
Little Smoky R. 3	1	0.167	0	.	0	.
Lesser Slave	2	0.186	8	0.26488	8	0.267
McLeod R. 2	4	0.134	2	0.2125	1	0.219
Pembina	4	0.21025	1	0.284	0	.
Peace River 1	1	0.286	2	0.2965	4	0.299
Peace River 2	1	0.173	3	0.295	5	0.2548
Peace River 3	0	.	1	0.144	5	0.2116
Smoky R. 1	3	0.16733	7	0.24814	8	0.25275
Smoky R. 2	1	0.184	0	.	0	.
Slave River Delta	10	0.1868	9	0.24989	0	.
Wabasca R. 1	2	0.1645	1	0.296	4	0.28725
Wapati R. 1	2	0.179	2	0.205	0	.
Wapati R. 2	5	0.1636	7	0.24271	0	.

Some comparison with burbot from outside the NRBS study area is appropriate because we have obtained a number of other collections over the past decade. The samples were collected by a number of agencies and individuals and analyzed by the same laboratory using methods comparable to those used here. Consequently, the results should be comparable directly in the same units as reported here. The map in Figure 6 shows the NRBS burbot EROD results in the wider context of those from throughout western and northern Canada. With the exception of high activities in burbot from collections at the A5 and WAB1 sites, the burbot MFO values are consistent with the wider data set. In addition to the values shown in Figure 6, Kloepper-Sams and Benton (1994) reported EROD activities in the 0.02 to 0.034 nmol/mg/min range for burbot from the Smoky/Wapiti system and from the North Saskatchewan River, values consistent with those reported here.

Failure to detect a clear response in burbot from reaches or regions close to pulp mills is a puzzle since the 1992 data showed clear responses downstream from the Hinton site in two other species, namely

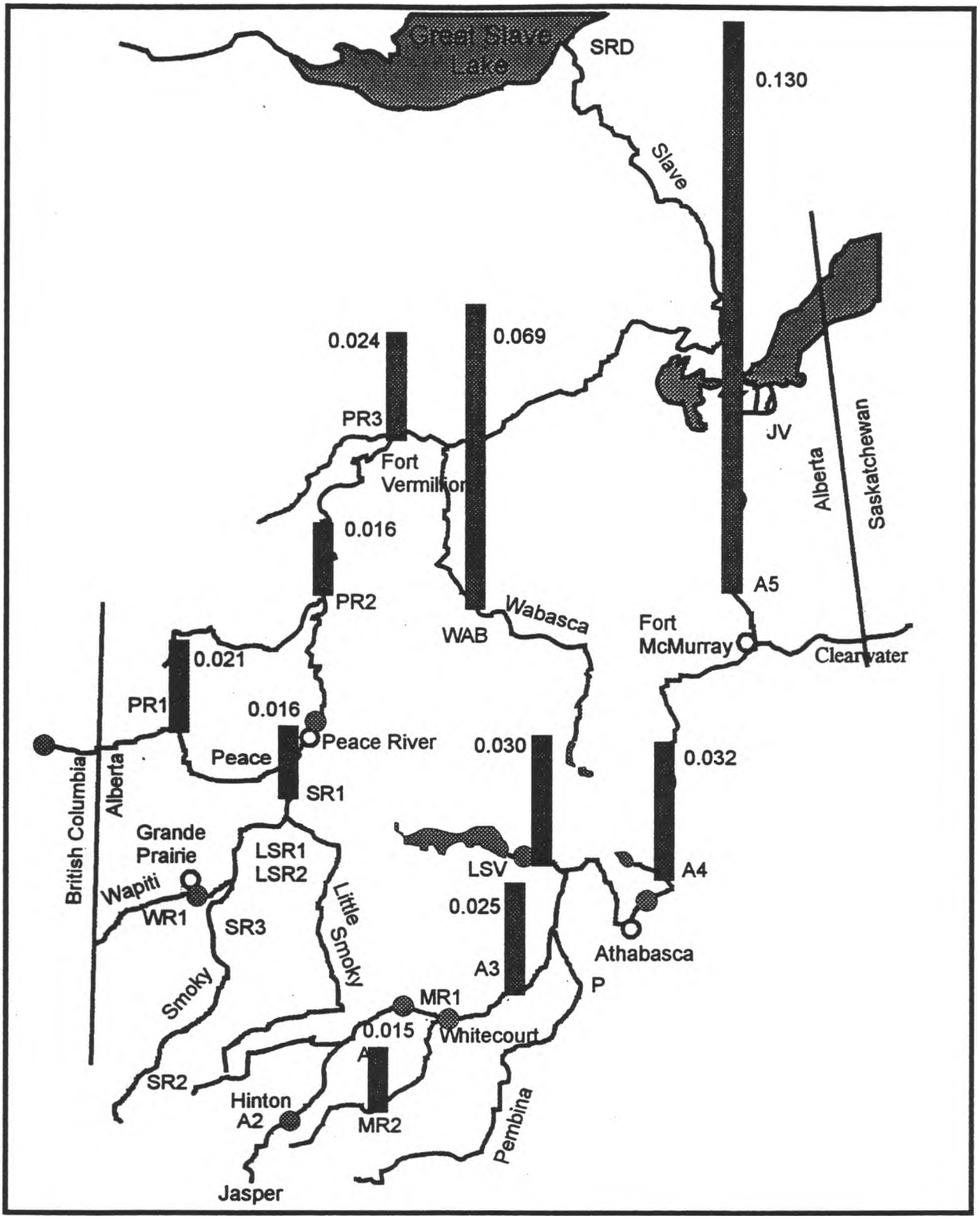

Figure 3. Relative EROD activities in immature burbot taken for the Northern River Basins Study, 1994. Bases of bars may have been shifted slightly from sampling locations to minimize overlapping of bars. Approximate locations of pulp mills (solid circles) are also shown.

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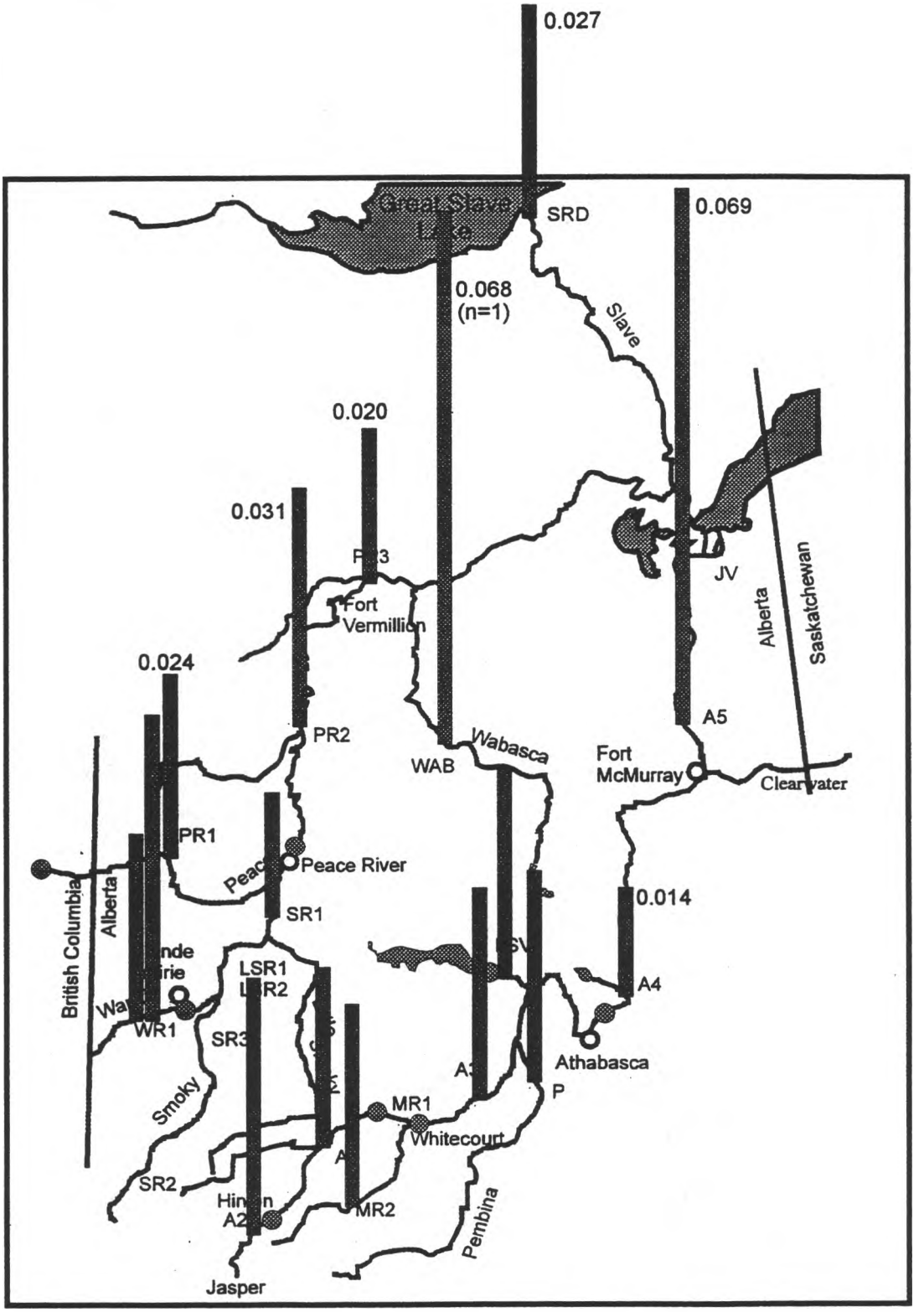

Figure 4. Relative EROD activities in mature male burbot taken for the Northern River Basins Study, 1994. Bases of bars may have been shifted slightly from sampling locations to minimize overlapping of bars. Approximate locations of pulp mills (solid circles) are also shown.

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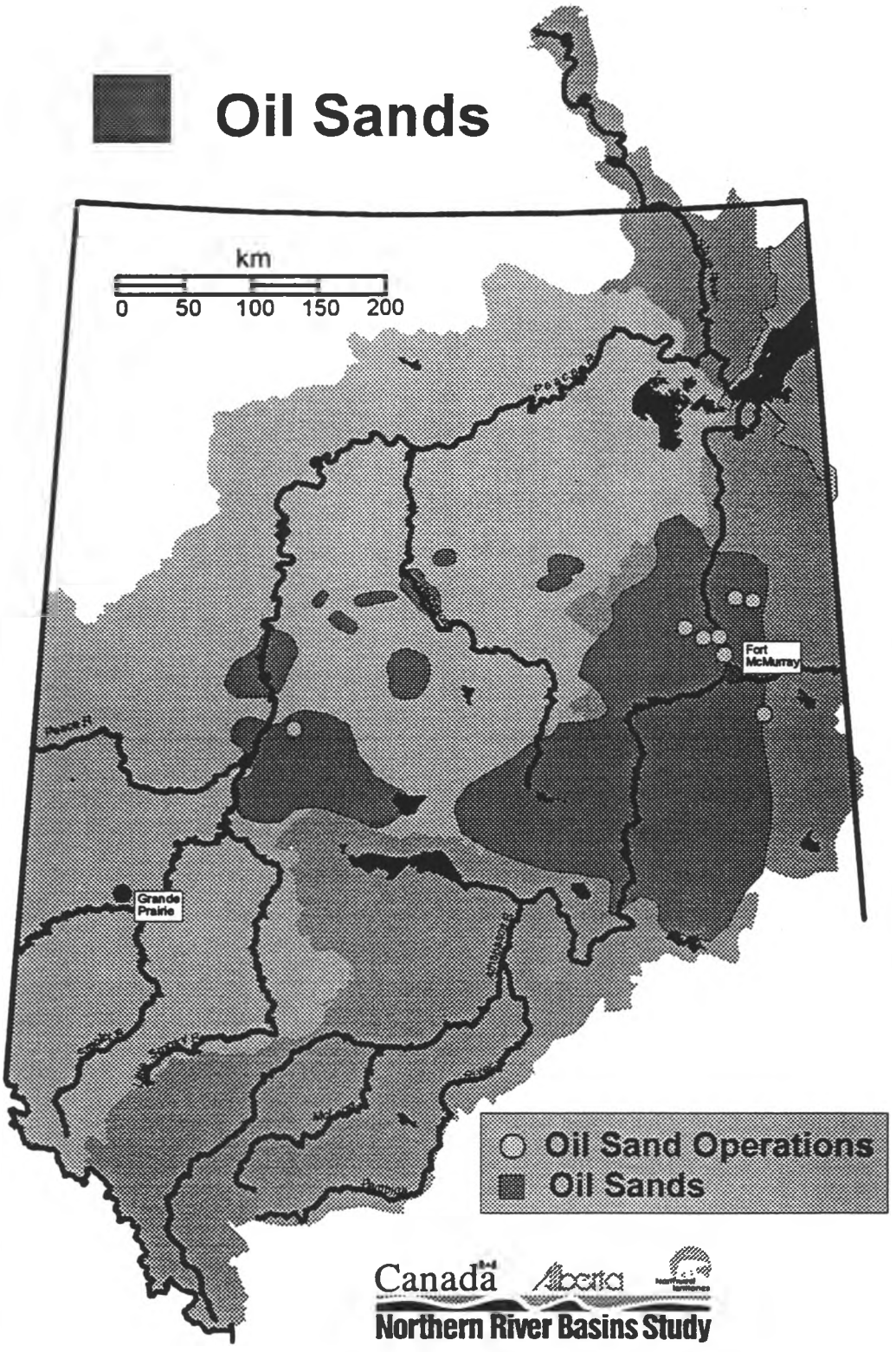

Figure 5. Distribution of oil sands in Alberta

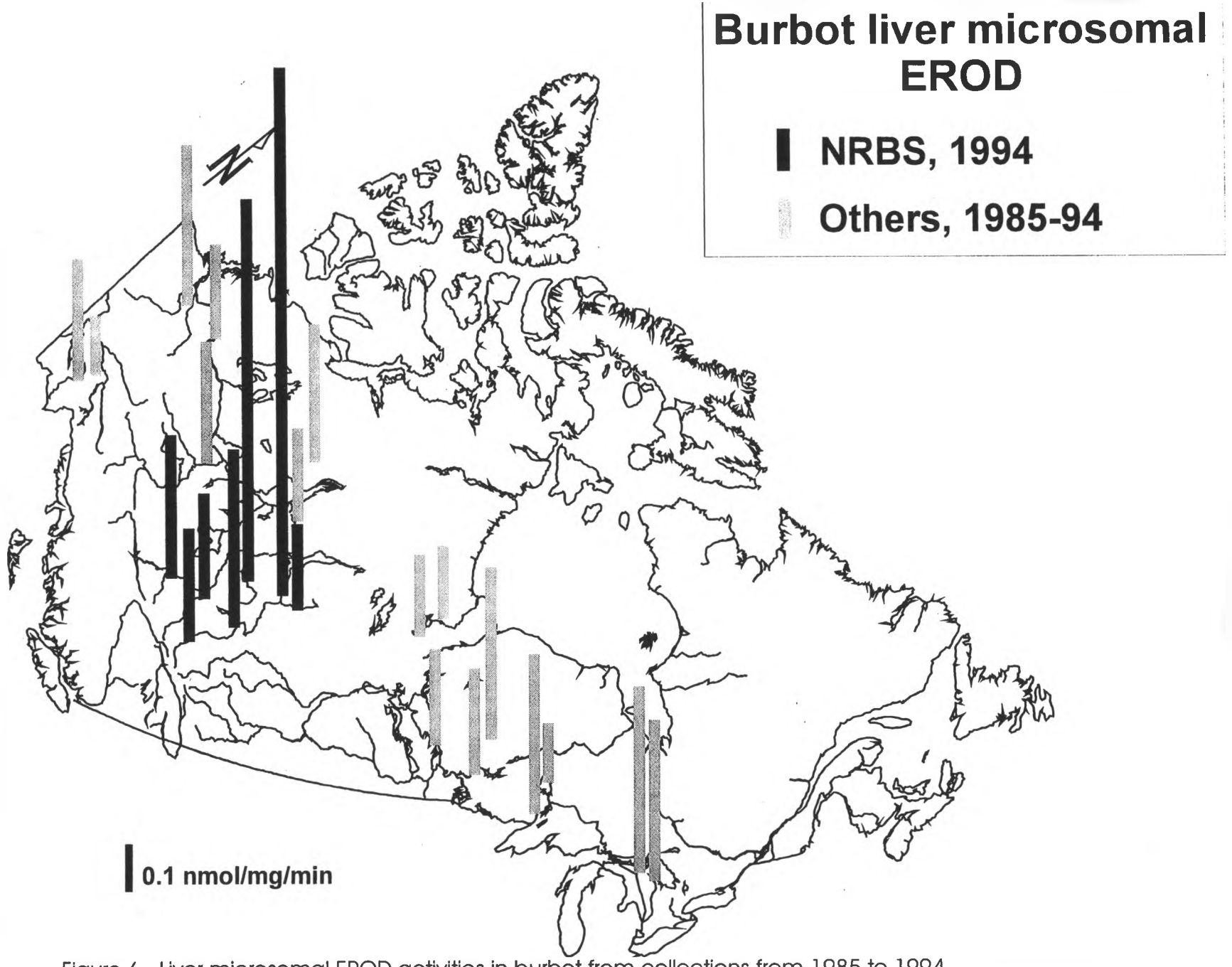

Figure 6. Liver microsomal EROD activities in burbot from collections from 1985 to 1994. Samples from different sexes and maturities were pooled. Some bars represent several collections from the same point over a number of years. Bases of bars have sometimes been shifted slightly to minimize overlaps.

mountain whitefish and longnose suckers. Kloepper-Sams and Benton (1994) also found burbot to be unresponsive in their study at Grande Prairie.

In experiments unrelated to NRBS studies, we have observed an interaction between the nutritional state of arctic char and their EROD response to PCBs. That is, fish given a low dosage of PCB and then restricted to sub-maintainance diets responded with greater induction than fish on maintainance diets (Delorme et al., 1995). Liver size has sometimes been found to increase in fish exposed to pulp mill effluents (Munkittrick et al., 1994; Kloepper-Sams et al., 1994). However, burbot use the liver for the storage of fat more than many other species, and the experiment with arctic char suggests that stored fat serves to insulate the fish from the effects of PCBs. Presumably stored fat was mobilized during the period of reduced feeding and PCB previously stored in the fat became free to act pharmacologically and induce the enzyme activities. Burbot typically store fat in the liver (Chen, 1969, Lockhart et al., 1989). We have no direct measures of fat in burbot collected for these analyses, however, EnviResource Consulting (1994) recorded liver weights and those may offer a surrogate for fat storage. Females had relatively larger livers. (Liver somatic index, LSI = (liver wt/total wt)*100), in percentage.) The LSI for females was 4.90 % while that for males was 3.73 % and for immatures 2.62 %. This may offer a simple mechanism to suggest why the males and immatures were apparently more responsive than the females; the larger livers in females may have offered a larger reservoir of fat to store inducing compounds and keep them from acting pharmacologically. Considering the responses of genders/maturities separately, there were negative correlations between EROD activities and LSI (males, r=-0.28, p=0.018, n=69; females, r=-0.44, p=0.0008 n=55; immatures, r =-0.26, p=0.069, n=50). The consistent negative sign of the correlations suggests that larger livers, presumably with larger lipid reservoirs, may have offered some protection from induction.

3.2 Flathead Chub

Twenty-one flathead chub (*Platiogobio gracilis*) were obtained from some of the sampling sites (Appendix 2) but liver weights were low and we were unable to obtain enough tissue for all the analyses. Priority was given to the EROD analysis and we obtained that value for all 21 individuals; AHH activities were obtained for only 5 fish. Gender was not reported for four of the fish and the remaining 17 comprised 9 mature females, 3 immature females and 5 immature males. The mean EROD activity for the 9 females was 0.019 nmol/mg/min while those for the immature females and males were 0.036 and 0.016 nmol/mg/min respectively. Statistical analysis using PROC GLM of SAS did not reveal differences among the gender/maturity groups at the p=0.05 level. The highest EROD value obtained was 0.063 nmol/mg/min for an immature female taken from the Little Smoky River site LSR2.

3.2.1 Enzyme Activities of Flathead Chub at a Regional Scale

In view of the small number of fish and the failure to detect gender differences in EROD activities, the entire sample of flathead chub was sorted into the three regional groups as defined by Table 3 without regard for gender or maturity. This yielded 7 fish from the control region with a mean EROD activity of 0.024 nmol/mg/min, 13 from the near-field region with a mean of 0.021 nmol/mg/min and 1 from the far-field region with an activity of 0.018 nmol/mg/min (Figure 7). Statistically, there were no differences indicated among the regions.

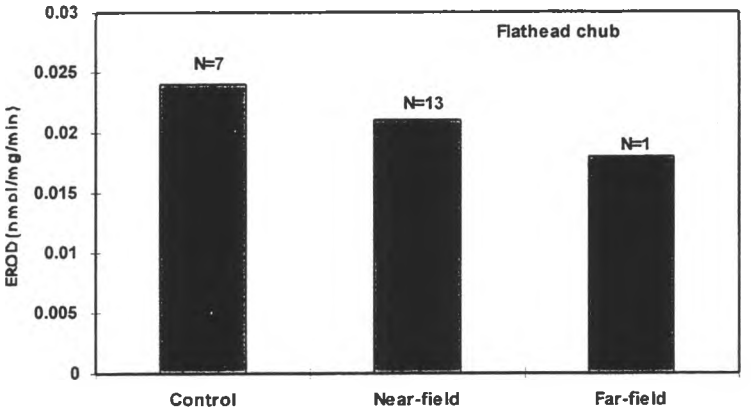

Figure 7. EROD activities of flathead chub from the three regions, not segregated for gender or maturity.

3.2.2 Enzyme Activities of Flathead Chub at a Reach Scale

The WR1 and SR1 sites offered the only site-by-site comparison of values upstream and downstream from the mill at Grande Prairie. There were 5 chub taken upstream at the WR1 site and these had a mean EROD activitiy of 0.017 nmol/mg/min as compared with 11 from the SR1 site downstream from Grande Prairie with a mean of 0.021 nmol/mg/min. These means did not differ statistically.

3.3 Longnose Suckers

Eighty-two longnose suckers (*Catostomus catostomus*) were obtained from several sites, mostly from the Peace River and the Smoky/Wapiti system, and the enzyme activities for each individual are shown in Appendix 3. These fish consisted of 16 mature males, 33 mature females, 30 immature fish and three for which neither sex nor maturity could be determined. About one third of the fish were immature and these were smaller in both length and weight than mature females (PROC GLM of SAS, least square means, $p < 0.01$), although they did not differ from males. The average length for the mature females was 429 mm as compared with 404 mm for males and 385 mm for immature fish. Ages were available for most of the fish and the mean age of females was 10.9 years while male and immature fish both averaged 9.2 years. Considering the group as a whole, an analysis of variance indicated that the females had lower activities than male or immature fish, and that the latter two groups did not differ from each other.

3.3.1 Enzyme Activities of Longnose Suckers at a Regional Scale

Enzymatic activities for the longnose suckers, pooled into control, near-field and far-field regions as defined in Table 3, are shown in Table 9. Although the EROD values suggest a pattern of response (Figure 8) in males, and to a lesser extent in females, consistent with the locations of pulp mills, the analysis of variance (PROC GLM of SAS) conducted separately on each of the three gender/maturity groups revealed no significant differences among regions for any of the measurements.

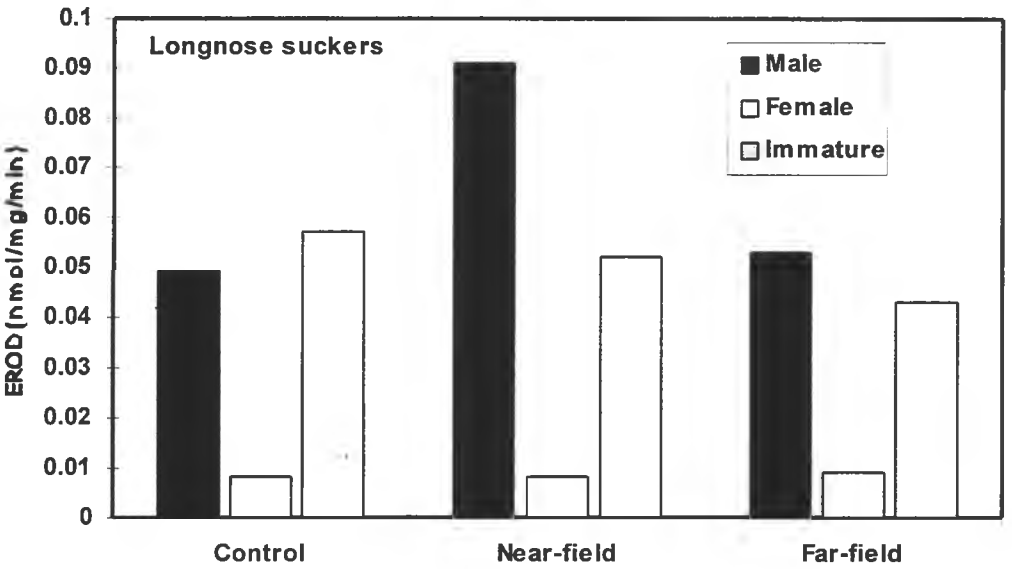

Figure 8. EROD activities of male, female and immature longnose suckers from the three regions.

Table 9. Biochemical values from longnose suckers from the three regions (control, near field and far field) segregated by gender and maturity

Region	Males only		Females only		Immatures only	
	N	EROD	N	EROD	N	EROD
Control	4	0.049	12	0.008	7	0.057
Near	4	0.091	7	0.008	9	0.052
Far	8	0.053	14	0.009	14	0.043
	N	AHH	N	AHH	N	AHH
Control	4	0.077	12	0.018	6	0.120
Near	4	0.137	7	0.027	9	0.084
Far	8	0.108	14	0.023	14	0.092
	N	P450	N	P450	N	P450
Control	4	0.216	12	0.184	7	0.337
Near	4	0.293	7	0.213	9	0.300
Far	8	0.268	14	0.183	14	0.284

3.3.2 Enzyme Activities of Longnose Suckers at a Reach Scale

The numbers of longnose suckers and the mean EROD activities for each site are shown in Table 10. There were four catches of females with at least four individuals per site, namely the Wapiti River 1, Smoky River 1, Peace River 1 and Peace River 2. The females from the Wapiti River 1 site had the lowest mean activity while that from the Peace River 1 site had the highest. The four sites were compared using analysis of variance (PROC GLM of SAS) and the results indicated considerable overlap among the sites, with only the highest and lowest differing from each other.

	PR1, SR1, PR3, WR1
Duncan group	a a a a a a a a a a a
(females, EROD)	b b b b b b b b b b

The most meaningful comparison among the females from these sites is probably the WR1 (upstream from the mill at Grande Prairie, mean EROD = 0.004 nmol/mg/min) *vs.* SR1 (downstream from the same mill, mean EROD = 0.007 nmol/mg/min). Again, although the values of the means suggest a response below the source, the values from these two sites were generally low and failed to differ statistically. Earlier studies with longnose suckers from the Wapiti River identified mild induction downstream from the mill at Grande Prairie (Kloepper-Sams and Benton, 1994; Kloepper-Sams et al., 1994).

Table 10. Mean liver microsomal EROD activities (nmol/mg protein/min) in mature female, mature male and immature longnose suckers from the Northern River Basins Study, 1994

Locations	Female		Male		Immature	
	N	EROD	N	EROD	N	EROD
Athabasca 1	0	-	1	0.110	0	-
Athabasca 2	1	0.013	0	-	2	0.020
Little Smoky R. 2	0	-	3	0.017	3	0.083
McLeod R.	0	-	1	0.061	0	-
Peace R. 1	7	0.009	3	0.055	6	0.067
Peace R. 2	2	0.012	1	0.220	4	0.078
Peace R 3	7	0.008	5	0.052	8	0.024
Smoky R. 1	5	0.007	2	0.016	5	0.031
Smoky R. 2	1	0.007	0	-	0	-
Wapiti R. 1	7	0.004	3	0.045	2	0.055

There was only one catch of male longnose suckers with four or more individuals, and so statistical comparisons were not made. There were, however, four catches of immature suckers with at least four individuals, and so these were compared using PROC GLM of SAS. There were highly significant differences with the PR1 and PR2 catches falling into one group with high activities

(0.067 and 0.078 nmol/mg/min respectively) and SR1 and PR3 falling into a second with lower activities (0.031 and 0.024 nmol/mg/min respectively).

	PR1, PR2, SR1, PR3
Duncan Groups (immature, EROD)	a a a a a a b b b b b b

When the same analysis was run using AHH values, no differences were detected among the four groups. There were small differences in cytochrome P-450 concentrations with site PR1 and PR3 differing from each other as shown:

	PR1, SR1, PR2, PR3
Duncan Groups	a a a a a a a a a a
(immature, P450)	b b b b b b b b b b

There does appear to be a real difference between at least PR1 and PR3. Reference to Table 3 and the map of sites (Figure 1) show that both these sites are in the 'far field' region and so the reason for their high values are probably not related to pulp mills. PR2 is in a region where some oil sand deposits are found (map, Figure 5) and so those deposits may offer an explanation of the high values at site PR2. Only one male sucker was taken at site PR2, but it had a high value of 0.22 nmol/mg/min. The reason for a high value as site PR1 is unknown since is far downstream from the nearest mill in British Columbia and since no oil sands deposits are shown in that area in Figure 5. Site Little Smoky River 2 had only three immature suckers and so it was excluded from the site-by-site comparison; however, it had a high activity of 0.083 nmol/mg/min, comparable with those from sites PR1 and PR2. In contrast to the immature suckers, the three mature males from LSR2 did not have high activities.

There were too few longnose suckers taken from the upper Athabasca River in 1994 to make meaningful comparisons with that species from the 'Representative Area Program' in 1992. The only longnose suckers taken in 1994 upstream from Hinton were two immature fish with a mean EROD activity of 0.020 nmol/mg/min, which is similar to the mean value of 0.025 nmol/mg/min obtained for males taken upstream in 1992 (Lockhart et al., 1996). One male was taken at site A1 near Whitecourt and it had a high EROD value of 0.11 nmol/mg/min which falls within the range of values found for males between Hinton and Whitecourt in 1992 (Lockhart et al., 1996). Taken together, these few fish suggest that the induction detected in 1992 catches downstream from Hinton was still present in 1994.

3.4 Northern Pike

Thirty-seven northern pike were available and their individual enzymatic activities are shown in Appendix 4. Two individuals (PR1NRPK4 and PR2NRPK3) were not characterized for sex or maturity; both were small fish (154 g and 112 g respectively) and are assumed to have been immature.

3.4.1 Enzyme Activities of Northern Pike at a Regional Scale

The catches of pike were pooled into the regional groups used for the other species (Table 3); 17 were from the control region, 9 from the near-field region and 11 from the far-field region. Segregating these

fish further into sex and maturity groups, the regional means are listed in Table 11. Inspection of the mean EROD values for males suggests higher values from the far-field region but the statistical comparison (PROC GLM of SAS) indicated regional differences were significant at $p=0.063$, just short of the statistical criterion of $p<0.05$. A similar pattern was indicated with AHH activities in males, but the differences were not significant statistically. Considering females, the mean EROD activities were again higher in the far-field region, but but again the difference ($p=0.06$) fell just short of the statistical criterion of $p<0.05$. No clear pattern was evident in the AHH activities or cytochrome P-450 levels from females and no statistical differences were indicated. There were only two immature fish in the near-field and far-field samples and so statistical comparisons were not made.

The mean EROD values for the regional gender and maturity groups are shown in Figure 9 and there is striking agreement among males, females and immatures with all three having higher mean activities in the far-field group. While these differences failed to meet the statistical criterion of $p<0.05$, they were very close to it. Inspection of the values from individual sites in Table 12 shows that the high regional values for far-field sites were driven by high values from pike taken at Jackfish Village in the Athabasca Delta.

Table 11. Biochemical values from northern pike from the three regions (control, near field and far field) segregated by gender and maturity

Region	Males only		Females only		Immatures only	
	N	EROD	N	EROD	N	EROD
Control	8	0.041	3	0.009	6	0.044
Near	4	0.042	3	0.011	2	0.041
Far	4	0.119	5	0.018	2	0.096
	N	AHH	N	AHH	N	AHH
Control	8	0.073	3	0.011	6	0.054
Near	4	0.050	3	0.019	2	0.046
Far	4	0.103	5	0.019	2	0.090
	N	P450	N	P450	N	P450
Control	4	0.330	3	0.276	6	0.330
Near	4	0.261	3	0.260	2	0.244
Far	8	0.346	5	0.259	2	0.260

3.4.2 Enzyme Activities of Northern Pike at a Reach Scale

Mean EROD activities for northern pike from each location are shown in Table 12. The numbers of fish obtained from most sites were very small and so no statistical comparisons were made. The only pattern suggested is one of high EROD activities from the three male and immature fish from the Athabasca Delta but there were too few samples available to support any firm conclusion. We are not aware of sedimentation properties but it seems possible that the fish from the Athabasca Delta may have been

exposed to material entering the system upstream and then transported down to the Delta and deposited there.

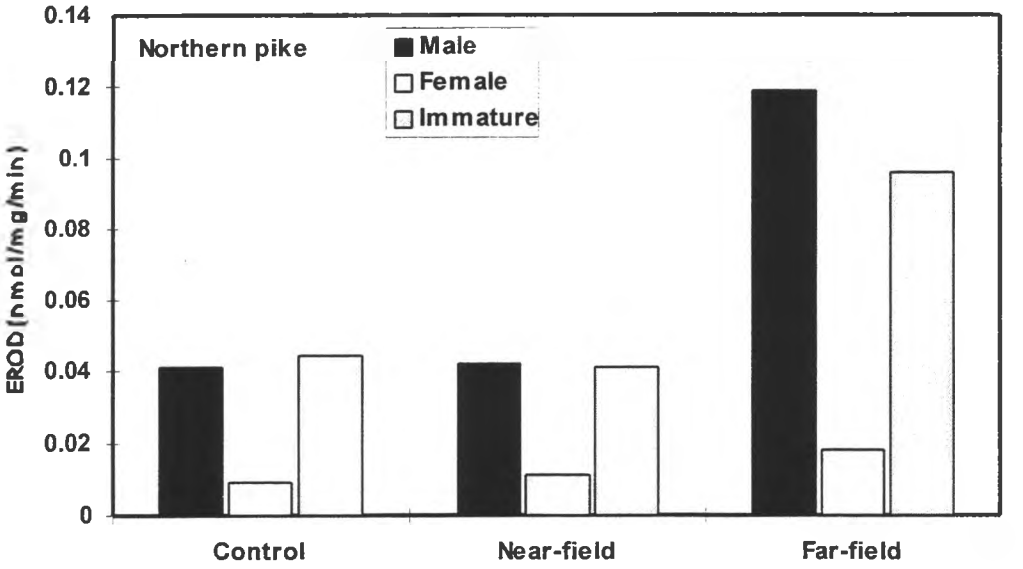

Figure 9. EROD activities of male, female and immature northern pike from the three regions.

Table 12. Mean liver microsomal EROD activities (nmol/mg protein/min) in mature female, mature male and immature northern pike from the Northern River Basins Study, 1994.

Locations	Females		Males		Immatures	
	N	EROD	N	EROD	N	EROD
Athabasca 1			2	0.036	1	0.066
Jackfish Village 2	2	0.019	1	0.175	1	0.173
Jackfish Village 3			1	0.188		
Peace River 1	1	0.016	2	0.056	1	0.018
Peace River 2	1	0.008	1	0.053	1	0.016
Smoky River 1	2	0.013	1	0.042		
Slave River Delta	2	0.018				
Wapiti River 1	2	0.009	7	0.036	3	0.014
Wapiti River 2	1	0.010				
McLeod River			1	0.079	3	0.074

A small number of northern pike were also taken in the 'Representative Area Program' in 1992 from the reach of the Athabasca River between Hinton and Whitecourt (Lockhart et al., 1996). The closest comparison we can make is with reach F (upstream from Whitecourt in 1992) which corresponds approximately to site A1 in 1994. The mean EROD activities for males in the spring and fall of 1992 were 0.077 (n=6) and 0.080 (n=4) nmol/mg/min, respectively. This is in good agreement with values of 0.036 nmol/mg/min for males in 1994 (n=2) and 0.066 (n=1) for immature pike in 1994.

4.0 CONCLUSIONS

1. The effects of pulp mill effluents on the mixed-function oxygenase enzymes of burbot were not evident at locations where the burbot would have been exposed to these effluents. There were sufficient samples of burbot available to allow detection of apparent effects in the lower Athabasca River and in the Wabasca River, and so it seems likely that pulp mill effects would have been detected if they were present.

2. Mild induction of mixed-function oxygenases was detected in male and immature burbot from the Athabasca River near the producing oil sands plants and from the Wabasca River. This is the fourth such indication for the oil sands area of the lower Athabasca River and the first indication for the Wabasca River. Attribution of the induction effect to oil sands is consistent with the chemistry of these materials in the Athabasca River, but is speculative in the Wabasca River.

3. Male and female burbot showed different geographic patterns of MFO enzyme activities. Male burbot had a weak but significant statistical relationship in which increasing EROD activities were associated with increasing gonad sizes. Females showed the opposite relationship in which increasing EROD activities were associated with decreasing gonad sizes. Immature burbot responded more like males than like females. A testable hypothesis to explain this difference is that females may have been protected from fat-soluble inducers by their larger livers.

4. Burbot and longnose suckers appear to respond somewhat differently. For example, differences detected in the longnose suckers between sites PR1 and PR3 were not evident in the burbot.

5. Too few longnose suckers were obtained from the upper Athabasca River in 1994 to confirm with confidence the effects reported for that reach of the river on the basis of collections of longnose suckers in the fall of 1992 and mountain whitefish in the spring of 1992. The limited samples available suggest that the induction present in 1992 was still present.

6. Taken together, the high MFO values from the burbot at sites A5 and WAB1, in northern pike from Jackfish Village, and the immature longnose suckers at sites PR1 and PR2 indicate the possibility of widespread, low-level exposures to inducing compounds in reaches near and downstream of oil sands. The effects of enzyme induction on natural populations are not known and these populations may offer an opportunity to define the effects of low-level exposures to inducing materials.

5.0 RECOMMENDATIONS

1. A historical and geological description of oil and oil sand deposits and activities in the general areas of sites A5, WAB1, PR1 and PR2 should be made to see whether this might offer some insight into why burbot from sites A5 and WAB1 and suckers from sites PR1 and PR2 had high enzymatic activities.

2. Analyses of suspended and bottom sediments and of fish from sites A5, WAB1, PR1 and PR2 for polycyclic aromatic hydrocarbons would help to test whether petroleum-related activities could be responsible for the enzyme activities in the fish.

3. The high enzymatic activities in the few northern pike from Jackfish Village in the Athabasca Delta are intriguing. The sedimentation patterns in the Delta should be examined for evidence of the delivery of chemicals from upstream. Similarly, the high values from sites PR1 and PR2 for immature longnose suckers and at WAB1 and A5 in burbot males and immatures all suggest low-level exposures to inducing materials. These populations should be useful to characterize the importance of low-level exposures.

4. The frequency of immature fish seems high in these collections suggesting that the onset of maturity may be delayed in burbot. Data from other collections should be examined to see whether this can be confirmed. The gonad size data suggest that male burbot may mature faster than females within a given year. This can probably be tested using other NRBS data such as histological examinations.

5. It was hoped that the 1994 collections would be useful to find out whether the induction reported for longnose suckers from the Representative Area Program in 1992 was still present. There were too few samples from the upper Athabasca River to test the argument rigourously although the samples available suggest that induction is still present. Efforts should be made to obtain more samples from the reach of the Athabasca River extending from above Hinton to below Whitecourt.

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APPENDICES

Appendix 1. Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in burbot from the Peace, Athabasca and Slave River drainages, 1994

Lab Number	Field Number	Species	Loc	Capture Date	Length	Weight	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P-450
9404191	A1BURB1	Burbot	A1	12-Sep-94	550	722	M	6	42.7	35.8	0.022	0.009	0.312
9404192	A1BURB2	Burbot	A1	12-Sep-94	498	700	M	8	45.5	38.7	0.024	0.001	0.274
9404196	A1BURB6	Burbot	A1	12-Sep-94	469	406	F	7	18.9	13.2	0.01	0.005	0.232
9404197	A1BURB7	Burbot	A1	12-Sep-94	450	438	F	7	26.8	11.9	.	0.005	0.214
9404198	A1BURB8	Burbot	A1	13-Sep-94	452	448	F	7	20	14.4	.	0.002	0.224
9404199	A1BURB9	Burbot	A1	13-Sep-94	498	624	F	8	30.4	19.7	0.008	0.002	0.205
9404200	A1BURB10	Burbot	A1	13-Sep-94	447	435	F	5	29.5	12.1	.	0.005	0.286
9404201	A1BURB11	Burbot	A1	14-Sep-94	485	635	M	7	44	56.5	.	0.012	0.251
9404281	A2BURB1	Burbot	A2	20-Sep-94	519	613	F	2	27	29.3	0.016	0.006	0.133
9404283	A2BURB3	Burbot	A2	20-Sep-94	681	1508	F	11	84.8	59	0.014	0.004	0.241
9404284	A2BURB4	Burbot	A2	21-Sep-94	768	2530	F	13	119.6	107	0.012	0.008	0.208
9404285	A2BURB5	Burbot	A2	22-Sep-94	431	440	M	5	17.6	27.1	0.033	0.019	0.231
9404287	A2BURB7	Burbot	A2	22-Sep-94	843	3890	F	13	268.2	170.2	0.006	0.001	0.184
9404288	A2BURB8	Burbot	A2	23-Sep-94	720	1962	F	11	82.5	85.9	0.008	0.012	0.186
9404351	A3BURB1	Burbot	A3	26-Sep-94	424	378	M	7	14.2	14.2	0.023	0.017	0.291
9404352	A3BURB2	Burbot	A3	26-Sep-94	529	744	M	10	12.1	26.4	0.023	0.015	0.289
9404353	A3BURB3	Burbot	A3	26-Sep-94	440	458	M	5	13.7	18.4	0.036	0.016	0.295
9404354	A3BURB4	Burbot	A3	26-Sep-94	395	342	M	5	8.6	8.1	0.039	0.025	0.288
9404355	A3BURB5	Burbot	A3	26-Sep-94	416	323	FI	4	10	1.6	0.027	0.016	0.216
9404356	A3BURB6	Burbot	A3	26-Sep-94	463	519	M	5	20.3	25.3	0.018	0.003	0.292
9404357	A3BURB7	Burbot	A3	26-Sep-94	450	362	M	4	4.4	9.5	0.032	0.013	0.376
9404358	A3BURB8	Burbot	A3	26-Sep-94	515	704	M	10	18.1	26.5	0.032	0.013	0.314
9404359	A3BURB9	Burbot	A3	26-Sep-94	613	1242	F	9	80.5	49.3	0.009	0.004	0.219
9404360	A3BURB10	Burbot	A3	26-Sep-94	536	743	M	11	20.4	26.8	0.027	0.006	0.3
9404361	A3BURB11	Burbot	A3	26-Sep-94	467	508	MI	5	21.4	0.3	0.021	0.013	0.207
9404362	A3BURB12	Burbot	A3	26-Sep-94	462	496	F	6	17.2	10.9	0.024	0.011	0.268
9404363	A3BURB13	Burbot	A3	26-Sep-94	583	1094	F	11	63.2	24.9	0.012	0.016	0.188
9404364	A3BURB14	Burbot	A3	26-Sep-94	590	931	M	11	33.2	32.3	0.017	0.007	0.272

Appendix 1. Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in burbot from the Peace, Athabasca and Slave River drainages, 1994

Lab Number	Field Number	Species	Loc	Capture Date	Length	Weight	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P-450
9404365	A3BURB15	Burbot	A3	26-Sep-94	458	445	FI	5	11.2	2.1	0.026	0.002	0.389
9404366	A3BURB16	Burbot	A3	26-Sep-94	480	594	MI	5	15.4	0.6	0.031	0.007	0.368
9404367	A3BURB17	Burbot	A3	26-Sep-94	487	529	FI	6	11.8	2.6	0.019	0.013	0.233
9404368	A3BURB18	Burbot	A3	26-Sep-94	475	514	M	6	13	24.2	0.047	0.018	0.359
9404369	A3BURB19	Burbot	A3	26-Sep-94	455	493	M	.	16.5	22	0.026	0.013	0.266
9404370	A3BURB20	Burbot	A3	26-Sep-94	514	736	M	7	28.8	32.2	0.02	0.013	0.358
9404371	A3BURB21	Burbot	A3	26-Sep-94	787	2774	M	13	115.2	107.7	0.01	0.007	0.224
9404372	A3BURB22	Burbot	A3	26-Sep-94	459	432	M	6	9.1	14.3	0.031	0.013	0.315
9404661	A4BURB1	Burbot	A4	06-Oct-94	570	982	MI	8	50.7	0.8	0.03	0.021	0.208
9404662	A4BURB2	Burbot	A4	07-Oct-94	558	896	F	12	38.6	17.5	0.014	0.001	0.138
9404663	A4BURB3	Burbot	A4	07-Oct-94	559	896	F	7	48.9	21.9	0.011	0.009	0.211
9404664	A4BURB4	Burbot	A4	07-Oct-94	523	615	MI	4	14.3	0.4	0.034	0.022	0.266
9404665	A4BURB5	Burbot	A4	07-Oct-94	575	1059	FI	8	34.1	3.1	0.033	0.019	0.289
9404666	A4BURB6	Burbot	A4	07-Oct-94	620	1523	M	11	115.7	85	0.014	0.007	0.128
9404667	A4BURB7	Burbot	A4	07-Oct-94	593	1050	FI	7	35.3	5	0.03	0.014	0.261
9404668	A4BURB8	Burbot	A4	07-Oct-94	603	1128	FI	7	49.3	4.6	0.017	0.015	0.309
9404669	A4BURB9	Burbot	A4	07-Oct-94	391	274	MI	4	6.5	.	0.025	0.003	0.312
9404670	A4BURB10	Burbot	A4	07-Oct-94	374	245	FI	5	3.4	0.8	0.046	0.038	0.223
9404674	A4BURB14	Burbot	A4	08-Oct-94	425	356	FI	4	5.7	1.6	0.038	0.025	0.267
9404675	A4BURB15	Burbot	A4	08-Oct-94	645	1213	FI	8	29.9	5	0.031	0.014	0.24
9404861	A5BURB1	Burbot	A5	13-Oct-94	571	1103	M	7	17.5	95.8	0.065	0.076	0.229
9404862	A5BURB2	Burbot	A5	13-Oct-94	457	468	F	5	23.1	30.5	0.01	0.013	0.121
9404863	A5BURB3	Burbot	A5	13-Oct-94	377	260	MI	3	3.2	0.1	0.053	0.068	0.267
9404864	A5BURB4	Burbot	A5	13-Oct-94	567	805	M	7	22.8	49.7	0.05	0.045	0.222
9404865	A5BURB5	Burbot	A5	14-Oct-94	435	912	M	9	19	60.9	0.048	0.037	0.211
9404866	A5BURB6	Burbot	A5	14-Oct-94	410	326	FI	4	5.1	1.6	0.18	0.164	0.42
9404867	A5BURB7	Burbot	A5	14-Oct-94	514	726	M	7	10.7	70.4	0.076	0.092	0.229
9404868	A5BURB8	Burbot	A5	14-Oct-94	513	813	M	5	28.4	64.2	0.089	0.066	0.2
9404869	A5BURB9	Burbot	A5	14-Oct-94	462	399	FI	4	5.2	0.5	0.164	0.174	0.308

Appendix 1. Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in burbot from the Peace, Athabasca and Slave River drainages, 1994

Lab Number	Field Number	Species	Loc	Capture Date	Length	Weight	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P-450
9404870	A5BURB10	Burbot	A5	14-Oct-94	463	530	MI	3	10.7	0.6	0.121	0.134	0.265
9404871	A5BURB11	Burbot	A5	14-Oct-94	443	920	M	6	35.4	82.2	0.061	0.054	0.191
9404872	A5BURB12	Burbot	A5	14-Oct-94	560	945	M	7	19.8	82.4	0.074	0.075	0.2
9404873	A5BURB13	Burbot	A5	14-Oct-94	451	477	M	5	6.8	56	0.049	0.057	0.264
9404874	A5BURB14	Burbot	A5	14-Oct-94	513	758	F	6	26.6	20.6	0.027	0.025	0.151
9404875	A5BURB15	Burbot	A5	14-Oct-94	599	1162	M	1	52.9	81.7	0.049	0.034	0.255
9404876	A5BURB16	Burbot	A5	14-Oct-94	403	373	M	5	15.7	51.4	0.124	0.105	0.342
9404877	A5BURB17	Burbot	A5	14-Oct-94	613	1358	F	7	47.4	72.6	0.01	0.01	0.163
9404911	CWBURB1	Burbot	CW	10-Oct-94	520	688	F	7	25.6	33.5	0.006	0.008	0.12
9404912	CWBURB2	Burbot	CW	10-Oct-94	374	317	F	3	12.1	15.5	0.017	0.014	0.171
9404913	CWBURB3	Burbot	CW	10-Oct-94	423	432	F	6	15.6	22.6	0.017	0.016	0.134
9404914	CWBURB4	Burbot	CW	10-Oct-94	427	404	F	6	13.1	18.3	0.01	0.01	0.177
9404915	CWBURB5	Burbot	CW	12-Oct-94	469	586	F	7	24.2	33.6	0.011	0.015	0.185
9404151	LSR2BURB1	Burbot	LSR2	19-Sep-94	471	590	F	8	13	14.5	0.031	0.023	0.276
9404186	LSR3BURB1	Burbot	LSR3	20-Dec-94	450	508	F	.	.	.	0.009	0.005	0.167
9404551	LSVBURB1	Burbot	LSV	02-Oct-94	460	555	M	9	31.2	34.8	0.03	0.018	0.237
9404552	LSVBURB2	Burbot	LSV	02-Oct-94	549	715	FI	9	18.8	3.1	0.033	0.026	0.248
9404553	LSVBURB3	Burbot	LSV	02-Oct-94	465	566	M	8	33.9	32.8	0.039	0.016	0.297
9404554	LSVBURB4	Burbot	LSV	02-Oct-94	470	538	M	7	17.7	23.7	.	0.016	0.276
9404555	LSVBURB5	Burbot	LSV	02-Oct-94	552	802	FI	10	46.3	5.8	0.011	0.005	0.2
9404557	LSVBURB7	Burbot	LSV	03-Oct-94	446	405	M	6	10.8	21.7	.	0.019	0.276
9404558	LSVBURB8	Burbot	LSV	03-Oct-94	515	718	M	8	46	36	0.018	0.004	0.252
9404559	LSVBURB9	Burbot	LSV	03-Oct-94	621	926	FI	9	33.2	4.3	0.024	0.004	0.313
9404560	LSVBURB10	Burbot	LSV	03-Oct-94	520	631	MI	10	20	0.9	.	0.016	0.252
9404561	LSVBURB11	Burbot	LSV	03-Oct-94	540	800	F	8	44.3	23.9	0.01	0.001	0.173
9404562	LSVBURB12	Burbot	LSV	03-Oct-94	565	793	FI	9	18.3	3.7	.	0.014	0.288
9404563	LSVBURB13	Burbot	LSV	03-Oct-94	629	1226	M	1	50.3	58.4	0.018	0.01	0.202
9404564	LSVBURB14	Burbot	LSV	03-Oct-94	583	970	FI	10	28.7	6.4	0.035	0.018	0.254
9404565	LSVBURB15	Burbot	LSV	03-Oct-94	423	392	F	7	15.5	15.2	0.008	0.009	0.199

Appendix 1. Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in burbot from the Peace, Athabasca and Slave River drainages, 1994

Lab Number	Field Number	Species	Loc	Capture Date	Length	Weight	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P-450
9404566	LSVBURB16	Burbot	LSV	03-Oct-94	387	593	M	1	22.4	27.2	0.022	0.013	0.293
9404567	LSVBURB17	Burbot	LSV	03-Oct-94	312	169	FI	3	3.1	0.5	0.045	0.03	0.367
9404568	LSVBURB18	Burbot	LSV	03-Oct-94	525	835	MI	8	21.5	1.5	0.031	0.009	0.214
9404569	LSVBURB19	Burbot	LSV	03-Oct-94	547	805	M	9	27.2	46.7	0.035	0.007	0.286
9404971	MCR2BURB1	Burbot	MCR2	15-Dec-94	490	702	F	8	37.5	59.8	0.003	0.001	0.131
9404972	MCR2BURB2	Burbot	MCR2	15-Dec-94	452	514	M	9	9.1	78	0.023	0.014	0.204
9404973	MCR2BURB3	Burbot	MCR2	15-Dec-94	481	530	F	7	16.1	48.9	0.004	0.002	0.137
9404974	MCR2BURB4	Burbot	MCR2	16-Dec-94	430	386	F	5	10	34.7	0.004	0.001	0.175
9404975	MCR2BURB5	Burbot	MCR2	16-Dec-94	474	513	FI	7	13.6	5.8	0.015	0.012	0.219
9404976	MCR2BURB6	Burbot	MCR2	16-Dec-94	513	579	F	7	31	67.7	0.004	0.001	0.093
9404977	MCR2BURB7	Burbot	MCR2	16-Dec-94	463	563	M	8	10.7	91.5	0.029	0.02	0.221
9404381	MRBURB1	Burbot	MR		.	.		.	.	.	0.026	0.018	0.225
9404581	PBURB1	Burbot	P	28-Sep-94	380	225	F	5	3.9	8.7	0.027	0.028	0.239
9404582	PBURB2	Burbot	P	28-Sep-94	517	671	F	11	19.4	15.9	0.016	0.006	0.225
9404583	PBURB3	Burbot	P	29-Sep-94	500	490	F	10	11.9	17	0.017	0.011	0.195
9404584	PBURB4	Burbot	P	29-Sep-94	538	806	M	4	11.9	24	0.027	0.008	0.284
9404585	PBURB5	Burbot	P	30-Sep-94	394	290	F	5	4.8	8.7	0.028	0.023	0.182
9404492	PR1BURB2	Burbot	PR1	28-Sep-94	590	1063	F	12	23.7	27	0.011	0.002	0.286
9404493	PR1BURB3	Burbot	PR1	29-Sep-94	645	1468	FI	8	38.7	8.1	0.015	0.003	0.352
9404494	PR1BURB4	Burbot	PR1	29-Sep-94	517	642	FI	7	13.2	5.2	0.025	0.015	0.312
9404495	PR1BURB5	Burbot	PR1	29-Sep-94	411	382	M	6	14.2	13	0.019	0.003	0.36
9404496	PR1BURB6	Burbot	PR1	30-Sep-94	541	734	FI	12	13	5	0.03	0.015	0.291
9404497	PR1BURB7	Burbot	PR1	30-Sep-94	582	1108	FI	8	29	9.1	0.012	0.004	0.241
9404498	PR1BURB8	Burbot	PR1	30-Sep-94	508	557	M	8	14.6	19	0.028	0.011	0.233
9404611	PR2BURB1	Burbot	PR2	02-Oct-94	618	1137	FI	11	17	7	.	0.005	0.296
9404612	PR2BURB2	Burbot	PR2	02-Oct-94	474	507	MI	7	6	1	0.015	0.004	0.274
9404614	PR2BURB4	Burbot	PR2	02-Oct-94	513	604	MI	13	4	2	0.018	0.007	0.216
9404615	PR2BURB5	Burbot	PR2	02-Oct-94	804	3672	M	13	162	167	0.029	0.012	0.321
9404616	PR2BURB6	Burbot	PR2	02-Oct-94	756	2108	F	15	51	46	.	0.004	0.173

Appendix 1. Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in burbot from the Peace, Athabasca and Slave River drainages, 1994

Lab Number	Field Number	Species	Loc	Capture Date	Length	Weight	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P-450
9404617	PR2BURB7	Burbot	PR2	03-Oct-94	643	1452	FI	11	58	9	0.018	0.009	0.248
9404618	PR2BURB8	Burbot	PR2	03-Oct-94	559	827	M	8	22	36	0.04	0.009	0.313
9404619	PR2BURB9	Burbot	PR2	03-Oct-94	580	931	M	7	22	40	0.023	0.011	0.251
9404620	PR2BURB10	Burbot	PR2	05-Oct-94	645	1312	FI	9	16	6	0.014	0.015	0.24
9404712	PR3BURB2	Burbot	PR3	06-Oct-94	635	1242	FI	7	10	5	0.01	0.001	0.207
9404714	PR3BURB4	Burbot	PR3	06-Oct-94	442	454	FI	6	3	2	0.036	0.021	0.25
9404715	PR3BURB5	Burbot	PR3	06-Oct-94	569	859	FI	8	10	6	0.034	0.007	0.253
9404716	PR3BURB6	Burbot	PR3	06-Oct-94	473	452	M	6	4	13	0.02	0.021	0.144
9404718	PR3BURB8	Burbot	PR3	08-Oct-94	398	268	FI	6	2	1	0.008	0.014	0.103
9404719	PR3BURB9	Burbot	PR3	08-Oct-94	570	972	MI	7	8	1	0.034	0.011	0.245
9404011	SR1BURB1	Burbot	SR1	12-Sep-94	444	411	MI	9	17	2	0.01	0.012	0.253
9404012	SR1BURB2	Burbot	SR1	12-Sep-94	495	612	F	8	21	17	0.006	0.001	0.121
9404014	SR1BURB4	Burbot	SR1	13-Sep-94	391	364	M	8	18	5.8	0.018	0.025	0.254
9404015	SR1BURB5	Burbot	SR1	13-Sep-94	502	675	MI	9	17	5.7	0.014	0.011	0.206
9404016	SR1BURB6	Burbot	SR1	13-Sep-94	480	577	F	8	25	17	0.007	0.01	0.206
9404017	SR1BURB7	Burbot	SR1	13-Sep-94	402	306	FI	7	2.4	1.8	0.022	0.015	0.266
9404018	SR1BURB8	Burbot	SR1	13-Sep-94	412	352	M	6	22	2.2	0.016	0.001	0.176
9404019	SR1BURB9	Burbot	SR1	13-Sep-94	441	483	FI	6	11	5.1	0.024	0.018	0.275
9404020	SR1BURB10	Burbot	SR1	13-Sep-94	475	597	MI	10	32	18	0.007	0.013	0.22
9404021	SR1BURB11	Burbot	SR1	13-Sep-94	518	756	FI	9	55	4	0.005	0.001	0.129
9404022	SR1BURB12	Burbot	SR1	13-Sep-94	545	749	M	11	23.4	16.6	0.016	0.018	0.323
9404023	SR1BURB13	Burbot	SR1	13-Sep-94	486	628	M	11	26	29	0.006	0.005	0.132
9404024	SR1BURB14	Burbot	SR1	13-Sep-94	449	479	M	7	16	6.2	0.012	0.002	0.229
9404025	SR1BURB15	Burbot	SR1	13-Sep-94	465	495	F	8	14.8	10	0.009	0.004	0.175
9404026	SR1BURB16	Burbot	SR1	14-Sep-94	352	244	M	4	7	4	0.023	0.013	0.325
9404027	SR1BURB17	Burbot	SR1	14-Sep-94	375	252	MI	4	16	1	0.03	0.046	0.355
9404028	SR1BURB18	Burbot	SR1	14-Sep-94	369	284	MI	4	14	1	0.021	0.007	0.318
9404029	SR1BURB19	Burbot	SR1	16-Sep-94	466	553	M	7	22.4	16	0.021	0.014	0.298
9404853	SR2BURB1	Burbot	SR2	21-Dec-94	456	581	F	.	19.9	76.4	0.009	0.002	0.184

Appendix 1. Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in burbot from the Peace, Athabasca and Slave River drainages, 1994

Lab Number	Field Number	Species	Loc	Capture Date	Length	Weight	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P-450
9404771	SRBURB1	Burbot	SR	14-Oct-94	536	840	M	8	23	54.9	0.027	0.015	0.32
9404772	SRBURB2	Burbot	SR	14-Oct-94	575	1170	F	8	91	39.3	0.008	0.003	0.16
9404773	SRBURB3	Burbot	SR	14-Oct-94	642	1100	F	10	118.4	13	0.006	0.005	0.23
9404774	SRBURB4	Burbot	SR	14-Oct-94	454	465	M	8	44	76.6	0.037	0.014	0.149
9404776	SRBURB6	Burbot	SR	15-Oct-94	454	646	M	8	38.8	66	0.032	0.003	0.19
9404777	SRBURB7	Burbot	SR	15-Oct-94	470	642	M	6	99.1	45.4	0.028	0.001	0.322
9404778	SRBURB8	Burbot	SR	15-Oct-94	463	630	M	8	18.8	42.5	0.024	0.014	0.245
9404779	SRBURB9	Burbot	SR	15-Oct-94	540	1120	F	8	81.3	64.5	0.009	0.01	0.155
9404780	SRBURB10	Burbot	SR	15-Oct-94	598	1740	F	11	176	60.5	0.006	0.004	0.189
9404781	SRBURB11	Burbot	SR	15-Oct-94	495	760	M	7	39.6	56.1	0.019	0.001	0.239
9404782	SRBURB12	Burbot	SR	15-Oct-94	472	650	M	7	43.5	106.9	0.02	0.013	0.29
9404783	SRBURB13	Burbot	SR	15-Oct-94	465	724	F	10	72.4	28.8	0.008	0.005	0.205
9404784	SRBURB14	Burbot	SR	15-Oct-94	455	675	M	8	20.5	69.2	0.03	0.012	0.246
9404785	SRBURB15	Burbot	SR	15-Oct-94	490	699	F	9	42.7	46.3	0.008	0.001	0.209
9404786	SRBURB16	Burbot	SR	15-Oct-94	495	842	M	8	43.3	65.3	0.029	0.012	0.248
9404787	SRBURB17	Burbot	SR	15-Oct-94	482	710	F	7	38.7	43	0.007	0.002	0.164
9404788	SRBURB18	Burbot	SR	15-Oct-94	490	886	F	9	70	36.9	0.016	0.001	0.219
9404790	SRBURB20	Burbot	SR	16-Oct-94	695	2500	F	14	241	111.5	0.006	0.001	0.178
9404791	SRBURB21	Burbot	SR	16-Oct-94	525	936	F	10	101.1	62.6	0.009	0.003	0.159
9404811	WAB1BURB1	Burbot	WAB1	09-Oct-94	381	211		6	4	1	0.047	0.051	0.239
9404812	WAB1BURB2	Burbot	WAB1	10-Oct-94	340	232	FI	4	4	2	0.078	0.077	0.296
9404813	WAB1BURB3	Burbot	WAB1	10-Oct-94	314	135	FI	4	2	2	0.062	0.041	0.296
9404814	WAB1BURB4	Burbot	WAB1	10-Oct-94	395	334	MI	5	12	4	0.045	0.024	0.248
9404815	WAB1BURB5	Burbot	WAB1	10-Oct-94	320	159	FI	5	3	1	0.091	0.061	0.309
9404816	WAB1BURB6	Burbot	WAB1	10-Oct-94	423	381	F	7	14	18	0.021	0.019	0.181
9404818	WAB1BURB8	Burbot	WAB1	11-Oct-94	426	447	M	6	10	34	0.068	0.034	0.296
9404819	WAB1BURB9	Burbot	WAB1	11-Oct-94	380	317	F	5	11	13	0.013	0.007	0.148
9404421	WR1BURB1	Burbot	WR1	23-Sep-94	509	712	F	12	29	15.7	0.013	0.013	0.183
9404422	WR1BURB2	Burbot	WR1	23-Sep-94	560	824	M	11	30.2	55	0.009	0.016	0.14

Appendix 1. Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in burbot from the Peace, Athabasca and Slave River drainages, 1994

Lab Number	Field Number	Species	Loc	Capture Date	Length	Weight	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P-450
9404423	WR1BURB3	Burbot	WR1	25-Sep-94	446	468	M	7	10	27.7	0.039	0.021	0.27
9404424	WR1BURB4	Burbot	WR1	25-Sep-94	628	1277	F	10	55	38.3	0.011	0.01	0.175
9404941	WR2BURB1	Burbot	WR2	18-Oct-94	472	756	M	9	23	126	0.037	0.012	0.233
9404942	WR2BURB2	Burbot	WR2	18-Oct-94	576	1044	F	6	53	44	0.008	0.002	0.187
9404943	WR2BURB3	Burbot	WR2	18-Oct-94	510	758	M	7	29	85	0.041	0.013	0.195
9404944	WR2BURB4	Burbot	WR2	18-Oct-94	322	192	M	2	6	24	0.037	0.018	0.193
9404945	WR2BURB5	Burbot	WR2	18-Oct-94	505	676	F	10	35	26	0.011	0.003	0.151
9404946	WR2BURB6	Burbot	WR2	18-Oct-94	434	491	M	7	11	59	0.029	0.001	0.222
9404947	WR2BURB7	Burbot	WR2	18-Oct-94	549	831	F	8	39	30	0.008	0.001	0.152
9404948	WR2BURB8	Burbot	WR2	18-Oct-94	527	821	F	7	39	33	0.009	0.006	0.165
9404949	WR2BURB9	Burbot	WR2	18-Oct-94	465	491	M	7	10	55	0.037	0.007	0.33
9404950	WR2BURB10	Burbot	WR2	18-Oct-94	472	601	M	8	12	78	0.053	0.012	0.283
9404952	WR2BURB12	Burbot	WR2	18-Oct-94	518	825	M	10	19	94	0.04	0.016	0.243
9404953	WR2BURB13	Burbot	WR2	18-Oct-94	483	641	F	9	25	32	0.007	0.002	0.163

Appendix 2. Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in flathead chub from the Peace and Athabasca River drainages, 1994

Lab Number	Field Number	Species	Loc	Capture Date	Length (mm)	Weight (g)	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P-450
9404041	SR1FLCH1	Flathead	SR1	13-Sep-94	287	230	F	8	1	8	0.005	0.025	0.27
9404042	SR1FLCH2	Flathead	SR1	14-Sep-94	245	156	F	8	1	4	0.024	.	.
9404043	SR1FLCH3	Flathead	SR1	14-Sep-94	300	274	F	8	1.8	5.7	0.003	0.001	0.28
9404044	SR1FLCH4	Flathead	SR1	16-Sep-94	275	206	F	7	4	10	0.028	0.106	0.25
9404045	SR1FLCH5	Flathead	SR1	16-Sep-94	264	191	F	8	1.8	5	0.007	.	.
9404046	SR1FLCH6	Flathead	SR1	16-Sep-94	196	77	F	4	1	1	0.032	.	.
9404048	SR1FLCH8	Flathead	SR1	18-Sep-94	242	136	MI	8	0.6	0.4	0.018	.	.
9404049	SR1FLCH9	Flathead	SR1	18-Sep-94	153	31	FI	3	0.5	1	0.026	.	.
9404050	SR1FLCH10	Flathead	SR1	18-Sep-94	176	51		4	0.2	0	0.042	.	.
9404051	SR1FLCH11	Flathead	SR1	18-Sep-94	200	75	MI	5	0.2	0.3	0.015	.	.
9404052	SR1FLCH12	Flathead	SR1	18-Sep-94	200	79	F	5	0.3	1.5	0.032	.	.
9404161	LSR2FLCH1	Flathead	LSR2	20-Sep-94	165	47	FI	3	1.8	2.9	0.063	.	.
9404431	WR1FLCH1	Flathead	WR1	22-Sep-94	150	26	FI	3	0.3	0.4	0.019	.	.
9404432	WR1FLCH2	Flathead	WR1	25-Sep-94	146	26	MI	3	0.2	0.2	0.018	.	.
9404433	WR1FLCH3	Flathead	WR1	25-Sep-94	158	36	MI	4	0.3	0.2	0.012	.	.
9404434	WR1FLCH4	Flathead	WR1	25-Sep-94	134	22		3	0.3	0.1	0.009	.	.
9404435	WR1FLCH5	Flathead	WR1	25-Sep-94	132	21		3	0.4	0.1	0.029	.	.
9404511	PR1FLCH1	Flathead	PR1	30-Sep-94	151	33.2		2	1.6	.	0.018	.	.
9404632	PR2FLCH2	Flathead	PR2	03-Oct-94	261	164	F	6	1	3	0.028	0.127	0.38
9404633	PR2FLCH3	Flathead	PR2	05-Oct-94	294	285	F	8	3	8	0.016	0.064	0.22
9404821	WAB1FLCH1	Flathead	WAB1	11-Oct-94	191	75	MI	4	4	4	0.017	.	0.39

Appendix 3. Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in longnose suckers from the Peace and Athabasca River drainages, 1994

Lab number	Field number	Species	Loc	Capture date	Length (mm)	Weight (g)	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P450
9404061	SR1LNSC1	longnose	SR1	16-Sep-94	381	664	F	.	8	35	0.01	0.037	0.228
9404062	SR1LNSC2	longnose	SR1	16-Sep-94	393	704	FI	8	2	7	0.031	0.07	0.347
9404063	SR1LNSC3	longnose	SR1	16-Sep-94	296	245	FI	.	1.2	1	0.038	0.074	0.296
9404064	SR1LNSC4	longnose	SR1	18-Sep-94	425	762	FI	.	8	10	0.032	0.065	0.316
9404065	SR1LNSC5	longnose	SR1	17-Sep-94	395	787	M	.	3.8	31	0.023	0.08	0.266
9404066	SR1LNSC6	longnose	SR1	18-Sep-94	410	752	M	.	5.2	30	0.009	0.062	0.221
9404067	SR1LNSC7	longnose	SR1	17-Sep-94	470	1115	F	.	16.2	66	0.008	0.014	0.173
9404068	SR1LNSC8	longnose	SR1	18-Sep-94	487	1200	F	.	9	36	0.006	0.016	0.231
9404069	SR1LNSC9	longnose	SR1	18-Sep-94	415	771	FI	.	5.3	6	0.02	0.036	0.188
9404070	SR1LNSC10	longnose	SR1	18-Sep-94	445	866	F	.	9	46	0.003	0.038	0.225
9404071	SR1LNSC11	longnose	SR1	18-Sep-94	381	638	FI	.	4.6	4	0.035	0.089	0.407
9404072	SR1LNSC12	longnose	SR1	18-Sep-94	376	592	F	.	5.6	24.6	0.009	0.036	0.23
9404171	LSR2LNSC1	longnose	LSR2	19-Sep-94	405	807	F	.	12	42	0.017	0.032	0.195
9404172	LSR2LNSC2	longnose	LSR2	20-Sep-94	432	885	F	.	11	47	0.023	0.039	0.239
9404173	LSR2LNSC3	longnose	LSR2	20-Sep-94	380	623	F	.	11	33	0.01	0.036	0.162
9404175	LSR2LNSC4	longnose	LSR2	20-Sep-94	268	210	FI	.	2.2	1.8	0.11	0.217	0.377
9404176	LSR2LNSC5	longnose	LSR2	20-Sep-94	252	171	FI	.	3.5	2	0.039	0.085	0.377
9404177	LSR2LNSC6	longnose	LSR2	20-Sep-94	196	85	FI	.	1.6	0.8	0.1	0.142	0.288
9404211	A1LNSC1	longnose	A1	11-Sep-94	375	691	M	11	6.7	40	0.11	0.178	0.322
9404313	A2LNSC3	longnose	A2	21-Sep-94	311	332	MI	6	2.8	0.3	0.027	0.073	0.33
9404314	A2LNSC4	longnose	A2	23-Sep-94	355	503	FI	8	6.1	5.7	0.013	.	0.235
9404315	A2LNSC5	longnose	A2	23-Sep-94	434	1013	F	14	10.7	71.6	0.013	0.037	0.326
9404391	MRLNSC1	longnose	MR	15-Sep-94	381	655	M	9	5.7	32.2	0.061	0.105	0.226
9404441	WR1LNSC1	longnose	WR1	22-Sep-94	420	884	F	11	14	74.6	0.008	0.009	0.179
9404442	WR1LNSC2	longnose	WR1	22-Sep-94	341	515	M	7	7.4	21.3	0.06	0.034	0.234
9404443	WR1LNSC3	longnose	WR1	22-Sep-94	378	648	F	.	12.7	37	0.003	0.003	0.14

Appendix 3 (continued) Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in longnose suckers from the Peace and Athabasca River drainages, 1994													
Lab number	Field number	Species	Loc	Capture date	Length (mm)	Weight (g)	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P450
9404444	WR1LNSC4	longnose	WR1	22-Sep-94	363	490	FI	.	5.8	6	0.071	0.117	0.403
9404445	WR1LNSC5	longnose	WR1	22-Sep-94	352	452	FI	.	4.2	3.3	0.039	0.087	0.352
9404446	WR1LNSC6	longnose	WR1	22-Sep-94	412	808	F	8	9.2	53.5	0.003	0.01	0.152
9404447	WR1LNSC7	longnose	WR1	22-Sep-94	418	865	F	.	12.3	59	0.003	0.014	0.135
9404448	WR1LNSC8	longnose	WR1	22-Sep-94	392	653	F	10	8.1	30.3	0.004	0.001	0.163
9404449	WR1LNSC9	longnose	WR1	23-Sep-94	390	580	F	8	9.2	26	0.005	0.015	0.148
9404450	WR1LNSC10	longnose	WR1	23-Sep-94	430	702	F	15	8.1	28.8	0.002	0.003	0.185
9404451	WR1LNSC11	longnose	WR1	23-Sep-94	363	469	M	7	6.1	25.3	0.008	0.025	0.164
9404452	WR1LNSC12	longnose	WR1	23-Sep-94	312	292		5	3.4	0.5	0.015	0.031	0.225
9404453	WR1LNSC13	longnose	WR1	24-Sep-94	475	1165	M	11	10	59.7	0.066	0.145	0.238
9404521	PR1LNSC1	longnose	PR1	27-Sep-94	481	1217	F	11	25.8	52	0.006	0.028	0.2
9404522	PR1LNSC2	longnose	PR1	27-Sep-94	397	759	F	7	11	52.5	0.011	0.033	0.186
9404523	PR1LNSC3	longnose	PR1	27-Sep-94	370	563	FI	7	8.3	3	0.089	0.16	0.359
9404524	PR1LNSC4	longnose	PR1	27-Sep-94	405	755	FI	8	14.2	11	0.086	0.135	0.286
9404525	PR1LNSC5	longnose	PR1	27-Sep-94	422	934	F	10	18	67.5	0.008	0.028	0.221
9404526	PR1LNSC6	longnose	PR1	27-Sep-94	335	458	MI	5	5.9	0.4	0.064	0.133	0.436
9404527	PR1LNSC7	longnose	PR1	27-Sep-94	363	590	FI	7	10	6.1	0.072	0.134	0.425
9404528	PR1LNSC8	longnose	PR1	27-Sep-94	455	1002	F	9	15.6	39	0.007	0.025	0.249
9404529	PR1LNSC9	longnose	PR1	27-Sep-94	402	738	M	8	9	18	0.053	0.106	0.354
9404530	PR1LNSC10	longnose	PR1	27-Sep-94	387	726	FI	8	13	8	0.036	0.049	0.274
9404532	PR1LNSC12	longnose	PR1	27-Sep-94	408	724	M	8	11.4	33	0.068	0.117	0.279
9404533	PR1LNSC13	longnose	PR1	27-Sep-94	414	760	M	9	13	5.9	0.044	0.089	0.261
9404534	PR1LNSC14	longnose	PR1	27-Sep-94	437	916	F	9	18	68.2	0.005	0.012	0.211
9404535	PR1LNSC15	longnose	PR1	29-Sep-94	431	973	F	12	19.8	66	0.012	0.027	0.213
9404536	PR1LNSC16	longnose	PR1	29-Sep-94	407	739	FI	9	10	1.2	0.056	0.114	0.329
9404537	PR1LNSC17	longnose	PR1	29-Sep-94	415	905	F	9	14.6	53.6	0.016	0.001	0.203
9404641	PR2LNSC1	longnose	PR2	02-Oct-94	476	1304	FI	10	20	17	0.041	0.061	0.187

Appendix 3 (continued) Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in longnose suckers from the Peace and Athabasca River drainages, 1994

Lab number	Field number	Species	Loc	Capture date	Length (mm)	Weight (g)	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P450
9404642	PR2LNSC2	longnose	PR2	02-Oct-94	462	1246	F	10	21	60	0.011	0.011	0.171
9404643	PR2LNSC3	longnose	PR2	02-Oct-94	446	1107	F	9	18	75	0.012	0.035	0.231
9404644	PR2LNSC4	longnose	PR2	02-Oct-94	393	634	FI	8	7	3	0.063	0.104	0.36
9404645	PR2LNSC5	longnose	PR2	02-Oct-94	474	1202	FI	13	17	14	0.03	0.038	0.199
9404647	PR2LNSC7	longnose	PR2	05-Oct-94	401	714	M	8	8	31	0.22	0.228	0.363
9404648	PR2LNSC8	longnose	PR2	05-Oct-94	399	697	FI	8	9	3	0.176	0.222	0.4
9404731	PR3LNSC1	longnose	PR3	06-Oct-94	458	1221	M	10	22	55	0.029	0.064	0.208
9404732	PR3LNSC2	longnose	PR3	06-Oct-94	453	1106	F	11	18	45	0.001	0.001	0.001
9404733	PR3LNSC3	longnose	PR3	06-Oct-94	445	1067	F	10	19	72	0.007	0.025	0.182
9404734	PR3LNSC4	longnose	PR3	06-Oct-94	416	954	M	13	14	39	0.056	0.113	0.291
9404735	PR3LNSC5	longnose	PR3	06-Oct-94	468	1131	F	15	22	71	0.013	0.039	0.175
9404736	PR3LNSC6	longnose	PR3	06-Oct-94	485	1252	FI	15	19	11	0.019	0.052	0.244
9404737	PR3LNSC7	longnose	PR3	06-Oct-94	362	587	MI	7	12	1	0.026	0.102	0.279
9404738	PR3LNSC8	longnose	PR3	06-Oct-94	463	1430	F	10	31	85	0.013	0.029	0.218
9404739	PR3LNSC9	longnose	PR3	06-Oct-94	411	943	F	10	17	70	0.006	0.024	0.159
9404740	PR3LNSC10	longnose	PR3	06-Oct-94	446	1124	FI	13	16	10	0.024	0.07	0.2
9404741	PR3LNSC11	longnose	PR3	06-Oct-94	378	650	M	10	8	30	0.078	0.176	0.289
9404742	PR3LNSC12	longnose	PR3	07-Oct-94	411	725	M	9	9	26	0.019	0.064	0.173
9404743	PR3LNSC13	longnose	PR3	07-Oct-94	458	1304	F	14	29	79	0.008	0.02	0.184
9404744	PR3LNSC14	longnose	PR3	07-Oct-94	450	915	FI	12	18	8	0.009	0.033	0.215
9404745	PR3LNSC15	longnose	PR3	07-Oct-94	452	1066	F	12	25	76	0.006	0.024	0.163
9404746	PR3LNSC16	longnose	PR3	07-Oct-94	410	871	MI	10	15	2	0.023	0.054	0.205
9404747	PR3LNSC17	longnose	PR3	07-Oct-94	441	1016	MI	10	19	4	0.031	0.096	0.251
9404748	PR3LNSC18	longnose	PR3	07-Oct-94	500	1433	FI	12	24	16	0.022	0.07	0.233
9404749	PR3LNSC19	longnose	PR3	07-Oct-94	446	1154	M	9	19	39	0.078	0.134	0.291
9404750	PR3LNSC20	longnose	PR3	07-Oct-94	435	1198	FI	10	29	11	0.038	0.084	0.246
9404751	PR3LNSC21	longnose	PR3	07-Oct-94	332	426		.	.	.	0.008	0.027	0.174

Appendix 3 (continued) Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in longnose suckers from the Peace and Athabasca River drainages, 1994

Lab number	Field number	Species	Loc	Capture date	Length (mm)	Weight (g)	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P450
9404753	PR3LNSC23	longnose	PR3	07-Oct-94	340	431		6	6	1	0.037	0.096	0.262
9404851	SR2LNSC1	longnose	SR2	15-Oct-94	402	746	F	17	12	64	0.007	0.014	0.182

Appendix 4. Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in northern pike from the Peace, Athabasca and Slave River drainages, 1994

Lab number	Field number	Species	Loc	Capture date	Length (mm)	Weight (g)	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P450
9404001	JV2NRPK1	nr pike	JV2	18-Oct-94	562	1820	M	4	9.6	17.5	0.175	0.154	0.379
9404002	JV2NRPK2	nr pike	JV2	18-Oct-94	625	1660	F	5	21.3	45	0.016	0.016	0.232
9404003	JV2NRPK3	nr pike	JV2	18-Oct-94	705	1975	FI	8	25.3	19.3	0.173	0.149	0.292
9404004	JV3NRPK4	nr pike	JV3	18-Oct-94	580	1536	M	4	30.7	25.7	0.188	0.155	0.369
9404005	JV2NRPK5	nr pike	JV2	18-Oct-94	535	1190	F	4	18.5	43.2	0.022	0.024	0.256
9404081	SR1NRPK1	nr pike	SR1	12-Sep-94	610	.	F	5	22	25	0.009	0.02	0.263
9404082	SR1NRPK1	nr pike	SR1	13-Sep-94	490	740	M	4	3.3	21.6	0.042	0.073	0.223
9404083	SR1NRPK3	nr pike	SR1	18-Sep-94	460	733	F	3	8	13	0.017	0.026	0.255
9404231	A1NRPK1	nr pike	A1	12-Sep-94	489	773	M	4	7.6	12.1	0.058	0.068	0.373
9404232	A1NRPK2	nr pike	A1	12-Sep-94	498	830	FI	3	9.8	9.2	0.066	0.071	0.31
9404234	A1NRPK4	nr pike	A1	14-Sep-94	568	1273	M	4	10.8	24.9	0.014	0.012	0.183
9404401	MRNRPK1	nr pike	MR	17-Sep-94	370	322	FI	2	1.5	1.1	0.056	0.068	0.327
9404402	MRNRPK2	nr pike	MR	17-Sep-94	494	785	M	3	3.9	15.8	0.079	0.105	0.322
9404403	MRNRPK3	nr pike	MR	18-Sep-94	393	350	FI	2	2.5	1.3	0.098	0.116	0.3
9404404	MRNRPK4	nr pike	MR	18-Sep-94	429	474	FI	3	2.8	2.4	0.069	0.093	0.33
9404461	WR1NRPK1	nr pike	WR1	21-Sep-94	345	282	M	3	4	3.6	0.042	0.055	0.207
9404462	WR1NRPK2	nr pike	WR1	21-Sep-94	542	1095	M	4	15	68	0.038	0.05	0.295
9404463	WR1NRPK3	nr pike	WR1	22-Sep-94	475	720	M	4	5.4	22	0.06	0.072	0.456
9404464	WR1NRPK4	nr pike	WR1	24-Sep-94	710	3431	F	6	68.2	130.6	0.003	0.001	0.264
9404465	WR1NRPK5	nr pike	WR1	24-Sep-94	356	283	M	3	2.3	4.5	0.024	0.023	0.297
9404466	WR1NRPK6	nr pike	WR1	24-Sep-94	464	681	M	3	5.4	12	0.025	0.034	0.399
9404467	WR1NRPK7	nr pike	WR1	24-Sep-94	341	272	FI	2	3.1	2.4	0.019	0.014	0.313
9404468	WR1NRPK8	nr pike	WR1	24-Sep-94	353	299	FI	2	2.2	2	0.013	0.013	0.343
9404469	WR1NRPK9	nr pike	WR1	25-Sep-94	586	1187	F	4	17.7	30.1	0.014	0.024	0.289
9404470	WR1NRPK10	nr pike	WR1	25-Sep-94	543	1085	M	4	12.2	18.6	0.059	0.241	0.427
9404471	WR1NRPK11	nr pike	WR1	25-Sep-94	357	262	FI	2	2.5	1.6	0.011	0.018	0.367

Appendix 4. (continued)Liver mixed function oxygenase activities (EROD and AHH) (nmol/mg protein/min at 25C) and cytochrome P-450 (nmol/mg protein) in northern pike from the Peace, Athabasca and Slave River drainages, 1994

Lab number	Field number	Species	Loc	Capture date	Length (mm)	Weight (g)	Sex	Age	Liver weight	Gonad weight	EROD	AHH	P450
9404472	WR1NRPK12	nr pike	WR1	25-Sep-94	290	128	M	2	2	2.3	0.003	0.005	0.234
9404541	PR1NRPK1	nr pike	PR1	27-Sep-94	504	923	M	6	15	18	0.032	0.027	0.301
9404542	PR1NRPK2	nr pike	PR1	27-Sep-94	446	611	F	3	8.3	6.5	0.016	0.014	0.325
9404543	PR1NRPK3	nr pike	PR1	27-Sep-94	590	1678	M	5	31.3	40.9	0.079	0.075	0.334
9404544	PR1NRPK4	nr pike	PR1	29-Sep-94	264	154		2	1.6	1	0.018	0.031	0.227
9404651	PR2NRPK1	nr pike	PR2	02-Oct-94	565	1307	F	4	17	32	0.008	0.012	0.261
9404652	PR2NRPK2	nr pike	PR2	02-Oct-94	441	543	M	4	6	9	0.053	0.046	0.263
9404653	PR2NRPK3	nr pike	PR2	02-Oct-94	264	112		2	1	1	0.016	0.02	0.178
9404802	SRNRPK2	nr pike	SR	16-Oct-94	540	913	F	3	16.1	21.1	0.026	0.03	0.24
9404803	SRNRPK3	nr pike	SR	16-Oct-94	870	.	F	.	153.8	49.5	0.01	0.01	0.243
9404962	WR2NRPK2	nr pike	WR2	19-Oct-94	512	933	F	4	16	31	0.01	0.008	0.274

NORTHERN RIVER BASINS STUDY

APPENDIX 5 - TERMS OF REFERENCE

Project 3144-D2: 1994 Fall Basin-Wide Burbot Collection - Liver Mixed Function Oxygenase Analyses

I. BACKGROUND & OBJECTIVES

Mixed function oxygenases (MFOs) are a family of membrane-bound, detoxification enzymes found in the liver which increase the water solubility of aromatic and lipophilic compounds. Natural substrates for some MFOs include endogenous compounds such as steroid hormones and fatty acids, and others aid in drug metabolism and the breakdown and excretion of other exogenous compounds. MFO activity includes various reactions which add molecular oxygen to lipophilic compounds. The terminal oxidase enzyme of the MFO system is the iron-containing hemoprotein cytochrome P-450. One group of cytochrome P-450s, called P-450IA (usually measured as ethoxyresorufin-O-deethylase (EROD) and arylhydrocarbon hydrolase (AHH) activity), is "induced" by the presence of several foreign compounds. That is, in the presence of these foreign compounds, animals synthesize new amounts of P-450IA proteins and enzyme activity is measurably increased. Induction is initiated when a foreign compound binds to the cellular Aryl hydrocarbon (Ah) receptor. Binding triggers the expression of the gene coding for P-450IA leading to increased RNA transcription and eventual synthesis of new P-450IA protein.

Experimental treatments with pure compounds have established that some polynuclear aromatic hydrocarbons and some chlorinated aromatic hydrocarbons induce, and turpenoid hydrocarbons possibly induce liver P-450IA in several fish species. The ability to induce MFO activity appears to be related to molecular shape, i.e., the co-planarity of connected aromatic rings and the distribution of substituents such as chlorine atoms. Complex mixtures such as Aroclors, petroleum oils and pulp mill effluents also have inducing properties, probably because these mixtures contain specific inducers.

The biological significance of P-450IA induction is not completely known. Induction is an adaptive response and can result in the metabolism and excretion of exogenous substrates. Studies demonstrating increases in P-450IA activity in fish, birds and mammals have also documented changes in performance, including altered steroid hormone profiles, changes in thyroxine and vitamin A metabolism, impairment of reproductive and immune system, and an increased prevalence of diseases. However, there has not yet been a demonstration of causal links between altered P-450IA activity and all of the other biochemical responses.

The simultaneous occurrence of adverse effects and MFO induction indicates that measures of MFO induction justify further studies of biological impacts. MFO induction is one of the easiest and most sensitive responses to detect and has therefore been adopted in a wide range of environmental monitoring programs. If induction is not detected, then presumably other Ah-associated biological

responses are also not occurring. If induction is detected, more detailed studies are indicated on the bioaccumulation of inducers by local fish populations and on their survival, growth and reproduction. It must be recognized, however, that a lack of induction does not mean "no effect" - other effects may be produced by biochemical actions independent of the Ah receptor. Measurement of MFO induction signals only an increased probability of a suite of associated responses.

The aquatic fauna of the northern river basins are exposed to BKME and other types of municipal and industrial effluents. In the spring and fall of 1992 the Northern River Basins Study (NRBS) collected four fish species from six sites upstream, near and downstream from the bleached kraft mill located at Hinton on the Athabasca River (Barton *et al.* 1992a&b). These fish were analyzed for MFO induction. The results of these analyses were somewhat inconclusive, however, mountain whitefish from sites downstream of Hinton showed small increases in liver microsomal enzyme activity relative to fish collected from upstream sites (Lockhart *et al.* 1993). Swanson (1993) also reported elevated EROD activity in mountain whitefish and longnose suckers exposed to pulp mill effluents in the Wapiti/Smoky river system.

In September and October 1994 the NRBS initiated a basin-wide fish collection to further determine the effects of pulp mill and other effluents on fish populations in the Peace, Athabasca and Slave river basins. Results from the study will also be used to develop a long-term environmental monitoring program. The collection and sampling protocols for the project were designed to allow biochemical, physiological, contaminant and histological analyses to be performed on the fish. Because of its wide-ranging distribution and relatively sedentary behaviour, burbot were targeted for collection and analyses. However, provisions were also made for the collection of longnose sucker, flathead chub and northern pike for a broad suite of analyses.

The purpose of this project is to conduct MFO analyses on liver samples from burbot, flathead chub, northern pike and longnose sucker collected during the fall of 1994.

II. GENERAL REQUIREMENTS

1. Various sample sizes of fish species were collected at a number of different sites in the fall of 1994. The contractor is to conduct hepatic MFO analyses on all burbot, northern pike, longnose sucker and flathead chub submitted from each collection site. The contractor is to contact Dr. Don Metner regarding the location, disposition and number of liver samples for each of the four fish species.

2. Liver samples, stored and transported at -60°C have been supplied to Don Metner by EnviResource Consulting Limited, Calgary. The contractor 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 (Hodson *et al.* 1991). 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 (Lockhart *et al.* 1983) and Slave (L. Lockhart, unpublished data) rivers.

3. The contractor will record all information supplied with each liver sample and code laboratory record numbers with NRBS sample numbers (see Boag in prep.) so that the enzyme analyses can be compared with other data generated on the same fish.

4. The contractor will report the mean activity of the 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. Digital spectral data will be retained by the laboratory, but will be available upon request to the NRBS.

5. Details of all calculations will be retained by the laboratory, but will be made available to the NRBS upon request.

III. ANALYTICAL REQUIREMENTS

Hepatic MFO analyses carried out under this contract are to conform to the methods outlined in Lockhart *et al.* (1993). Specifically, the methodology is as follows:

Preparation of Microsomes

Pieces are to be cut from the frozen liver and allowed to thaw partially at 2-4°C until they can be cut with scissors. The small pieces of liver are then to be suspended in cold 0.02M HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid)/0.15M KCl (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 12,000 x g at 2°C for 20 minutes; the supernatant is further centrifuged at 105,000 x g at 2°C for 75 minutes to obtain a microsomal pellet. The pellet is then washed and resuspended in Tris/ glycerol buffer to a protein concentration between 5 and 10 mg/L. Actual protein concentration in each microsomal suspensions is to be measured by the method of Lowry *et al.*. (1951) as modified by Markwell *et al.*. (1981) using a bovine serum albumin as a standard. The microsomes are then to be resuspended in a buffer consisting of 0.05 M tris(hydroxymethyl)methylamine (tris), 1 mM dithiothreitol, 1 mM ethylenediamine tetraacetic acid (EDTA), and 20% glycerol (υ/υ) adjusted to pH 7.4. Resuspended microsomes are then to be frozen and stored in liquid nitrogen until analysis (Stegment *et al.* 1981).

Analysis of Microsomes for AHH Activity

The AHH assay is to be based on the methods 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) is to be 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; sodium isocitrate 10 µL, 154.86 mg/mL; isocitrate dehydrogenase, q.s. 1 U per incubation tube) and tritiated benzo(*a*)pyrene (20 µL, 15-20 µCi μM^{-1} in acetone, 1.135 to 1.140 mg/mL) for 30 minutes at 25°C. The reaction is to be stopped by adding 2 ml 0.15 M potassium hydroxide (KOH) in 85% dimethyl sulfoxide (DMSO). Unreacted benzo(*a*)pyrene is to be extracted with two washes, each with 3 ml hexane. Radioactivity (benzo(*a*)pyrene metabolites) is to be counted in a 200-µL aliquot 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. When amounts of tissue permit, triplicate sample incubations are to be conducted for each microsomal suspension, and in each case triplicate blank incubations to which KOH-DMSO has been added before the addition of substrate. Radioactivity remaining in the aqueous phase in the blanks is to be subtracted from that in the active preparations for calculation of activity as nanomoles of product per mg of microsomal protein per minute.

Analysis of Microsomes for EROD Activity

Ethoxyresorufin-O-deethylase is to be measured by the deethylation of 7-ethoxyresorufin to yield resorufin which is to be detected by the fluorometric procedure described by Pohl and Fouts (1980). The reaction mixture is to be 1100 µL of HEPES buffer (0.1 M, pH 7.8), 10 µL of magnesium sulfate (154 mg/mL), 10 µL NADP (98.4 mg/mL), isocitrate dehydrogenase (q.s. 1 U per incubation tube), and 50 µL bovine serum albumin (40 mg/ mL), all mixed in a Corex centrifuge tube where the mixture has been incubated at room temperature for at least 10 minutes. The microsomal suspension (50 µL) is to be added and the reaction is initiated by the addition of 10 µL ethoxyresorufin (0.03 mg/mL in DMSO); tubes are to be incubated for an accurately timed period of two minutes at 25°C. Then the reaction is to be stopped by the addition of 2.5 mL methanol. The samples are then to be centrifuged at 3500 x g for 15 minutes to remove precipitated protein and the resorufin in the supernatant is to be measured with a spectrofluorometer at an excitation wavelength of 530 nm and an emission wavelength of 585 nm. Standards are to 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 before the addition of substrate are to be run for each sample, and the final result corrected for any non-enzymatic production of resorufin. When the amount of tissue allows, triplicate incubation mixtures of each sample are to be run, along with triplicate blanks for each sample. Results are to be 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 to be based on methods described by Omura and Sato (1964a&b). Estabrooke *et al.*. (1972), and Johannesen and DePierre (1978) have described some of the problems with this assay. The microsome suspension is to be diluted to a concentration of 1 mg/mL 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 then bubbled

for a minimum of 20 seconds with carbon monoxide gas to saturate the sample with CO. The absorbance (which is stored as the background reference for the instrument - the instrument supplies its own background for the reference) of the carbon monoxide-saturated microsomes is then to be recorded from 400 to 500 nm using a single beam spectrophotometer. Several milligrams of dithionite are to be added to the cuvette to reduce the cytochrome P-450 and the absorbance spectrum is to be recorded again, using the carbon monoxide spectrum as the reference. The absorbance readings for the difference spectrum at 450 and 475 nm are to be 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) and the appropriate protein concentration. Analyses are to be done in triplicate.

IV. REPORTING REQUIREMENTS

1. Prepare a comprehensive report outlining the results of the hepatic MFO analyses carried out under this contract. To the extent possible, the results should also be discussed in relation to the possible effects of industrial and municipal effluents on the health of the fish populations examined.

   The report is to indicate that the details pertaining to the collection of fish analyzed under this contract are outlined in Boag (in prep.). The report is also to include a table or appendix outlining the results of EROD, AHH and Cytochrome P-450 analyses for each fish. Sample numbers indicated in the report are to conform to those outlined in Boag (in prep.).

2. Ten copies of the draft report plus an electronic disk copy are to be submitted to the Component Coordinator by **March 31, 1995**.

3. Three weeks after the receipt of review comments on the draft report, the Contractor is to provide the Component Coordinator with two unbound, camera ready copies and ten cerlox bound copies of the final report along with an electronic version.

4. The Contractor is to provide draft and final reports in the style and format outlined in the NRBS document, "A Guide for the Preparation of Reports," which will be supplied upon execution of the contract.

   The final report is to include the following: an acknowledgement section that indicates any local involvement in the project, Report Summary, Table of Contents, List of Tables, List of Figures and an Appendix with the Terms of Reference for this project.

   Text for the report should be set up in the following format:

   a) Times Roman 12 point (Pro) or Times New Roman (WPWIN60) font.
   b) Margins; are 1" at top and bottom, 7/8" on left and right.
   c) Headings; in the report body are labelled with hierarchical decimal Arabic numbers.

d) Text; is presented with full justification; that is, the text aligns on both left and right margins.

e) Page numbers; are Arabic numerals for the body of the report, centred at the bottom of each page and bold.

- If photographs are to be included in the report text they should be high contrast black and white.
- All tables and figures in the report should be clearly reproducible by a black and white photocopier.
- Along with copies of the final report, the Contractor is to supply an electronic version of the report in Word Perfect 5.1 or Word Perfect for Windows Version 6.0 format.
- Electronic copies of tables, figures and data appendices in the report are also to be submitted to the Project Liaison Officer along with the final report. These should be submitted in a spreadsheet (Quattro Pro preferred, but also Excel or Lotus) or database (dBase IV) format. Where appropriate, data in tables, figures and appendices should be geo-referenced.

5. All figures and maps are to be delivered in both hard copy (paper) and digital formats. Acceptable formats include: DXF, uncompressed Eøø, VEC/VEH, Atlas and ISIF. All digital maps must be properly geo-referenced.

6. All sampling locations presented in report and electronic format should be geo-referenced. This is to include decimal latitudes and longitudes (to six decimal places) and UTM coordinates. The first field for decimal latitudes / longitudes should be latitudes (10 spaces wide). The second field should be longitude (11 spaces wide).

7. A presentation package of 35 mm slides is to comprise of one original and four duplicates of each slide.

IV. DELIVERABLES

1. A data interpretation report that that includes the methods and results for the hepatic MFO analyses on NRBS fish samples collected in the fall of 1994.

2. Ten to twenty-five 35 mm slides that can be used at public meetings to summarize the project, methods and key findings.

VI. CONTRACT ADMINISTRATION

This contract is being conducted under the Contaminants Component of the NRBS. The Contaminants Component leader is:

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The Component Coordinator for this contract is:

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VII. LITERATURE CITED

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