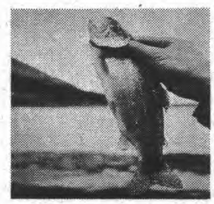
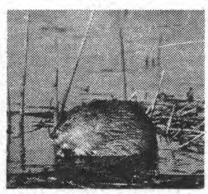
Canada Alberta Northwest Territories

Northern River Basins Study





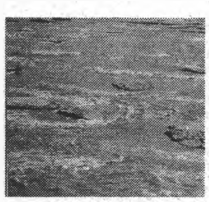


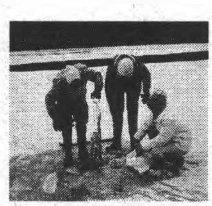




NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 93

CONCENTRATIONS OF METALLOTHIONEIN IN FISH, PEACE, ATHABASCA AND SLAVE RIVER BASINS, SEPTEMBER TO DECEMBER, 1994






DATE DUE

Prepared for the
Northern River Basins Study
under Project 3144-D5

by

J. F. Klaverkamp and C. L. Baron,
Department of Fisheries and Oceans, Freshwater Institute

NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 93

CONCENTRATIONS OF METALLOTHIONEIN IN FISH, PEACE, ATHABASCA AND SLAVE RIVER BASINS, SEPTEMBER TO DECEMBER, 1994

Published by the
Northern River Basins Study
Edmonton, Alberta
February, 1996

CANADIAN CATALOGUING IN PUBLICATION DATA

Klaverkamp, J. F.

Concentrations of metallothionein in fish, Peace, Athabasca
and Slave River Basins, September to December, 1994

(Northern River Basins Study project report,
ISSN 1192-3571 ; no. 93)
Includes bibliographical references.
ISBN 0-662-24590-3
Cat. no. R71-49/3-93E

1. Fishes -- Effect of water pollution on -- Alberta --
Athabasca River Watershed.
2. Fishes -- Effect of water pollution on -- Peace River
Watershed (B.C. and Alta.)
3. Fishes -- Effect of water pollution -- Slave River
Watershed (Alta. And N.W.T.)
4. Wood-pulp industry -- Waste disposal -- Environmental
aspects -- Alberta-- Athabasca River Watershed.
5. Wood-pulp industry -- Waste disposal -- Environmental
aspects -- Peace River Watershed (B.C. and Alta.)
6. Wood-pulp industry -- Waste disposal -- Environmental
aspects (Alta. and N.W.T.)
I. Baron, C. L.
II. Northern River Basins Study (Canada)
III. Title.
IV. Series.

QL626.K52 1996 597'.024'0971231 C96-980188-2

PREFACE:

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

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

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

NORTHERN RIVER BASINS STUDY
PROJECT REPORT RELEASE FORM

This publication may be cited as:

Klaverkamp, J. F. and Baron, C. L. 1996. ***Northern River Basins Study Project Report No. 93, Concentrations of Metallothionein in Fish, Peace, Athabasca and Slave River Basins, September to December, 1994,*** **Northern River Basins Study, Edmonton, Alberta.**

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.

F. J. Wrona — 14 Feb 96

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

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

P. A. Larkin — Feb 14/96

(Dr. P. A. Larkin, Ph.D., Chair) — (Date)

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 — 23 Feb /96

(Lucille Partington, Co-chair) — (Date)

Robert McLeod — 23 Feb/96

(Robert McLeod, Co-chair) — (Date)

CONCENTRATIONS OF METALLOTHIONEIN 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 contaminants from pulp mill effluent, and other types of industrial and municipal effluents, as well as atmospheric sources. Preliminary surveys by the Northern River Basins Study have detected physiological stress and external abnormalities in fish downstream of effluent sources in the upper Athabasca River. An analysis of metallothioneins would be useful in understanding whether metals or organic contaminants are responsible. Metallothioneins are a family of proteins that can occur in a variety of animal organs and tissues. Synthesis of these proteins is induced by exposure to heavy metals such as zinc, copper, mercury and cadmium. Metallothionein induction is correlated with metal toxicity in fish, and has been proposed as a promising biochemical indicator of heavy metal exposure in fish. In 1994, NRBS initiated a basin-wide program to collect and analyze fish for a variety of biochemical and morphological parameters including contaminants, liver enzymes, vitamins A and E, sex steroid hormones, gonad morphology, gross pathology and metallothioneins.

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 metallothionein concentrations in fish collected from the Peace, Athabasca and Slave rivers and their major tributaries. Metallothionein levels were obtained for the kidney, liver, intestine and gill of each fish collected. Metallothionein concentrations in gill and intestine can be useful in differentiating whether accumulation of metals is directly via water, versus from sediments and subsequent accumulation through the food chain.

Results from a total of 187 burbot, 79 longnose sucker, 34 northern pike and 20 flathead chub were incorporated into a portion or all of these analyses. Results from the collection sites were organized into reference (upstream locations and tributaries receiving no inputs form pulp mills), near-field (<100 km downstream of a pulp mill source) and far-field (>100 km downstream of a pulp mill source). Metallothionein concentrations in tissues of both burbot and longnose suckers tended to be higher at far-field locations than near-field or reference areas. Kidney in burbot collected from the Slave River Delta recorded the highest metallothionein levels, ranging from 7-times to 26-times higher than levels found in burbot from other sites. These same fish also had the highest observed metallothionein concentrations in gill tissue, compared with other sites or fish species. Metallothionein levels in burbot kidney showed a small but progressive increase from upstream to downstream sites in both the Peace and Athabasca rivers and their tributaries. Metallothionein concentrations were generally higher in gill of burbot and in gill and kidney of northern pike collected from the Pembina River, when compared with the majority of mainstem sites.

Results from this project indicate that metallothionein levels in fish were not significantly related to pulp mill discharges, suggesting a number of heavy metal sources. This project is one component of a study representing a large-scale effort to simultaneously evaluate contaminants levels, reproductive parameters and possible physiological effects of potential contaminant exposure. 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

Fish from Peace, Athabasca and Slave rivers and their tributaries are exposed to a variety of pulp mill, municipal and industrial effluents (EnviResource 1995; Brown and Vandenbyllaardt, 1996). Assessments of effects of contaminants have focussed on chlorinated organic compounds, such as dioxins and furans (Pastershank and Muir, 1995), and on alterations of parameters affecting reproduction physiology in individual fish (Brown et al., 1993; Brown et al., 1996; Lockhart et al., 1996). These studies have demonstrated that there is exposure to organic contaminants because mixed function oxidase activities are elevated (Lockhart, et al., 1996; Lockhart and Metner, 1996); and that fish collected downstream from the pulp mills may be stressed, because they exhibit a high percentage of sexually immature individuals, and they have depressed circulating concentrations of gonadal steroid hormones (Brown et al., 1993; Brown et al., 1996).

The purpose of the research described in this report was to initiate studies to see if metals may be contibuting to these stresses. The objective was to evaluate whether the metal-binding protein, metallothionein, was elevated in organs of burbot, longnose sucker, northern pike or flathead chub collected downstream from pulp mills and other effluent discharge points, and whether there was evidence of cumulative impacts with progression downstream in these rivers. An increase in MT concentrations in fish represents a molecular response that generally indicates exposure and development of resistance to toxicity to metals, especially Cd, Cu, Hg and Zn (Klaverkamp et al. 1991; Roesijadi, 1992). The study was designed by the Northern River Basins Study Science Directors and the Contaminants Component Leader, and was based on selecting fish collection sites on their proximity to discharges from pulp mills. Additional information on fish collection sites and on general biological parameters of fish collected in 1994 is presented in other reports (EnviResource 1995; Brown et al. 1996).

Two observations were made, both in burbot, which may indicate exposure to elevated metal concentrations and the presence of cumulative impacts. First, the greatest difference in MT concentrations between collection sites was observed in kidney of burbot collected in the Slave River Delta (SRD) of Great Slave lake. MT concentrations in kidneys from these fish ranged from approximately 7-times to 26-times higher than those concentrations found in kidneys of burbot from other collection sites. MT concentrations in gill of burbot from SRD were also the highest observed. The SRD burbot may be exposed to metals due to natural conditions of high mineralization in the Great Slave Lake Delta or other parts of the lake; or these fish may be exposed to metals discharged by mining operations, such as the decommissioned lead-zinc mine at Pine Point. The counterclockwise current in this portion of the lake could transport metals from a western source, such as Pine Point, to the Slave Delta (English, 1984). Second, a progressive increase in MT concentration in proceeding from upstream fish collection sites to downstream sites was observed in concentrations of MT in burbot liver. In the Peace River and associated tributaries (Little Smoky, Smoky, and Wapiti), there is a progressive increase of up to 3.34-fold in burbot liver [MT] moving from upstream to downstream collection sites. In the upper Athabasca River system, there is a progressive increase of up to 2.33-fold in burbot liver [MT] moving from upstream to downstream collection sites.

In other cases, fish from some of the tributaries, especially the Pembina River, had elevated [MT] relative to fish collected from other sampling locations. For example, [MT] were generally higher in gill of burbot and in kidney and gill of northern pike collected from the Pembina River. Therefore, due to the overall variability of MT results between collection sites, especially the relatively high MT concentrations found in fish from some of the tributaries, and the lower MT concentrations found in burbot from sites in the lower Athabasca river, conclusions can not be made that pulp mill effluents are causing elevated MT concentrations.

Recommendations are provided for additional research to assist in understanding the cause of the high MT concentrations observed in kidney and gill of burbot from the SRD; and in verifying whether cumulative impacts are occurring in burbot and whether they are due to metals.

ACKNOWLEDGMENTS

The authors wish to thank R. Chabaylo, K. Crutchfield, P. Larkin, S. Jordan and D. Schindler for providing valuble comments on an earlier draft. We would also like to thank Marc Amaral for his analytical work.

TABLE OF CONTENTS

List of Tables

List of Figures

<u>List of Figures</u>

1.0 INTRODUCTION

Metallothioneins (MT) are a family of widely-distributed, metal-binding proteins that are characterized by low molecular weight, high cysteine concentration, absence of aromatic amino acids and histidine, and high content of Group IB and IIB metals, especially Cu and Zn (Hamer 1986; Kagi and Kojima 1987). Synthesis of MT is induced to greatest degree by exposure to heavy metals, including Cu, Zn, Cd, Hg, and Ag; and to a lesser degree by hormones and organic contaminants (Olsson et al. 1987; Overnell et al. 1987; Hyllner et al. 1989; Waalkes and Goering 1990; Steadman et al. 1991). MT functions are thought to include the regulation of the essential metals, Cu and Zn, the scavenging of free-radicals, and the development of tolerance to the toxicity of heavy metals (Cousins 1985; Halliwell and Gutteridge 1989; Lohrer and Robson 1989; Bremner and Beattie 1990; Sato and Bremner, 1993; Muller et al. 1994).

Increases in MT concentrations are associated with the development of acclimation to metal toxicity in fish (Klaverkamp et al. 1984; Klaverkamp et al. 1991; Roesijadi, 1992). The use of MT as a sensitive bio-indicator for detecting early, toxicity-acclimation responses to heavy metals in fish and large, benthic invertebrates (Hamilton and Mehrle, 1986; Haux and Forlin, 1988; Roesijadi, 1992; Campbell and Roy, 1993) is based upon the induction of MT synthesis resulting from exposures to these metals. An increase in MT concentrations in fish, therefore, represents a molecular response that generally indicates exposure and development of resistance to toxicity to metals, especially Cd, Cu, Hg and Zn.

Analyses of MT in fish from the Peace, Athabasca and Slave River Basins were also conducted for several reasons that are specific to the study area.. First, while the focus of other contaminant-related studies on fish from these rivers has been on organic contaminants discharged by pulp mills located near the rivers, this study on MT was undertaken to determine whether these fish were also responding to metals. Preliminary surveys, for example, have demonstrated that Cd and Zn present in effluents discharged by municipalities are released into these rivers (Crosley, 1994). Studies conducted in previous years and on fish collected during this study period demonstrated that these fish may be under stresses, such as depressed circulating concentrations of gonadal steroid hormones and failure to attain sexual maturity (Brown et al., 1993; Brown et al., 1996); and analyses of MT would be useful in understanding whether metal or organic contaminants are responsible. Second, information on increases of MT concentrations in gill and intestine can be useful in deducing whether accumulation of metals is directly from water or indirectly from deposition to sediments and subsequent accumulation through the food web. The route of uptake of metals in fish can occur directly from the water through the gills and/or from their diet through the intestinal tract (Hogstrand and Haux, 1991; Farag et al., 1994). Third, metal distributions in fish organs generally follow patterns that are specific for each metal, and MT determinations could be useful in assessing which metals are producing a response and in directing subsequent analyses for those specific metals. Cu, for example, concentrates to greatest extent in fish liver (Lauren and McDonald, 1987; Julshamn et al., 1988), whereas Cd accumulates in fish kidney (Calamari et al., 1982; Wicklund Glynn and Olsson, 1991). Finally, for evaluating effects on fish health it can be more cost effective first to establish whether MT concentrations are elevated before conducting larger, more expensive programs on metal analyses. Subsequent metal analyses, in other words, can be conducted on these tissues in a more reasonable and directed approach following patterns of elevated MT concentrations if they are observed.

2.0 METHODS

Liver, kidney, gill and intestine samples from burbot (*Lota lota*), longnose sucker (*Catostomus catostomus*), northern pike (*Esox lucius*) and flathead chub (*Platygobio gracilis*) were obtained as described in the Terms of Reference (Appendix A) in 1994 from sites in the Peace, Athabasca and Slave River basins by EnviResource Consulting Ltd., Calgary, Alberta. All fish collection sites, which are summarized in Appendix B, were determined in advance by the NRBS Science Directors and Contaminants Component Leader, and are described in detail by EnviResource (1995). Fish tissues were frozen on site using dry ice, and were transported to the Freshwater Institute in Winnipeg, Manitoba, where they were stored at -100^{0}C prior to analyses.

Analyses of MT in organs of these fish used the method described by Dutton et al. (1993) as modified by Klaverkamp and Wautier (1996). Metal-saturation methods for MT analyses are preferable in field bio-monitoring applications because they are sensitive and use simple analytical techniques. Earlier problems associated with using cadmium as the displacing metal in fish MT, which generally has higher copper concentrations than mammalian MT, have been overcome by using mercury as the displacing metal (Dutton et al., 1993; Klaverkamp and Wautier, 1996).

The mercury saturation assay was run in duplicate for each of the four tissue samples for each designated fish. Tissue samples were homogenized in an appropriate volume of 0.9% (w/v) NaCl and heat-treated at 95^{0}C for five minutes in 1.5 mL polypropylene microcentrifuge tubes. Heat-treated homogenates were cooled on ice for five minutes and centrifuged for ten minutes at 10,000 g at room temperature in a benchtop microcentrifuge. The resulting supernatants were stored at -100^{0}C until analyzed. Mercury was used to saturate the metal-binding sites of metallothionein. To each tube, 200 µL of ^{203}Hg-labelled $HgCl_2$ (containing 10 µg Hg and approximately 10,000 cpm for liver samples; 10 µg Hg and approximately 5,000 cpm for kidney, gill filaments and intestine) in 20% (w/v) trichloroacetic acid (TCA). After mixing, incubation continues for ten minutes at room temperature to saturate the metal binding sites with mercury. To end the saturation process, 400 µL of 50% (w/w) chicken egg white in 0.9% (w/v) NaCl is added to each tube. The egg white, which denatures on contact with the TCA, binds the excess non-metallothionein bound Hg, which was removed by centrifugation at 10,000 g for three minutes. The TCA supernatant was removed from the assay tubes by pipette and transferred to clean microcentrifuge tubes for determination of ^{203}Hg activity by gamma counting using a LKB Wallac Compugamma Model 1282. With each batch of samples, Total Activity vials and Blank vials were also analyzed. Known concentrations of rabbit liver metallothionein II (SIGMA, St. Louis, MO.) were also analyzed and used to convert the ^{203}Hg activity from the fish samples to µgMT/g of tissue.

Livers from burbot collected at the sites described in Appendix A, with the exceptions of sites PR2 and PR3 in the Peace River, and the Pembina River site (P), were also analyzed by CHEMEX Labs Alberta Inc. (2021 - 41st Ave. N. E., Calgary, AB., T2E 6P2) for Cd, Cu and Zn by inductively coupled plasma emission spectroscopy. Additional information is described in CHEMEX Project Number NORT130-0501, and is available from the Northern River Basins Study office in Edmonton, Alberta.

An approach for evaluating whether differences in MT concentrations may be due to contaminants in pulp mill discharges was to identify and group fish collection sites according to a general "Field" designation that used location and distance from pulp mill discharge points. "Reference" field sites were those that were upstream of pulp mill discharges; "Near Field" were less than 100 km from a point of mill discharge; and "Far Field" were greater than 100 km from effluent emissions. The site WB1 was designated by NRBS Science Directors and Contaminants Component Leader as a "Null" site because male and immature fish from that site showed an anomalous 2- to 4-fold elevation in Mixed Function Oxidase activity.

One-way analysis of variance was employed to determine whether there was a significant difference in MT concentration as a function of fish sex; and regression analysis was used to test whether sampling time (date of sampling) had a significant effect on MT concentration. Linear regression was also used to examine the relationship between MT and metals in livers of burbot. The confidence level was set at $\alpha = 0.05$ in all analyses. The effect of "Field" (location designation according to position and distance of fish collection sites from pulp mills; see "Results and Discussion") on MT concentration in fish tissues was evaluated by one-way analysis of variance on the mean MT concentrations from each sampling site. This design avoids pseudo-replication, which would result from performing an ANOVA on individual fish values. In addition, the unbalanced nature of the data precludes optimal analysis by means of a sub-sampling design. Fisher's least-significant-difference test was used to detect significant differences between means. All statistical analysis was performed using SAS v. 6.08 (SAS Institute Inc., Cary, NC, 1989).

3.0 RESULTS AND DISCUSSION

Table 1 presents results on MT concentrations (µg/g) in liver, kidney, gill and intestine from burbot, longnose sucker, and northern pike. To gain some appreciation of the variability in MT concentrations in fish from different collection sites, results are highlighted for each species from fish collection sites where the highest (**bold** type) and lowest (*italics* type) MT concentrations were observed. Differences between collection sites having the highest and lowest MT concentrations in burbot tissues ranged from approximately 26-fold in kidney (site SRD compared to site A2) to 3-fold in intestine (site PR3 compared to site SR1). Using similar comparisons for northern pike, these differences ranged from a high of 8.5-fold for MT concentrations in gill (site JV compared to site A1) to a low of 1.5-fold for liver (site MR compared to site JV). For longnose sucker, these differences between highest and lowest MT concentrations were least pronounced, ranging from a highest of 4.7-fold for kidney (site PR3 compared to site A2) to a lowest of about 2-fold for liver (site SR1 compared to LSR). In four cases, two with burbot and two with longnose sucker, concentrations of MT were highest in tissues from fish collected at the site (PR3) upstream of Fort Vermillion.

MT concentrations in kidney of burbot from the Slave River Delta (SRD) ranged from approximately 7-times (compared to SR1) to 26-times (compared to A2) higher than those concentrations found in kidney of burbot from other collection sites (Table 1). The biological significance of this to the health of the burbot is not clear, because burbot from the SRD had the highest condition factor of any collected in the basin (Brown et al., 1996) . The SRD fish are likely to be from the population in Great Slave Lake, and may be exposed to metals due to natural conditions of high mineralization in the Great Slave Lake Delta or other parts of the lake. The elevated MT concentrations in burbot kidney may also result because these fish may be exposed to metals discharged by mining operations, such as the decommissioned lead-zinc mine at Pine Point. The counterclockwise current in this portion of the lake could transport metals from a western source, such as Pine Point, to the Slave Delta (English, 1984). In this regard, it is noteworthy that MT concentrations in burbot gill were also highest in the SRD group (Table 1).

Linear regression analysis determined that the date of fish sampling accounted for very little of the variation in MT, therefore sampling date was not included in subsequent analyses. For example, the regression of sampling date on MT resulted in $r^2 < 0.05$ for all four tissues of burbot.

With two exceptions, there was no significant relationship between MT concentration and fish sexual state (male, female, immature). Sex accounted for a significant portion of the variation in MT concentration in the livers from burbot (p=0.0001) and longnose sucker (p=0.043). Males generally had higher liver MT concentrations than females (Fig. 1), although analysis of variance within sex revealed no significant differences between locations defined as "Reference", "Near Field", and "Far Field" as described below.

An approach for evaluating whether differences in MT concentrations were due to pulp mill discharges was to designate fish collection sites as "Reference", which were those that are upstream of pulp mill discharges, or "Near Field", which are less than 100 km from a point of mill discharge, or "Far Field", which are greater than 100 km from effluent emissions. The site WB1 was designated by NRBS as a "Null" site because male and immature fish from that site showed a 2- to 4-fold elevation in Mixed Function Oxidase activity (pers. comm. W. L. Lockhart). Analysis of variance was conducted on site MT means independent of fish sex, since sex was not a significant factor in determining MT, other than for those two exceptions noted above. ANOVA p-values are summarized in Table 2.

In other burbot tissues, MT concentrations in intestine were significantly higher in fish from Far Field sites than in those from Near Field sites (Fig. 2). This may reflect an increase in the dietary route of exposure of metals in burbot collected from the Far Field sites. Distribution of these metals to other internal organs, however, probably was not occurring because MT concentrations in other burbot tissues were not significantly related to these designated "Field" sites.

MT concentrations in liver of longnose suckers from Near Field sites (Fig. 3 a) were marginally higher than those from reference sites (p=0.055), when evaluated independently of fish sex. As previously noted, these differences were not significant when comparisons were made within sex.

Examination of the relative distribution of fish sexes (immature, male and female) indicated that, although the percentage of males was similar throughout Field sites (ranging from 19.1 % in reference sites to 21.6 % in Far Field sites), Reference sites had 20 % more immature fish and 18.3 % fewer females compared to Near Field sites. Given that MT concentrations in longnose sucker livers were lower in females than in males (Fig. 1), the apparent differences between Near Field and Reference sites (sexes combined) may be attributed to differences in fish sex distribution between those sites.

In other longnose sucker tissues fish sex was not a significant factor. MT concentrations in kidney (Fig. 3 b) of fish from Far Field sites were significantly higher than those respective concentrations in fish from Reference sites. Concentrations of MT in gill and intestine (Fig. 3 c,d) from longnose sucker, however, were not significantly related to pulp mill discharge points indicating other sources of metals accumulation.

For comparison to MT concentrations in fish species from other areas in Canada, results are presented in Table 3 on the grand mean of the combined MT data for each tissue from each species from the NRBS, and on MT analyses conducted in our laboratory on fish collected at sites in northern Saskatchewan, northwestern Ontario, and western Quebec. In most cases, direct comparisons can not be made because the fish species are different between locations. It appears, however, that MT concentrations in fish collected from the NRBS sites are generally within the range, or even less, of those observed in fish from uncontaminated Canadian lakes.

Table 3 also presents the results for MT concentrations in tissues of the 24 flathead chub collected in 1994. Meaningful comparisons can not be made with most of the flathead chub MT data, because only one fish was captured at 3 of the 6 total collection sites. Where more than one fish was caught, no differences were observed between collection sites in MT concentrations in kidney and gill. MT concentrations in intestine of this species (n = 12) collected at the site (SR1) downstream from the confluence of the Wapiti river were only 52 percent of MT concentrations measured in fish (n = 4) collected at site PR2, which is immediately downstream from the Diashowa mill near the Notikewin river.

In order to gain a visual appreciation of whether there were elevated MT concentrations in organs of burbot, longnose sucker and northern pike collected from sites immediately downstream from pulp mills, and whether there were cumulative impacts going from upstream to downstream sites, the results are also expressed by plotting them on schematic maps of the NRBS area in Figures 4 to 15. In several cases, a trend, which may indicate cumulative impacts, of increasing MT concentrations in fish collected from upstream to downstream sites in both the Peace and Athabasca rivers was observed.

This progressive increase in MT concentration in proceeding from upstream fish collection sites to downstream sites was especially pronounced in concentrations of MT in burbot liver (Fig. 4). In the Peace River and associated tributaries (Little Smoky, Smoky, and Wapiti), there is a progressive increase of up to 3.34-fold in burbot liver [MT] moving from upstream to downstream collection sites. In this case, [MT] is lowest (265 μg MT/g ± 49) in liver of fish from WR1 and highest (884 µg MT/g ± 107) in liver of fish from PR3 (Fig. 4). In the upper Athabasca River system, there is a progressive increase of up to 2.33-fold in burbot liver [MT] moving from upstream to downstream collection sites. In this case, [MT] is low (207 µg MT/g ± 56) in liver of fish from A2 and higher (482 µg MT/g ± 61) in liver of fish from A3 (Fig. 4).

In other cases, fish from some of the tributaries, especially the Pembina River, had elevated [MT] relative to fish collected from other sampling locations. For example, [MT] were generally higher in gill of burbot (Fig. 6) and in kidney (Fig. 13) and gill (Fig. 14) of northern pike collected from the Pembina River.

Due to the overall variability of MT results between collection sites conclusions can not be made that pulp mill effluents are causing elevated MT concentrations in fish. Much of this variability results from the relatively high MT concentrations found in fish from some of the tributaries and the lower MT concentrations found in burbot from sites in the lower Athabasca River. For example, MT concentrations in liver of burbot from site A5 are not higher than those found in fish from upstream sites A3 and A4 (Fig. 4).

The following two recommendations are presented; first, to evaluate whether the results described above are due to the cumulative impacts of metals, and, second, to understand the cause of the high MT concentrations observed in kidney and gill of burbot from the Slave River Delta.

1. Additional burbot should be collected from the Peace River and associated tributaries, as well as from the upper Athabasca River system, and the livers and kidneys of these fish should be analyzed for MT and metals, specifically, Cd, Cu, Hg and Zn. Although these fish may be mobile within these aquatic systems, analyses of these metals in surficial sediments, sampled at the fish collection sites, would likely assist in interpreting metals and MT data from liver and kidney. This would be useful in verifying whether cumulative impacts are occurring and whether they are due to the presence of these metals. An example of this approach is provided in Figure 16, which illustrates a correlation between metal content and MT concentrations in liver of burbot collected at many, but not all, of the 1994 sampling sites. Linear regression analysis demonstrates a coefficient of determination (r^2) of 0.717.

2. To evaluate whether the high MT concentrations in gill and the 7- to 26-fold elevation in MT concentrations observed in kidneys of burbot from the Slave River Delta (SRD) are due to elevated metal concentrations in these fish, additional burbot should be captured from SRD and other sites in Great Slave Lake such as the Pine Point area, and analyzed for MT and metals. Analyses of metals in sediment cores, sampled from burbot collection sites, would be useful in determining whether the cause of increased MT concentrations is due to natural conditions of high mineralization or to discharges from the abandoned mine.

4.0 REFERENCES

Bremner, I. and J.H. Beattie. 1990. Metallothionein and the trace minerals. Annual Reviews in Nutrition 10, 63-83.

Brown, S.B., and L. Vandenbyllaardt. 1996. Analyses of dehydroretinol, retinol, retinyl palmitate, and tocopherol in fish, Peace, Athabasca and Slave Rivers, September to December, 1994. Northern River Basins Study Report No. 90. Northern River Basins Study, Edmonton, AB. 47 pages.

Brown, S.B., R.E. Evans, L. Vandenbyllaardt and A. Bordeleau, 1993. Analysis and interpretation of steroid hormones and gonad morphology in fish, Upper Athabaska River, 1992. Northern River Basins Study Project Report No. 13. Northern River Basins Study, Edmonton, AB. 812 pages.

Brown, S.B., R.E. Evans, and L. Vandenbyllaardt. 1996. Analyses for circulating gonadal sex steroids and gonad morphology in Fish, Peace, Athabasca and Slave Rivers, September to December 1994. Northern River Basins Study Project Report No. 89. Northern River Basins Study, Edmonton, AB. 125 pages.

Calamari, D., G. F. Gaffino and G. Pacchetti. 1982. Toxicokinetics of low levels of Cd, Cr, Ni and their mixture in long-term treatment on *Salmo gairdneri*, Rich. Chemosphere 11, 59-70.

Campbell, P.G.C. and R. Roy. 1993. Literature review report: Possible means of evaluating the biological effects of sub-aqueous disposal of mine tailings. MEND Project 2.11.2 (a) INRS-Eau C.P. 7500 Ste-Foy, Quebec G1V 4C7 54 p.

Cousins, R.J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: Special reference to metallothionein and ceruloplasmin. Physiological Reviews 65, 238-310.

Crosley, R.W., 1994. Contaminants in water and sediment, upper Athabasca River, April 1992. Report prepared for the Northern River Basins Study. Report No. 108.

Dutton, M.D., M. Stephenson and J.F. Klaverkamp. 1993. A mercury saturation assay for measuring metallothionein in fish. Environmental Toxicology and Chemistry 12, 1193-1202.

English, M. C. 1984. Implications of upstream impoundment on the natural ecology and environment of the Slave River Delta, Northwest Territories. In: Northern Ecology and Resource Management. R. Olson et al., Eds. The University of Alberta Press. pp. 311-339.

EnviResource 1995. Basin-wide fish collections, Peace, Athabasca and Slave Rivers, September to December, 1994. Report prepared for the Northern River Basins Study. Report No. 61.

Farag, A. M., C. J. Boese, D. F. Woodward and H. L. Bergman. 1994. Physiological changes and tissue metal accumulation in rainbow trout exposed to foodborne and waterborne metals. Environ. Toxicol. Chem. 13, 2021-2029.

Halliwell B., and J.M.C. Gutteridge. 1989. Free radicals in biology and medicine. Second edition. Toronto: Oxford University Press. p 133.

Hamer, D.H. 1986. Metallothionein. Annual Reviews in Biochemistry 55, 913-951.

Hamilton, S.J. and P.M. Mehrle. 1986. Metallothionein in Fish: Review of its importance in assessing stress from metal contaminants. Transactions of the American Fisheries Society 115, 596-609.

Haux, C. and L. Forlin. 1988. Biochemical methods for detecting effects of contaminants on fish. Ambio 17, 376-380.

Hogstrand, C. and C. Haux. 1991. Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionein. Comp. Biochem. Physiol. 100C, 137-141.

Hyllner, J.S., C. Haux, T. Andersson and P-E. Olsson. 1989. Cortisol induction of metallothionein in primary cultures of rainbow trout hepatocytes. J. Cell Physiol. 139, 24-28.

Julshamn, K., K-J Andersen, O. Ringdal and J. Brenna. 1988. Effect of dietary copper on the hepatic concentration and subcellular distribution of copper and zinc in the rainbow trout. (*Salmo gairdneri*). Aquaculture 73, 143-155.

Kagi, J.H.R. and Y. Kojima. 1987. Chemistry and biochemistry of metallothionein. In: Experientia Supplementum Volume 52. Metallothionein II (eds. J.H.R. Kagi and Y.Kojima), Boston: Birkhauser Verlag, 25-62.

Klaverkamp, J.F., W.A. Macdonald, D.A. Duncan and R. Wagemann. 1984. Metallothionein and acclimation to heavy metals in fish: A review. In: Contaminant effects on fisheries. V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.) New York, New York: John Wiley and Sons, 99-113.

Klaverkamp, J.F., M.D. Dutton, H.S. Majewski, R.V, Hunt and L.J. Wesson. 1991. Evaluating the effectiveness of metal pollution controls in a smelter by using metallothionein and other biochemical responses in fish. In: Metal Ecotoxicology: Concepts and applications. M.C. Newman and A.W. McIntosh (eds.) Chelsea, Michigan: Lewis Publishers. 33-64.

Klaverkamp, J.F. and K. Wautier. 1996. Modifications to a mercury saturation assay for measuring metallothionein in fish. Aquatic Toxicology (In preparation).

Lauren, D. J. and D. G. McDonald. 1987. Acclimation to copper by rainbow trout *Salmo gairdneri*: Biochemistry. Can. J. fish. Aquat. Sci. 44, 105-111

Lockhart, W.L., D.A. Metner, D.F. Rawn, R.J. Boychuk and J.R. Toews. 1996. Liver microsomal mixed function oxidase activities in fish from the Athabasca River, Alberta, supplied under the Representative Area Program of the Northern River Basins Study, 1992. Northern River Basins Study Project 2351-B1, Northern River Basins Study, Edmonton, AB. 40 pages

Lockhart, W.L. and D.A. Metner. 1996. Liver microsomal mixed-function oxygenase activities in fish from the Peace, Athabasca and Slave River drainages, 1994. Northern River Basins Study Project 3144-D2. Northern River Basins Study, Edmonton, AB. 40 pages

Lohrer, H. and T. Robson. 1989. Overexpression of metallothionein in CHO cells and its effect on cell killing by ionizing radiation and alkylating agents. Carcinogenesis 10, 2279-2284.

Muller, T., R. Schuckelt and L. Jaenicke. 1994. Evidence for radical species as intermediates in cadmium zinc-metallothionein-dependent DNA-damage in-vitro. Environ. Health Perspect 102, 27-29.

Olsson, P-E., C. Haux, and L. Forlin. 1987. Variations in hepatic metallothionein, zinc and copper levels during an annual reproductive cycle in rainbow trout, *Salmo gairdneri*. Fish Physiol. Biochem. 3, 39-47.

Overnell, J.R. McIntosh and T.C. Fletcher. 1987. The levels of metallothionein and zinc in plaice, *Pleuronectes platessa L.*, during the breeding season, and the effect of oestradiol injection. J. Fish Biol. 30, 539-546.

Pastershank, G.M. and D.C.G. Muir. 1995. Contaminants in environmental samples: PCDDs and PCDFs downstream of bleached kraft mills Peace and Athabasca Rivers, 1992. Northern River Basins Study Report No. 44. Northern River Basins Study, Edmonton, AB. 80 pages.

Roesijadi, G. 1992. Review: Metallothioneins in metal regulation and toxicity in aquatic animals. Aquatic Toxicology 22, 81-114.

SAS Institute Inc., SAS Systems for Linear Models, 1986.

Sato, M. and I. Bremner. 1993. Oxygen free radicals and metallothionein. Free Radical Biology and Medicine. 14: 325-337.

Steadman B. L., A. M. Farag and H. L. Bergman. 1991. Exposure-related patterns of biochemical indicators in rainbow trout exposed to No. 2 Fuel Oil. Envion. Toxicol. Chem. 10: 365-374.

Waalkes, M. P., and P. L. Goering. 1990. Metallothionein and other cadmium-binding proteins: Recent developments. Chemical Research in Toxicology 3, 281-288.

Wicklund Glynn, A. and P-E Olsson. 1991. Cadmium turnover in minnows (*Phoxinus phoxinus*) preexposed to waterborne cadmium. Environ. Toxicol. Chem. 10, 383-394.

Table 1. Concentrations (µg/g) of metallothionein , with S.E.M. and number (n) of fish analyzed, in organs from burbot (BURB), longnose suckers (LNSU), and northern pike (PIKE) collected from sites defined in the Northern River Basins Study (see Appendix A). For each organ of each fish species, the highest concentration is presented in bold type, and the lowest in italics (for n>3).

Fish	Collection	Site	Liver			Kidney			Gill			Intestine		
Species	Site	Category	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n
BURB	A2	REFERENCE	207	56.0	6	*2.97*	0.983	6	8.16	1.48	7	2.84	0.255	7
BURB	CW	REFERENCE	*181*	15.9	5	.	.	0	15.5	2.48	5	5.13	0.640	5
BURB	LSR	REFERENCE	506	280	2	4.85	1.22	2	10.3	2.17	2	4.06	0.450	2
BURB	MR	REFERENCE	406	152	8	6.92	1.08	8	19.6	5.63	8	4.65	0.294	8
BURB	P	REFERENCE	748	169	5	8.99	2.62	4	34.1	12.3	5	4.72	0.660	5
BURB	SR2	REFERENCE	243	.	1	5.41	.	1	21.8	.	1	4.33	.	1
BURB	WR	REFERENCE	266	52.1	16	6.44	1.96	6	21.1	2.43	16	5.24	0.581	16
BURB	A5	FAR	377	64.9	17	7.98	2.18	7	30.6	4.82	17	4.16	0.402	17
BURB	PR1	FAR	609	123	7	9.14	2.44	4	10.2	1.50	7	4.46	0.234	7
BURB	PR3	FAR	**884**	107	6	5.41	0.98	3	18.7	4.96	6	**7.46**	1.11	6
BURB	SRD	FAR	209	22.4	19	**76.4**	10.9	18	**48.0**	7.62	19	5.44	0.681	19
BURB	A1	NEAR	317	35.7	8	9.78	2.56	5	*7.69*	0.829	8	3.49	0.264	8
BURB	A3	NEAR	482	60.7	22	10.1	1.94	17	15.0	2.22	22	2.63	0.134	22
BURB	A4	NEAR	411	73.6	12	9.75	2.63	6	17.9	2.42	12	4.36	0.231	12
BURB	LSV	NEAR	303	39.0	18	7.77	1.60	13	17.9	2.52	18	4.14	0.168	18
BURB	PR2	NEAR	800	140	9	3.42	0.392	7	13.8	3.56	9	4.59	0.257	9
BURB	SR1	NEAR	485	54.7	18	11.0	5.23	3	18.3	3.79	18	*2.46*	0.212	18
BURB	WB1	NULL	546	114	8	.	.	0	9.16	1.12	8	3.09	0.272	8
LNSU	A2	REFERENCE	148	9.45	2	*53.9*	12.5	5	22.1	3.94	5	18.8	6.21	4
LNSU	LSR	REFERENCE	*122*	25.1	5	78.0	10.7	6	21.9	5.30	6	37.4	2.70	6
LNSU	MR	REFERENCE	92.9	.	1	55.7	.	1	27.8	.	1	27.1	.	1
LNSU	SR2	REFERENCE	151	.	1	116	.	1	18.5	.	1	23.3	.	1
LNSU	WR	REFERENCE	174	42.0	13	109	34.7	13	*16.5*	2.73	13	25.0	3.75	13
LNSU	PR1	FAR	158	17.6	16	110	17.5	16	**59.7**	6.35	16	23.3	1.57	16
LNSU	PR3	FAR	169	17.7	22	**251**	28.0	22	17.2	1.35	22	**55.4**	6.67	22
LNSU	A1	NEAR	256	.	1	67.6	.	1	19.6	.	1	39.5	.	1
LNSU	PR2	NEAR	178	37.6	7	68.3	21.0	7	26.4	3.10	7	43.8	8.00	7
LNSU	SR1	NEAR	**235**	24.3	11	68.6	18.5	12	24.4	3.14	12	*18.4*	1.71	12
PIKE	CW	REFERENCE	.	.	0	245	.	1	1.69	.	1	28.4	.	1
PIKE	MR	REFERENCE	**239**	62.1	4	47.9	6.75	4	4.46	1.05	4	10.2	0.936	4
PIKE	P	REFERENCE	.	.	0	156	3.38	2	17.5	7.55	2	15.2	1.58	2
PIKE	WR	REFERENCE	198	44.6	12	64.2	6.79	13	1.90	0.287	13	9.44	0.91	13
PIKE	A5	FAR	.	.	0	91.2	.	1	6.36	.	1	8.16	.	1
PIKE	JV	FAR	*165*	27.0	5	**133**	30.3	5	**7.94**	1.66	5	**25.2**	11.8	5
PIKE	PR1	FAR	136	36.6	3	113	16.9	4	2.86	0.153	4	9.95	0.492	4
PIKE	SRD	FAR	240	164	2	52.1	16.4	2	2.70	0.530	2	4.28	1.27	2
PIKE	A1	NEAR	191	72.8	3	*34.5*	5.62	4	*0.930*	0.063	4	*6.93*	0.915	4
PIKE	PR2	NEAR	199	73.5	2	52.9	8.27	3	3.85	0.222	3	15.9	5.33	3
PIKE	SR1	NEAR	398	76.6	3	46.1	12.1	3	1.43	0.377	3	8.20	1.56	3

Table 2. P-values for one-way ANOVA evaluating the effect of field (near, far or reference) on metallothionein concentrations in fish.

Species	Tissue			
	Liver	Kidney	Gill	Intestine
Burbot	0.5073	0.2247	0.2065	0.0553
Longnose sucker	0.0235	0.0528	0.2829	0.4526
Northern pike	0.5180	0.2460	0.5397	0.6636
Flathead chub	N/A	0.2659	0.0481	0.5564

(N/A = not analyzed)

Table 3. Metallothionein concentrations, expressed as mean ($\mu g\ MT \cdot g^{-1}$) ± standard error mean, in organs of freshwater fish.

Location:	Liver	Kidney	Gill	Intestine
A. Northern Rivers Basin Study (combined data from all collection sites)				
Burbot	419 ± 22	18.9 ± 3.0	20.8 ± 1.4	4.1 ± 0.1
Longnose sucker	174 ± 11	131 ± 13	27.8 ± 2.3	34.1 ± 2.6
Northern pike	212 ± 23	79.3 ± 8.1	3.9 ± 0.7	12.0 ± 1.7
Flathead chub	N/A	69.9 ± 6.6	12.3 ± 1.2	27.4 ± 4.8
B. Northern Saskatchewan (near proposed uranium mining sites)				
Northern pike:				
Boomerang Lake	506 ± 119	90 ± 26	N/A	N/A
Lower Read Lake	417 ± 142	103 ± 23	N/A	N/A
Little Yalowega Lake	226 ± 20	90 ± 12	N/A	N/A
Toby Lake	548 ± 96	82 ± 25	N/A	N/A
White sucker:				
Boomerang Lake	362 ± 58	32.8 ± 3.3	N/A	N/A
Lower Read Lake	268 ± 38	24.8 ± 3.6	N/A	N/A
Little Yalowega Lake	365 ± 75	64.4 ± 11.6	N/A	N/A
Toby Lake	286 ± 84	38.6 ± 7.6	N/A	N/A
C. Northwestern Ontario (Experimental Lakes Area)				
White sucker:				
Reference Lake	412 ± 25	53.6 ± 7.1	22.8 ± 2.0	19.0 ± 2.6
Cadmium-polluted lake	725 ± 82	295 ± 28	31.5 ± 2.9	34.3 ± 6.1
D. Western Quebec (near mining area of Val d 'Or)				
White sucker:	887 ± 79	365 ± 36	82.2 ± 5.9	N/A
Northern pike:	298 ± 45	199 ± 24	11.9 ± 1.8	N/A

(N/A = not analyzed)

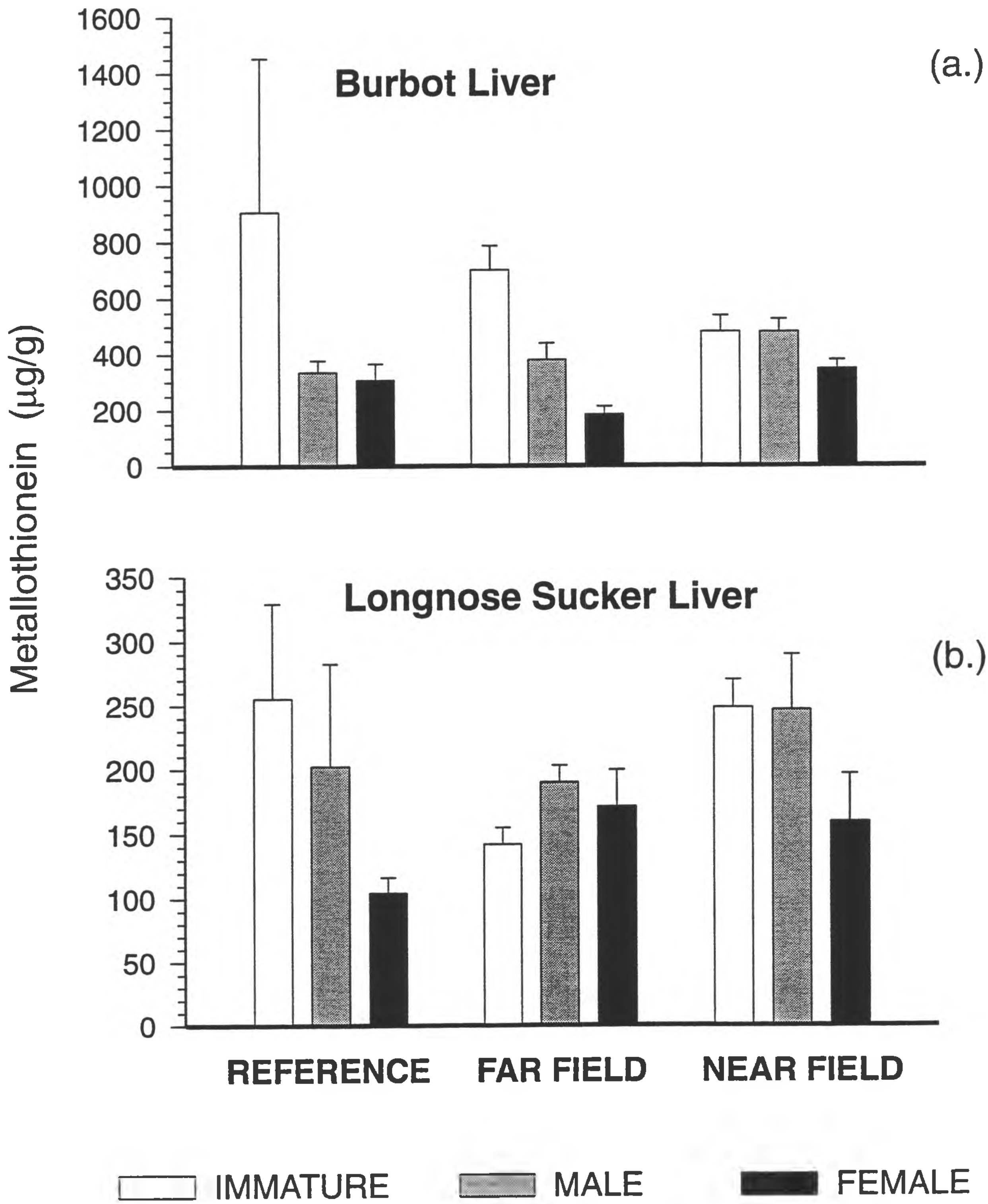


Figure 1. Metallothionein in liver of burbot (a.) and longnose sucker (b.), with associated standard error mean. Means are not significantly different (Fisher's LSD, $\alpha=0.05$) within sexes.

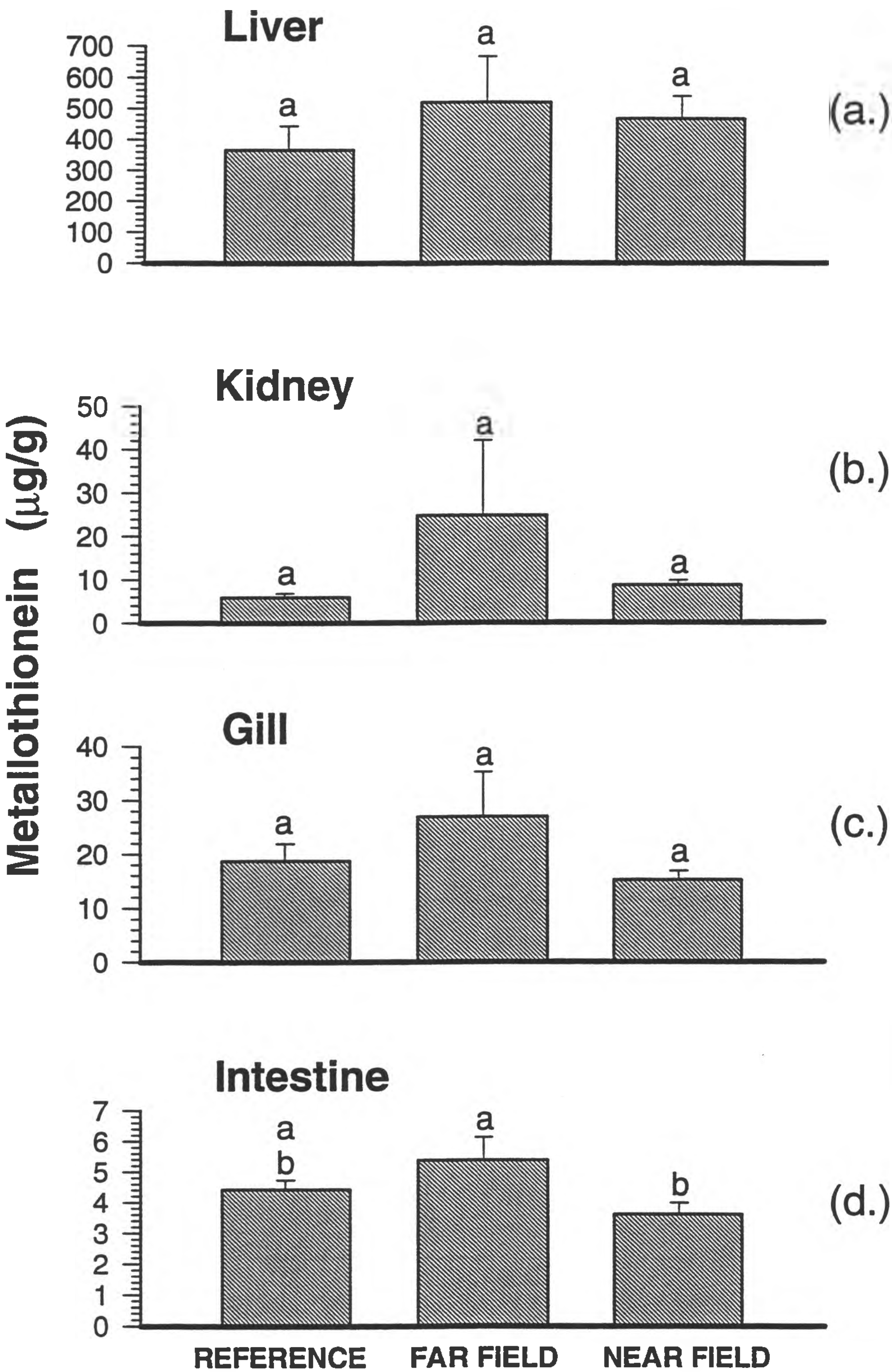


Figure 2. Metallothionein in burbot. Histogram bars represent mean value of sites within each field, with associated standard error mean. Means with the same letter are not significantly different (Fisher's LSD, $\alpha = 0.05$)

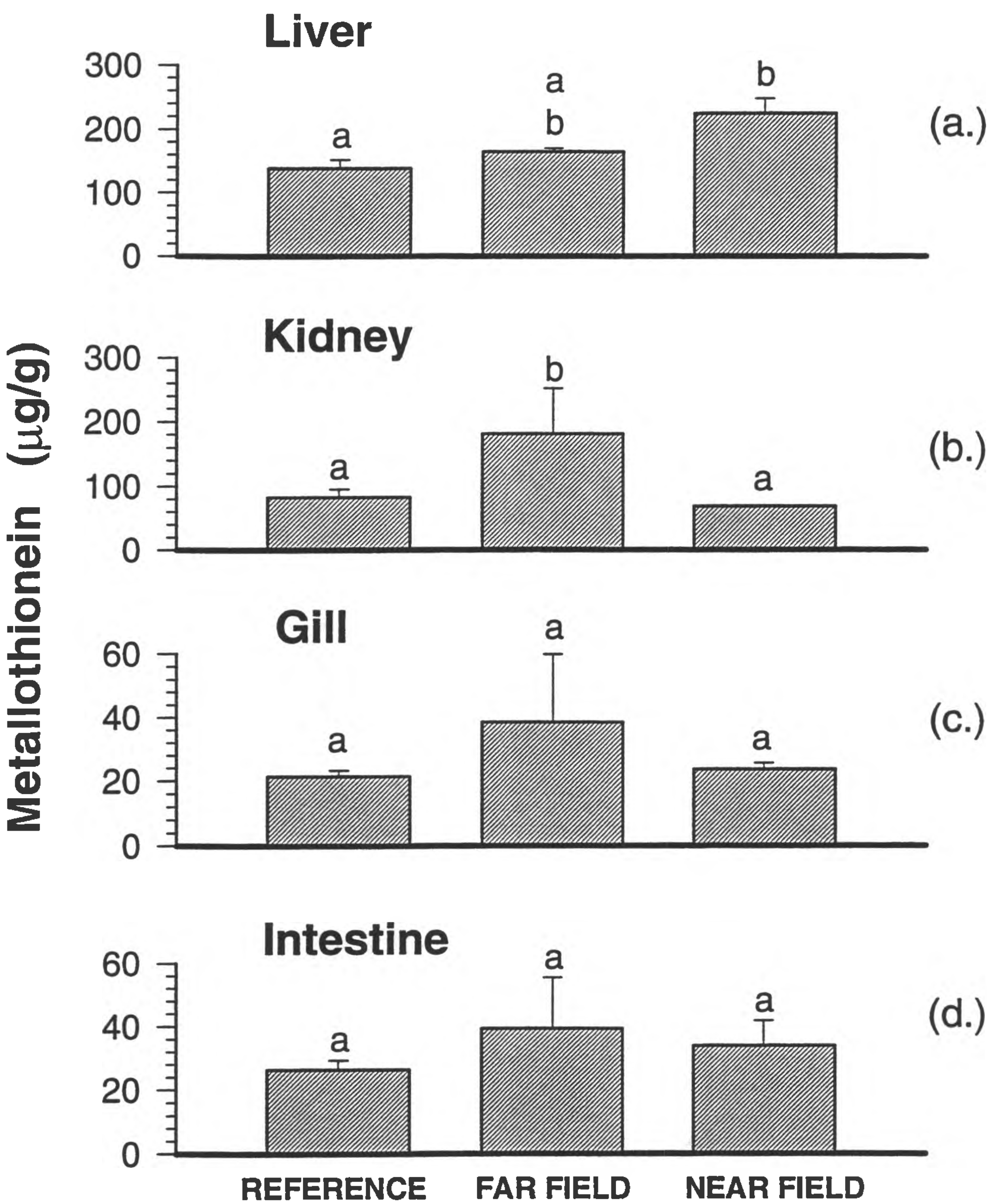


Figure 3. Metallothionein in longnose sucker. Histogram bars represent mean value of sites within each field, with associated standard error mean. Means with the same letter are not significantly different (Fisher's LSD, $\alpha = 0.05$).

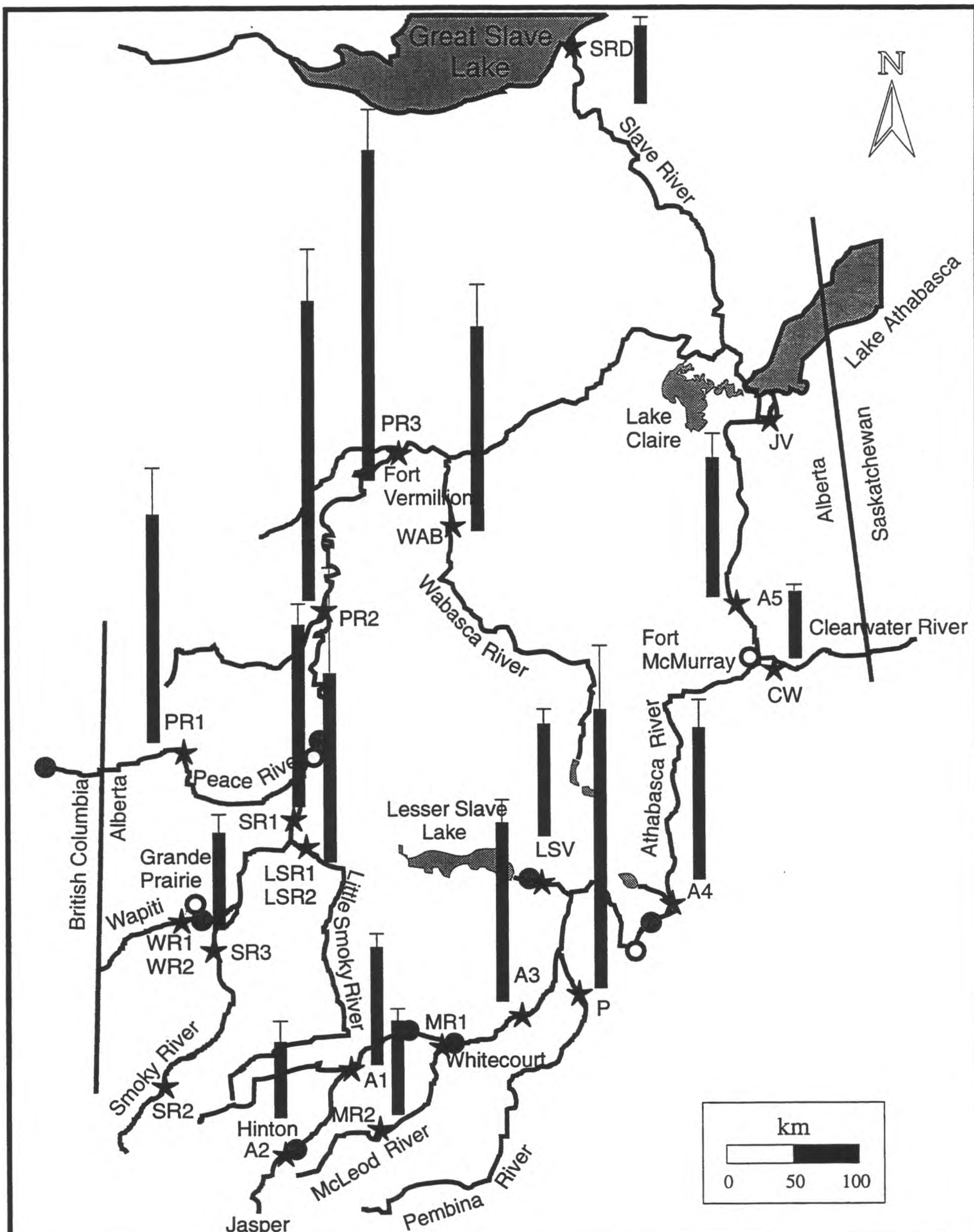


Figure 4. Metallothionein concentrations, expressed as mean +/- SEM, in liver of burbot collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills.

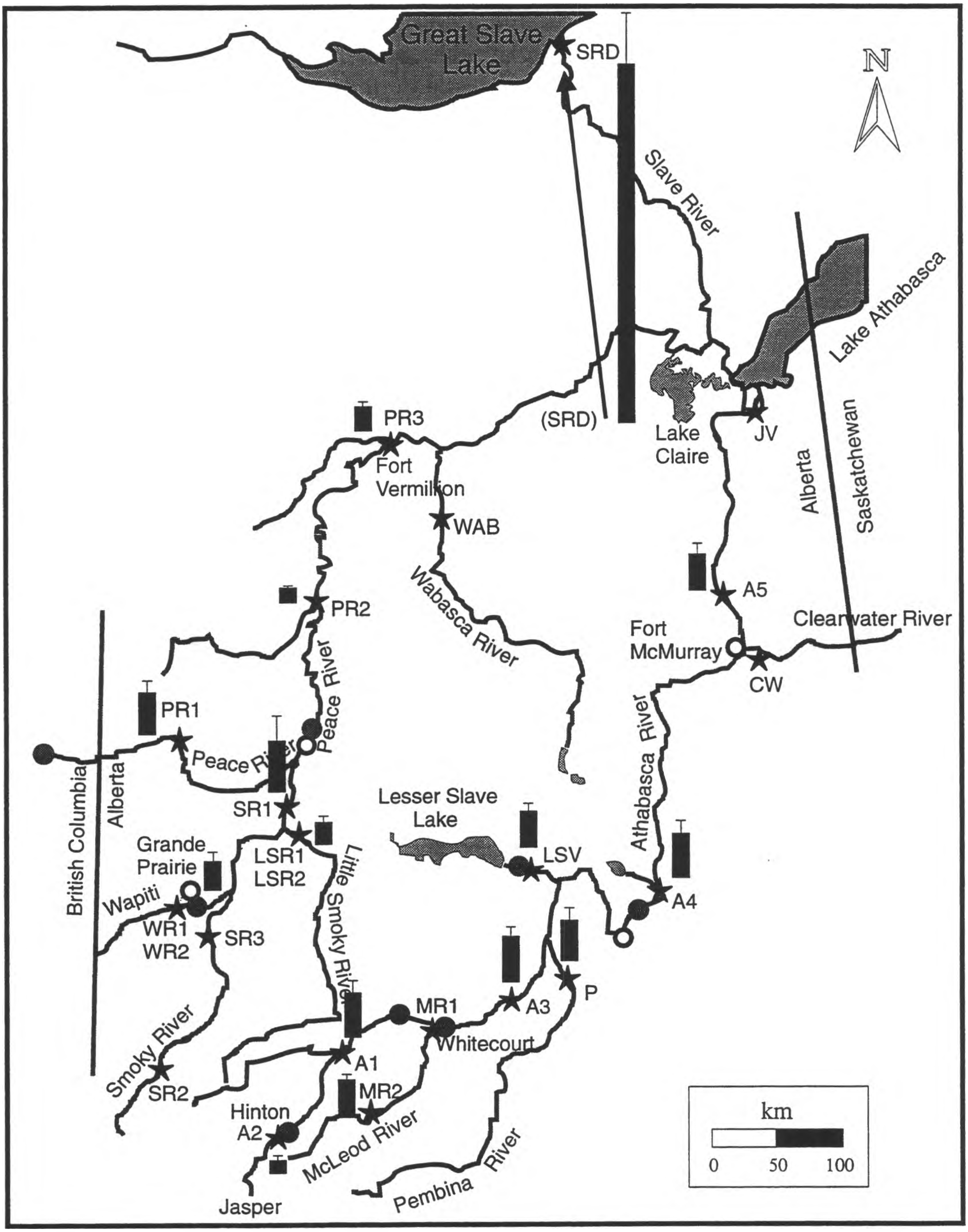


Figure 5. Metallothionein concentrations, expressed as mean +/- SEM, in kidney of burbot collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills.

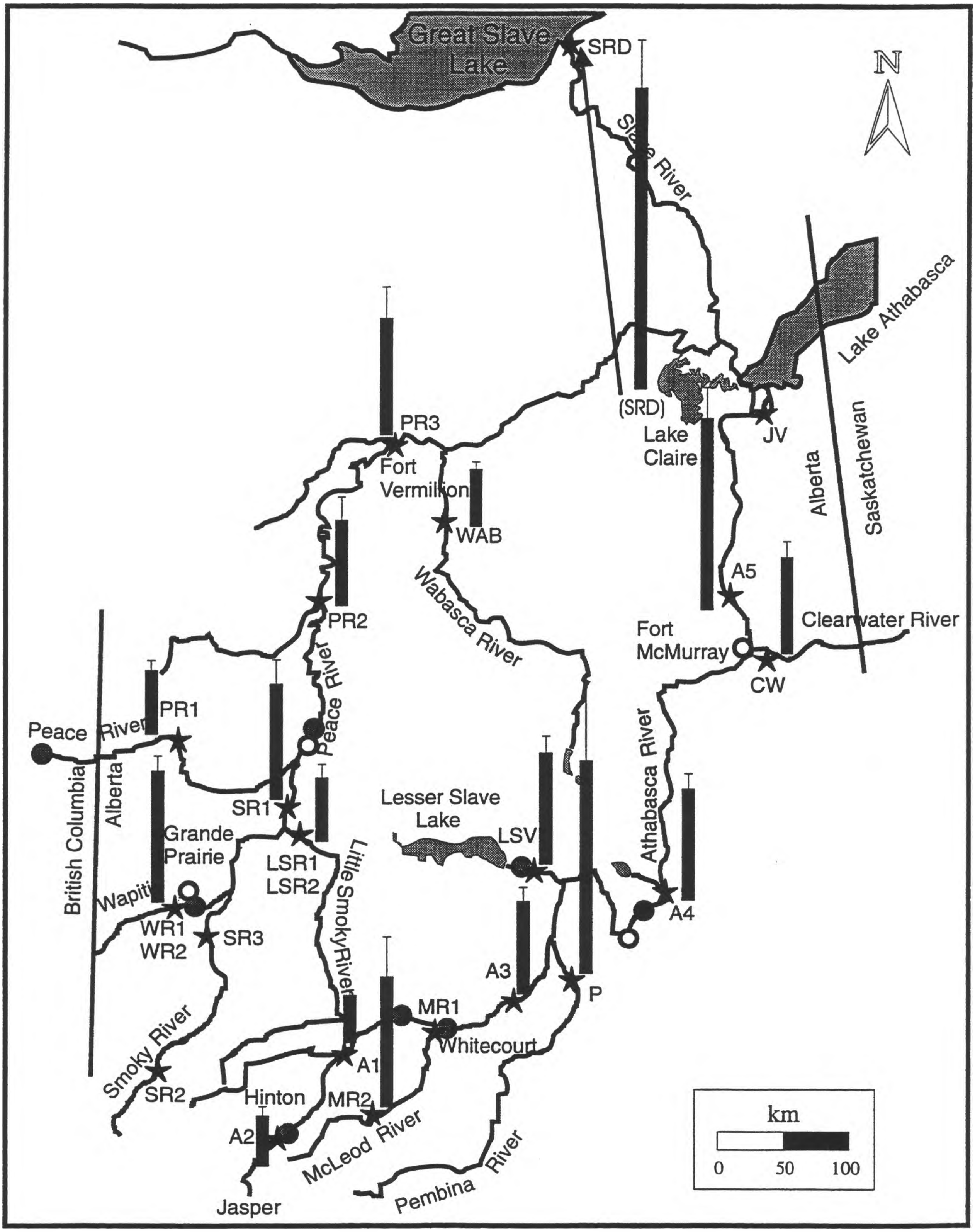


Figure 6. Metallothionein concentrations, expressed as mean +/- SEM, in gill of burbot collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills.

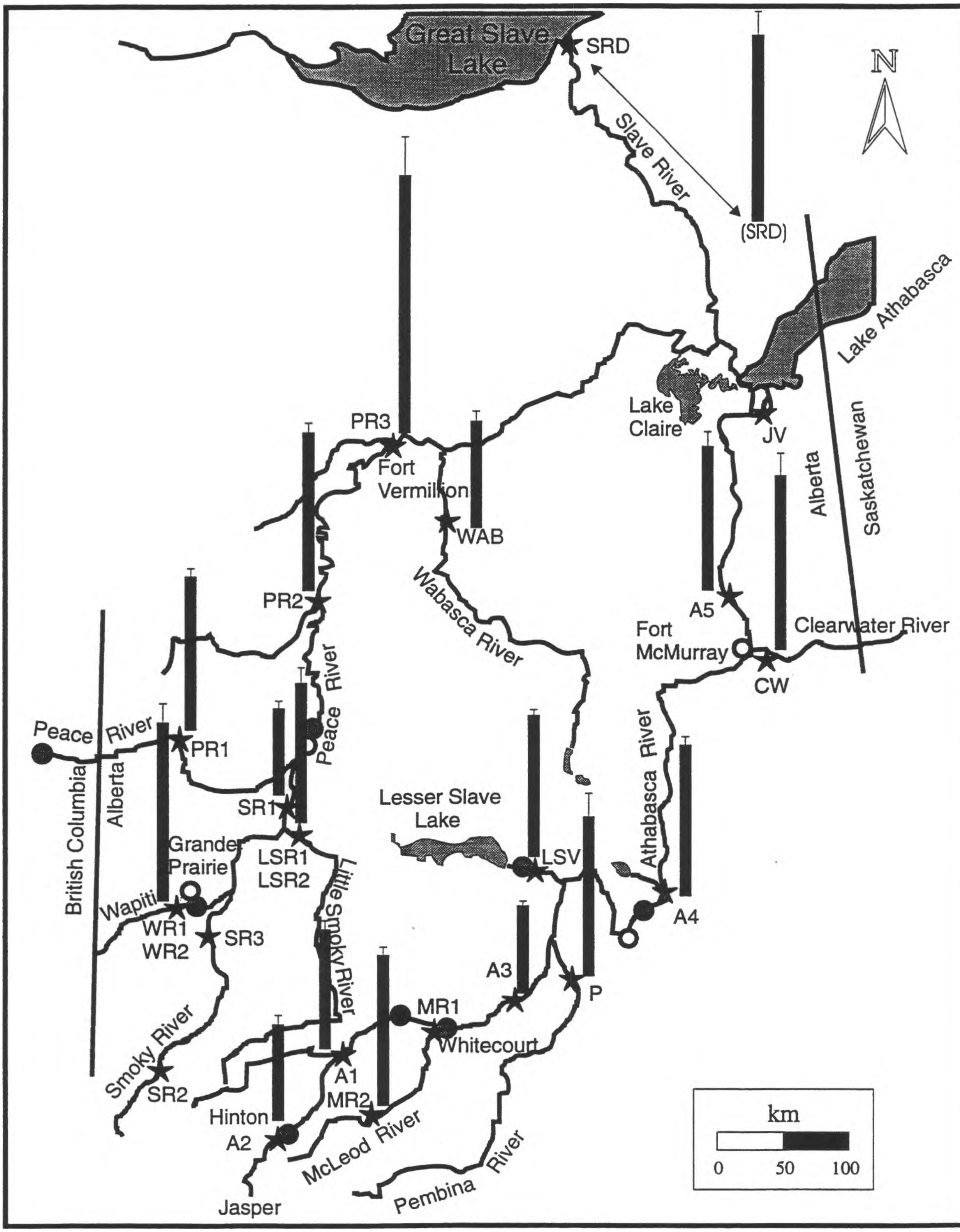


Figure 7. Metallothionein concentrations, expressed as mean +/- SEM, in intestine of burbot collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills.

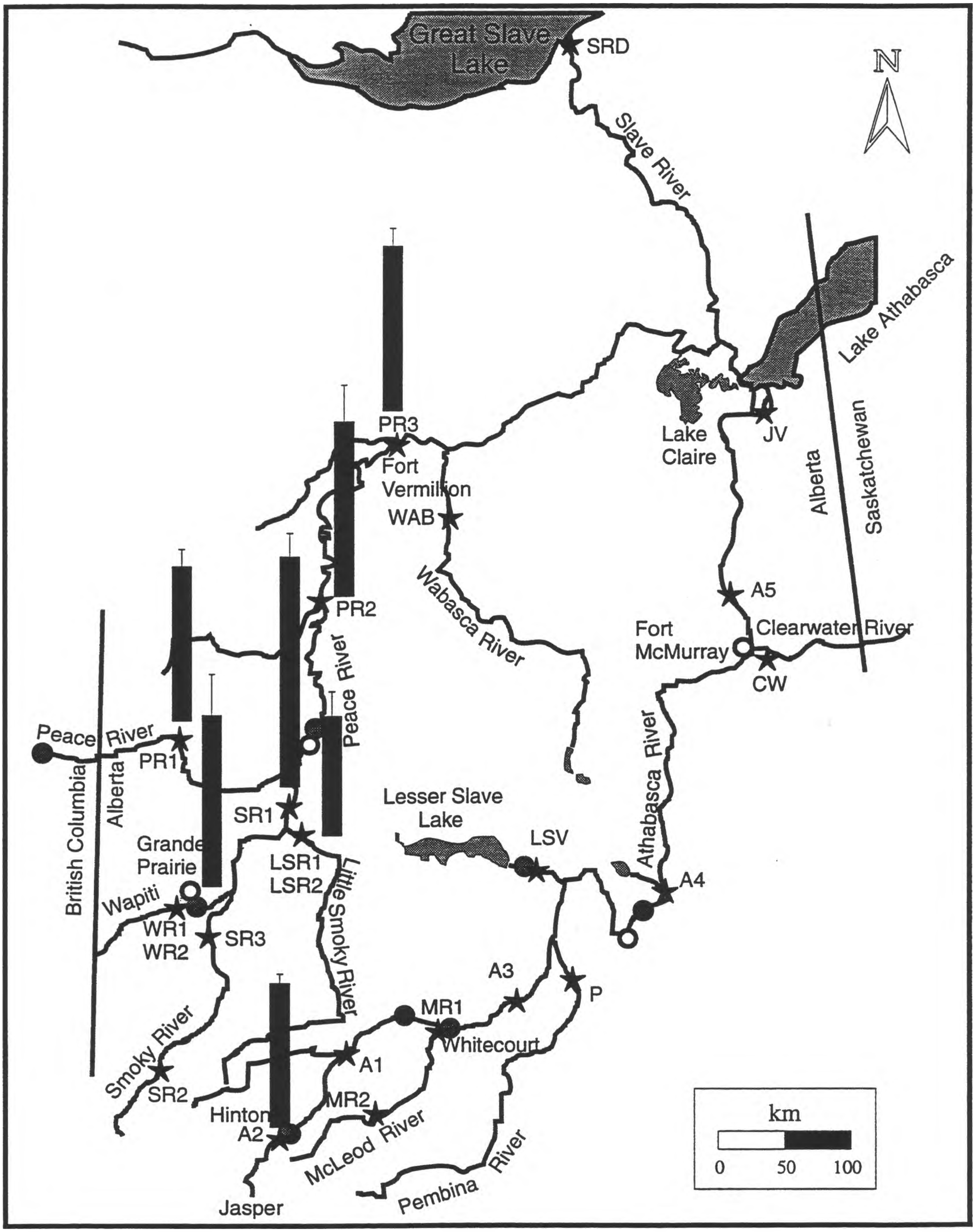


Figure 8. Metallothionein concentrations, expressed as mean +/- SEM, in liver of longnose suckers collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills.

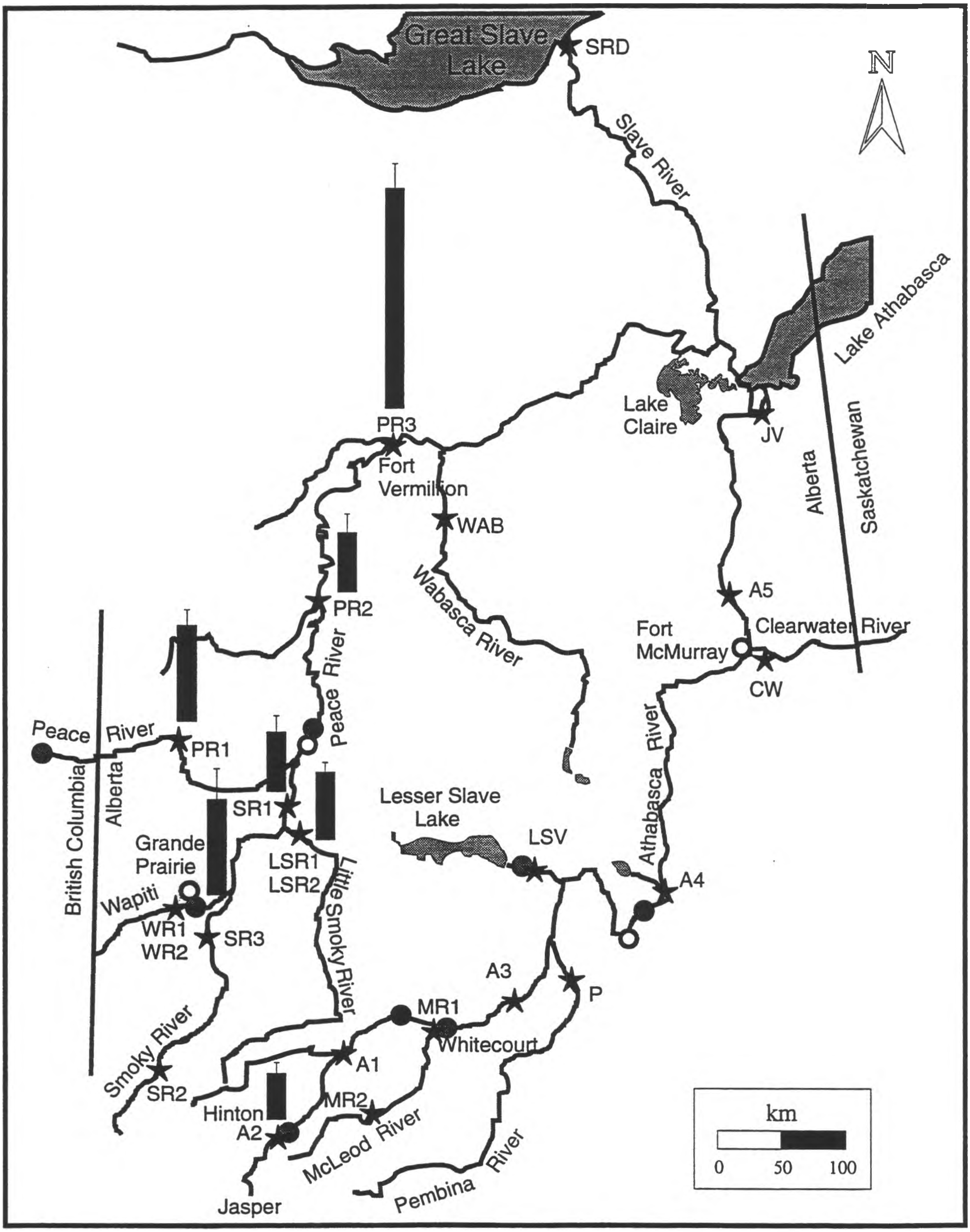


Figure 9. Metallothionein concentrations, expressed as mean +/- SEM, in kidney of longnose suckers collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills.

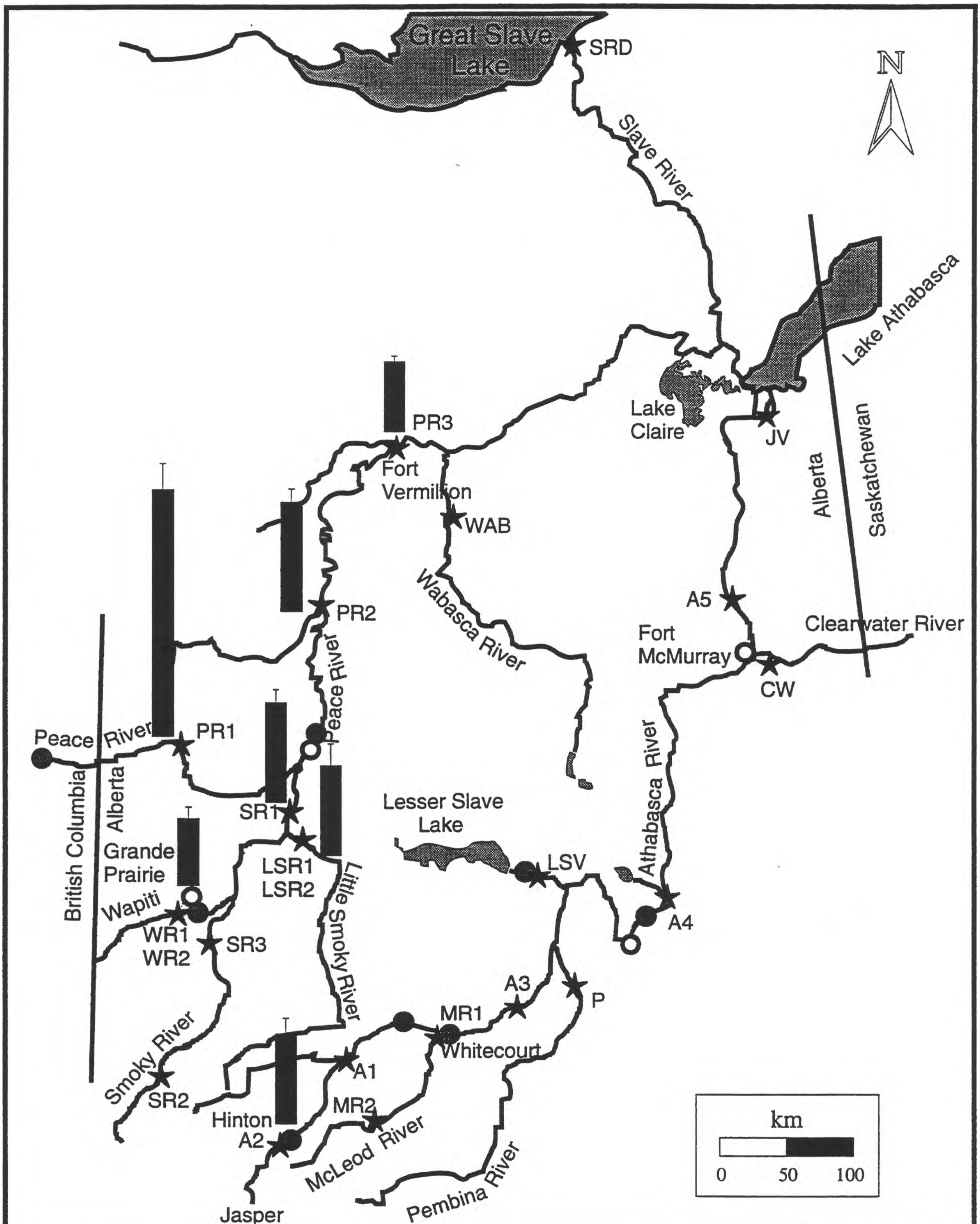


Figure 10. Metallothionein concentrations, expressed as mean +/- SEM, in gill of longnose suckers collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills

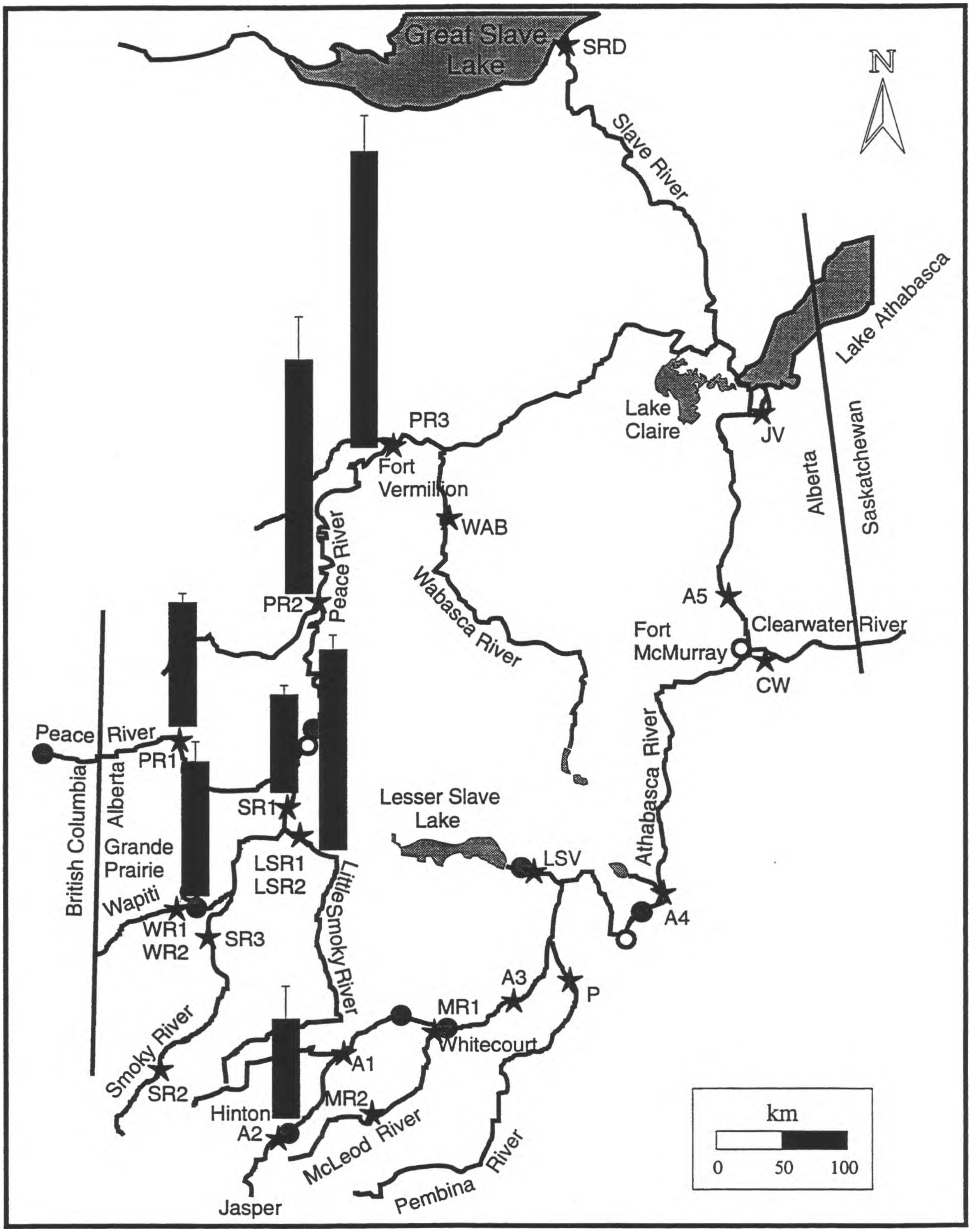


Figure 11. Metallothionein concentrations, expressed as mean +/- SEM, in intestine of longnose suckers collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills.

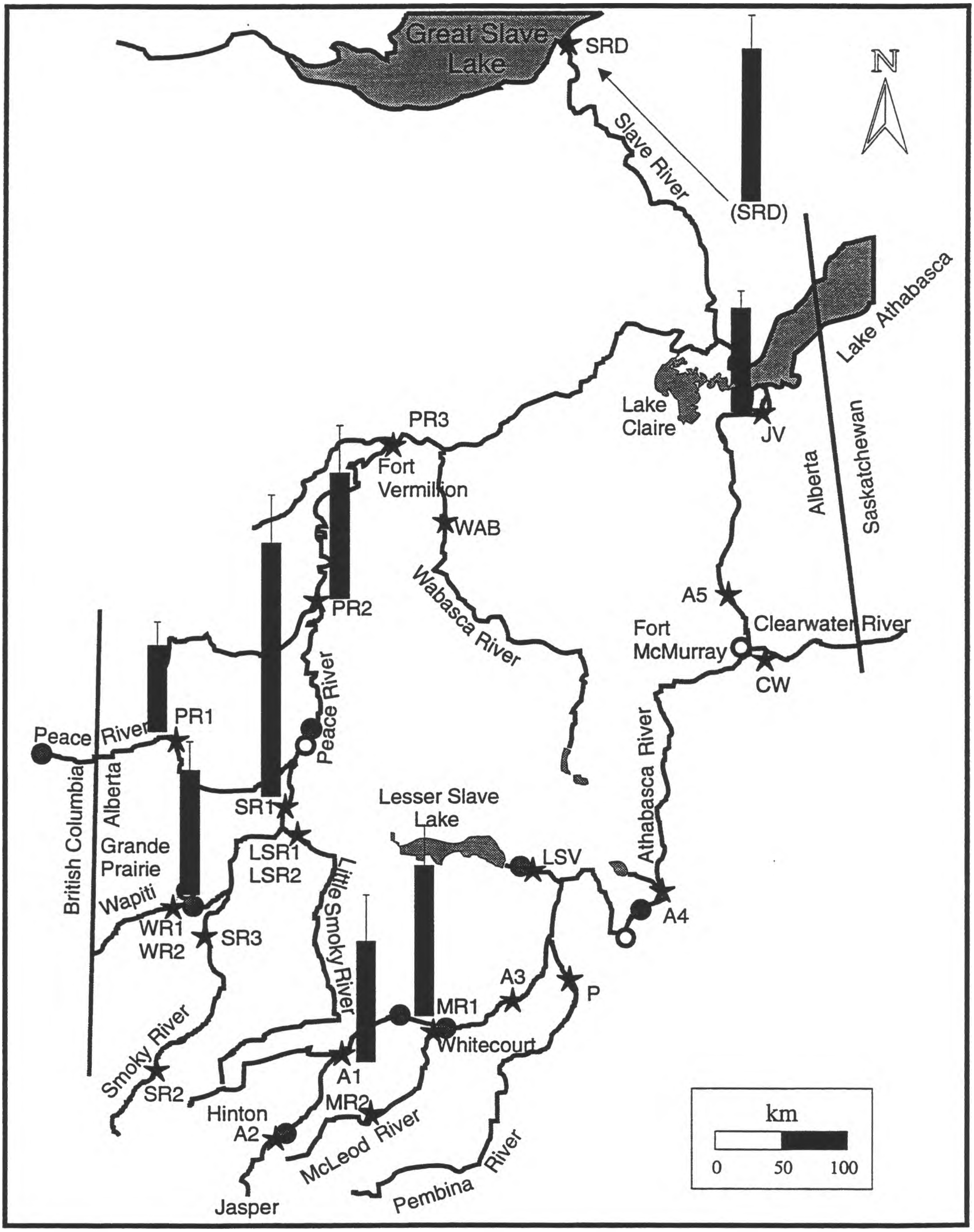


Figure 12. Metallothionein concentrations, expressed as mean +/- SEM, in liver of northern pike collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills.

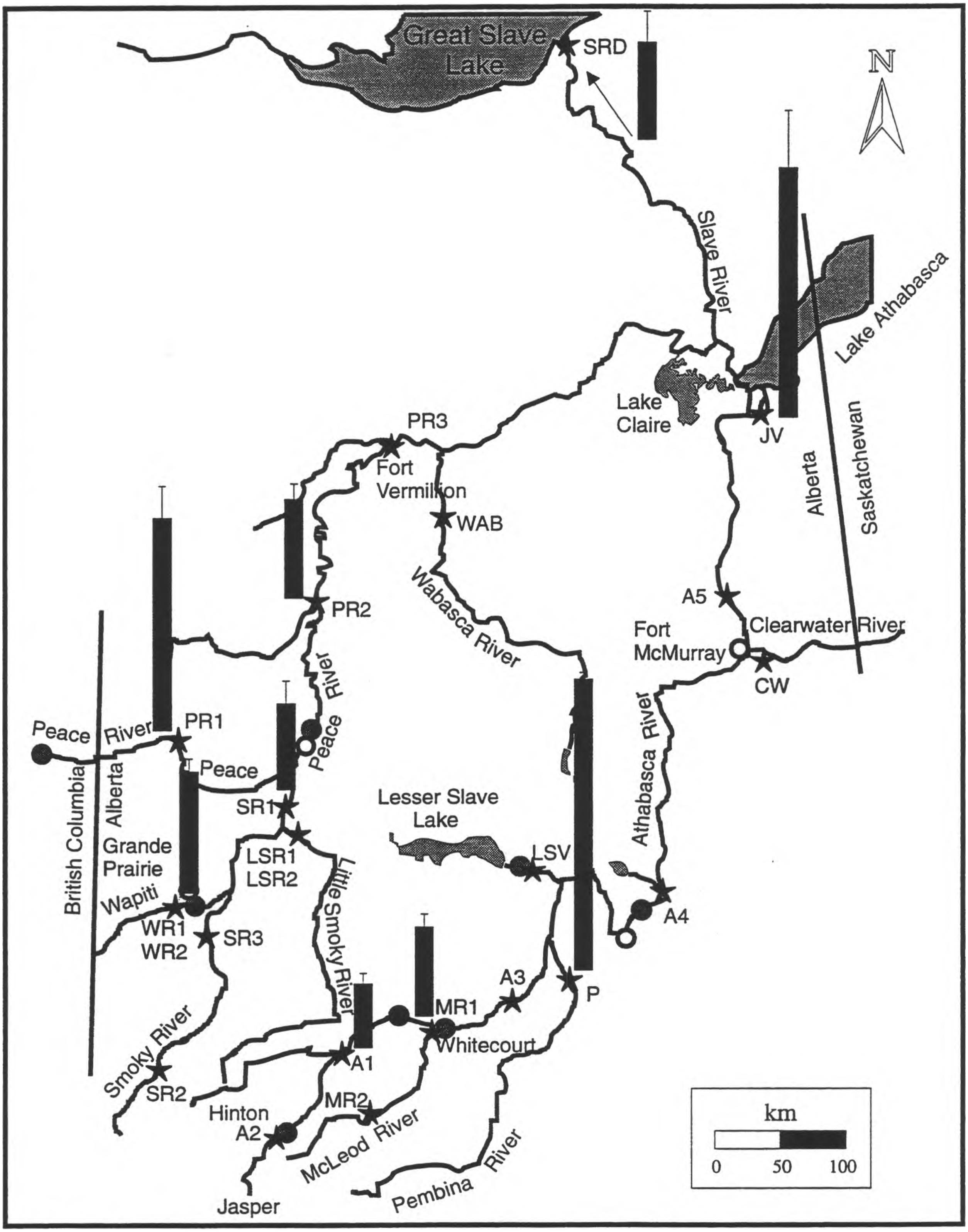


Figure 13. Metallothionein concentrations, expressed as mean +/- SEM, in kidney of northern pike collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills.

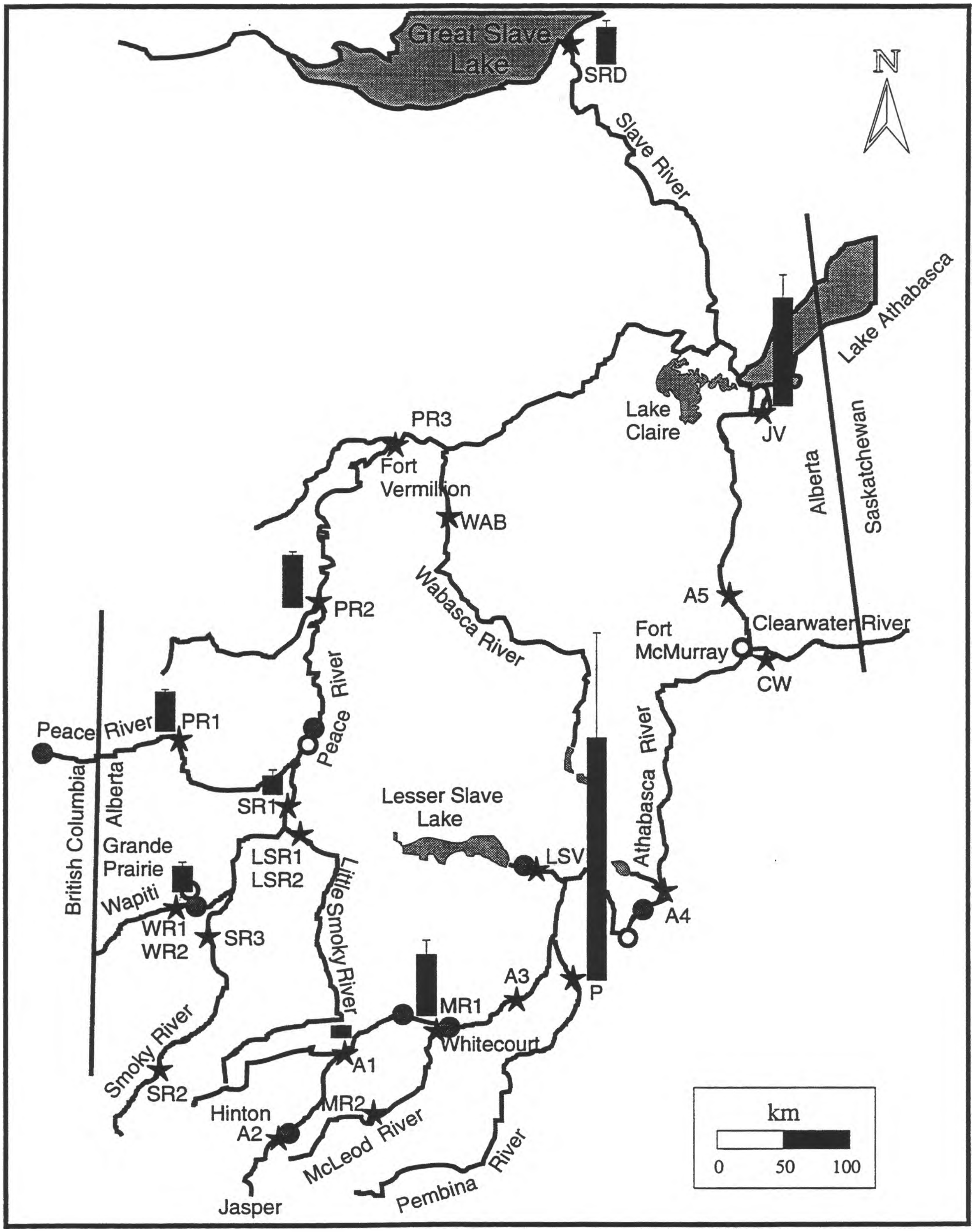


Figure 14. Metallothionein concentrations, expressed as mean +/- SEM, in gill of northern pike collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills.

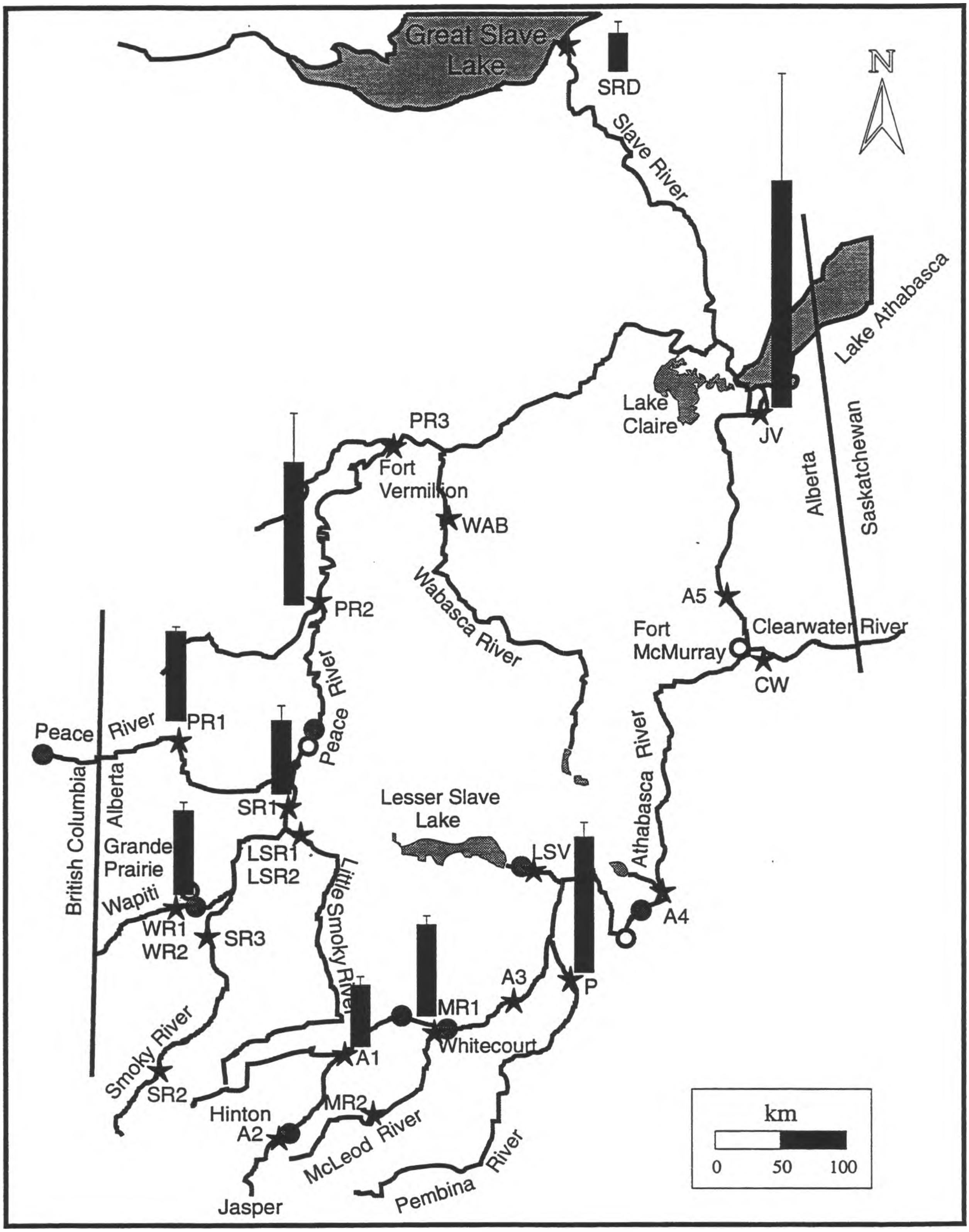


Figure 15. Metallothionein concentrations, expressed as mean +/- SEM, in intestine of northern pike collected from sites (stars) in the fall of 1994. Closed circles represent approximate locations of pulp mills

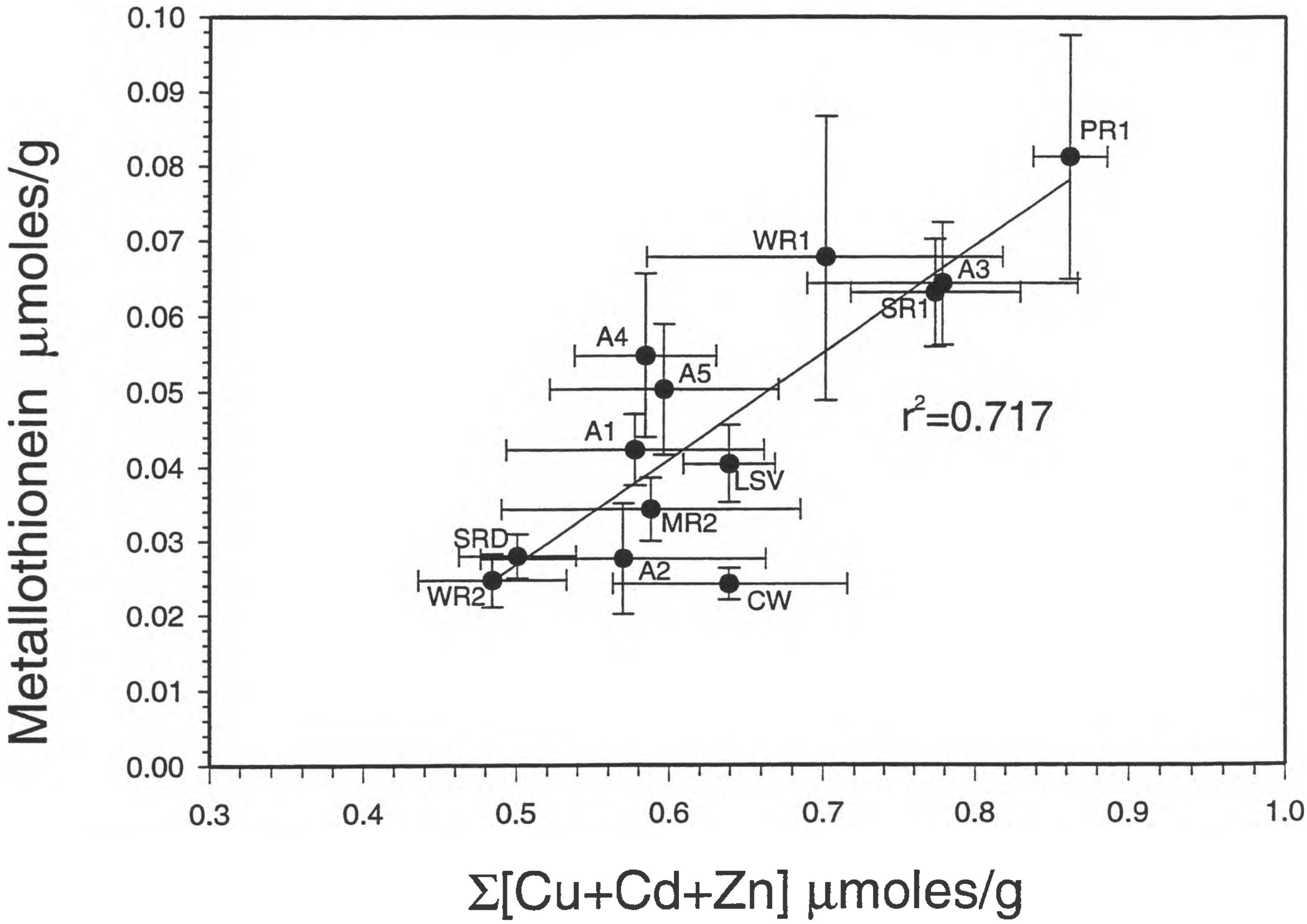


Figure 16. Regression of metal content (Cd, Cu and Zn) on metallothionein concentrations in liver of burbot.

Appendix A

Terms of Reference

NORTHERN RIVER BASINS STUDY

SCHEDULE A - TERMS OF REFERENCE

Project 3144-D5: 1994 Fall Basin-Wide Burbot Collection - Metallothionein Analyses

I. BACKGROUND & OBJECTIVES

"Metallothioneins (MTs) are a family of low-molecular-weight, cysteine-rich proteins that bind metals from groups IB and IIB of the periodic table (Kagi and Nordberg 1979, Hamer 1986, Kagi and Kojima 1987). These proteins appear to have evolved for intracellular zinc and copper regulation (Hamer 1986). MT synthesis is induced in animals by exposure to these and other heavy metals, and a capacity for the inducible synthesis of metallothionein has been demonstrated for most tissues and organisms studied to date (Palmiter 1987). MT induction and its correlation with toxicity acclimation in fish (Klaverkamp *et al.* 1984, Klaverkamp and Duncan 1987) indicate that functions may include detoxification of zinc, copper, mercury and cadmium. Metallothionein has been proposed as a promising biochemical indicator of heavy metal exposure in fish (Klaverkamp *et al.* 1984, Neff 1985, Hamilton and Mehrle 1986, Haux and Forlin 1988)" (Dutton *et al.* 1993).

The aquatic fauna of the northern river basins are exposed to pulp mill and other types of municipal and industrial effluents. The aquatic environments of the northern river basins may also be exposed to the long range transport of air borne contaminants from distant locations outside the basins. Specific studies have been conducted by the Northern River Basins Study (NRBS) to investigate these concerns, but to date the data from these studies has not been fully analyzed. Of interest, however, is a preliminary investigation of mercury levels in fish in the basins (NRBS, unpublished). This study revealed an increasing trend of mercury levels in fish tissues over time.

In September and October 1994, the NRBS initiated a basin-wide fish collection (Boag in prep.) to further determine the effects of pulp mill and other effluents, as well as airborne contaminants on fish populations. The collection and sampling protocols for the project were designed to allow a wide range of biochemical, 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 determine concentrations of metallothionein in the gill filaments, intestines, kidneys and livers of burbot, northern pike, longnose sucker and flathead chub collected in 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 metallothionein analyses on the gill filaments, intestines, kidneys and livers 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 samples for each of the four fish species.

2. Gill filaments, intestine, kidney and liver samples, stored and transported in the dark at -60°C have been supplied to Don Metner by EnviResource Consulting Limited, Calgary. The contractor is expected to maintain these tissue samples in a suitable condition to allow for metallothionein analyses to be carried out on these fish. Metallothionein assays are to be conducted on these tissues in accordance with the mercury saturation method presented in Dutton *et al.* (1993).

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

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

III. ANALYTICAL REQUIREMENTS

Metallothionein assays are to be conducted on the gill filaments, intestines, kidneys and livers of designated burbot, longnose sucker, northern pike and flathead chub in accordance with the mercury saturation assay outlined in Dutton *et al.* (1993) and Klaverkamp *et al.* (1991). Specifically, the methodology is as follows:

The mercury saturation assay is to be run in duplicate for each of the four tissues samples for each designated fish. Tissue samples are to be homogenized in an appropriate volume of 0.9% (w/v) NaCl and heat-treated at 95°C for five minutes in 1.5-mL polypropylene microcentrifuge tubes. The heat-treated homogenates are then to be cooled on ice for five minutes and then centrifuged for ten minutes at 10,000 g at room temperature in a benchtop microcentrifuge. The resulting supernatants are then to be stored at -120°C until analyzed.

The assay is to be performed on four serial dilutions (1:1, 1:3, 1:9 and 1:18) of each replicate. Four 1.5-mL microcentrifuge tubes are to be prepared for each dilution series. To the two tubes to be used for the 1:3 and 1:9 dilutions, 200 µL of 0.9% NaCl (w/v) is to be added and 100 µL of 0.9% (w/v) NaCl is to be added to a third tube (1:18 dilution). Heat-treated supernatant (300 µL) is to be added to the remaining microcentrifuge tube; 100 µL of this is to be withdrawn and transferred to the 1:3 dilution tube and mixed by several draws of the pipetter. After mixing, 100 µL are to be withdrawn from the 1:3 dilution tube and transferred to the 1:9 tube and mixed; 100

μL are then to be withdrawn from the 1:9 dilution tube and transferred to the 1:18 tube and mixed. This will provide 200 μL of 1:1, 1:3, 1:9 and 1:18 dilutions of the sample in the centrifuge tubes, respectively.

Mercury is then to be used to saturate the metal-binding sites of metallothionein. To each tube, 200 μL of ^{203}Hg-labelled $HgCl_2$ (containing 10 μg Hg and 10,000 cpm for liver analyses; 5 μg Hg and 5,000 cpm for kidney, gill filament and intestine analyses) in 20% (w/v) trichloroacetic acid (TCA) is to be added, mixed by vortex, and incubated for ten minutes at room temperature.

To end the saturation step, 400 L of 50% (w/w) chicken egg white in 0.9% (w/v) NaCl is to be added to each tube. The egg white denatures on contact with the acid and is to be removed from solution along with the non-metallothionein-bound Hg by centrifuging each tube at 10,000 g for three minutes. The TCA supernatant is then to be removed from the assay tubes by pipette and transferred to clean microcentrifuge tubes for determinations of ^{203}Hg activity.

Metallothionein concentrations are to be calculated as follows:

nmol MT/ml of sample =

[cpm (sample) - cpm (blank)]/[cpm (total)] * [10 μg Hg/(0.2-ml sample)]

* [1 nmol Hg/0.20059 μg Hg] * [1 nmol MT/7 nmol Hg] * [dilution (e.g., 1,3,9,18)]

Sample concentrations are to be based on the average concentration of the two replicates.

With each batch of samples, total activity vials and blank vials are also to be analyzed. Total activity samples are to consist of 200 μL of ^{203}Hg to determine the specific activity of ^{203}Hg. Blank samples are to consist of 200 μL of 0.9% NaCl. Percent recoveries for each batch of samples is to be determined from known concentrations of rabbit liver metallothionein II (Sigma, St. Louis, MO).

IV. REPORTING REQUIREMENTS

1. Prepare a comprehensive report outlining the results of the metallothionein assays carried out under this contract. To the extent possible, the results should also be discussed in relation to the uptake and removal of various metals from the body as well as the possible effects of industrial and municipal effluents and air-borne contaminants on the health of fish populations in the northern river basins. Specifically, the report is to include:

   a) a detailed description of the analytical methods employed and a summary of assay performance characteristics.
   b) an appendix or tables indicating the mean metallothionein concentrations in the gill filaments, intestines, kidneys and livers in each fish assayed.

The report is to indicate that the details pertaining to the collection of fish analyzed under this contract are outlined in Boag (in prep.). Sample numbers indicated in the report are to conform to those outlined in Boag (in prep.).

2. Ten copies of the draft report 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 Project Liaison Officer 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 E00, 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.

V. DELIVERABLES

1. A data interpretation report that includes the methods and results for the metallothionein analyses on NRBS fish samples collected in teh 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:

Dr. John Carey
National Water Research Institute
Environment Canada
867 Lakeshore Road
P.O. Box 5050
Burlington, Ontario L7R 4A6
phone: (905) 336-4913
fax: (905) 336-4972

The Component Coordinator for this contract is:

Richard Chabaylo
Northern River Basins Study
690 Standard Life Centre
10405 Jasper Avenue
Edmonton, Alberta T5J 3N4
phone: (403) 427-1742
fax: (403) 422-3055

VII. LITERATURE CITED

Barton, B. A., C. P. Bjornson and K. L. Egan. 1993a. Special fish collections, upper Athabasca river, May 1992. Northern River Basins Study Project Report No. 8. Prepared by: Environmental Management Associates, Calgary, Alberta. 37 pp. + appendices.

Barton, B. A., D. J. Patan and L. Seely. 1993b. Special fish collections, upper Athabasca River, September and October 1992. Northern River Basins Study Project Report No. 10. Prepared by: Environmental Management Associates, Calgary, Alberta. 50 pp. + appendices.

Boag, T. in prep. Collection of burbot from the Peace, Athabasca and Slave river basins, fall 1994. Prepared by: EnviResource Consulting Ltd., Calgary. Prepared for: the Northern River Basins Study.

Dutton, M. D., M. Stephenson and J. F. Klaverkamp. 1993. A mercury saturation assay for measuring metallothionein in fish. Environmental Toxicology and Chemistry 12: 1193-1202.

Hamer, D. H. 1986. Metallothionein. Annual Review of Biochemistry 55: 913-951.

Hamilton, S. J. and P. M. Mehrle. 1986. Metallothionein in fish: Review of its importance is assessing stress from metal contaminants. Transactions of the American Fisheries Society 115: 596-609.

Haux, C. and L. Forlin. 1988. Biochemical methods for detecting effects on contaminants on fish. Ambio 17: 376-380.

Kagi, J. H. R. and Y. Kojima. 1987. Chemistry and biochemistry of metallothionein. Exp. Suppl. (Basel) 52: 25-61.

Kagi, J. H. R. and M. Nordberg. 1979. Metallothionein. Proceedings, First International Meeting on Metallothionein and Other Low Molecular Weight Metal-Binding Proteins, Zurich, Switzerland, July 17-22, 1978. Birkhauser-Verlag, Boston.

Klaverkamp, J. F. and D. A. Duncan. 1987. Acclimation to cadmium toxicity by white suckers: Cadmium binding capacity and metal distribution in gill and liver cytosols. Environmental Toxicology and Chemistry 6: 275-289.

Klaverkamp, J. F., M. D. Dutton, H. S. Majewski, R. V. Hunt and L. J. Wesson. 1991. Evaluating the effectiveness of metal pollution controls in a smelter by using metallothionein and other biochemical responses in fish. pgs. 33-64, In: M. C. Newman and A. W. McIntosh (eds.). Metal ecotoxicology concepts and applications. Lewis Publishers, Chelsea, Michigan.

Klaverkamp, J. F., W. A. Macdonald, D. A. Duncan and R. Wagemann. 1984. Metallothionein and acclimation to heavy metals in fish: A review. Pgs. 99-113 in V. W. Cairns, P. V. Hodson and J. O. Nriagu (eds.). Contaminant effects on fisheries. John Wiley & Sons, N.Y.

Neff, J. M. 1985. Use of biochemical measurements to detect pollutant-mediated damage. Pgs. 155-183 in R. D. Cardwell, R. Purdy and R. C. Bahner (eds.). Aquatic Toxicity and Hazard Assessment (Seventh Symposium). STP 854. American Society for Testing and Materials, Philadelphia.

Palmiter, R. D. 1987. Molecular biology of metallothionein gene expression. Exp. Suppl. (Basel) 52:63-80.

Appendix B

Fish Sampling Sites

Appendix B. Fish collection sites for the 1994 basin-wide fish collections in the Northern River Basins Study area (modified from EnviResource 1995). Field and NRBS Group show the groupings used for analysis and presentation of burbot data. Possible discharge locations and approximate distances are indicated.

Drainage /Delta	River	Site Code	Date Sampled	Field	NRBS Group	General Location	Potential Effluent Exposure
Athabasca	Athabasca	A1a	11/09 to 13/09	NEAR	A1	Near Highway 947 crossing	D/S (approx. 95 km) of pulp mill-Hinton
		A1b	13/09 to 15/09	NEAR	A1	Near Berland River	D/S (approx. 80 km) of pulp mill-Hinton
		A2	21/09 to 24/09	REF	A2	U/S (approx. 10 km) of Hinton	D/S of town of Jasper
		A3	27/09	NEAR	A3	Near Fort Assiniboine	D/S (approx. 60 km) of pulp mills-Whitecourt
		A4	08/10 to 09/10	NEAR	A4	Near Calling River	D/S (approx. 25 km) of pulp mill-ALPAC
		A5	14/10 to 15/10	FAR	A5	Near Fort Mackay	D/S (approx. 20 km) of SUNCOR, D/S (approx. 310 km)
	McLeod	MR1	16/09 to 19/09	REF	MR	Near town of Whitecourt	D/S town of Edson
		MR2	15/12	REF	MR	U/S town of Edson	Tributary Reference
	Pembina	P	29/09 to 01/10	REF	P	Near town of Jarvie	D/S town of Barrhead
	Lesser Slave	LSV	03/10 to 04/10	NEAR	LSV	Near town of Slave Lake	D/S (approx. 10 km) Slave Lake Pulp
	Clearwater	CW	11/10 to 13/10	REF	CW	U/S of Fort McMurray	Tributary Reference
Peace	Peace	PR1	28/09 to 01/10	FAR	PR1	Near Many Islands Prov. Park	D/S (approx. 150 km) of pulp mills in BC
		PR2	03/10 to 05/10	NEAR	PR2	D/S Diashowa Near the Notikewan River	D/S (approx. 95 km) of pulp mill ans town of Peace River
		PR3	07/10 to 09/10	FAR	PR3	Near Fort Vermilion	Further D/S (approx. 230 km) of pulp mill -Peace River
	Wapiti	WR1	22/09 to 26/09	REF	WR	Near Pipestone Creek Prov. Park	U/S (approx. 20 km) pulp mill-Grande Prairie
		WR2	19/10 to 20/10	REF	WR	Near O'Brian Prov. Park	U/S (approx. 5 km) pulp mill-Grande Prairie
	Smoky	SR1	13/09 to 19/09	NEAR	SR1	Near Highway 49 Near Watino	D/S (approx. 90 km) pulp mill - Grande Prairie
		SR2	16/10	REF	SR2	Near Grande Cache	U/S Reference
		SR3	21/12	REF	SR3	U/S confluence of Wapiti near Canfor bridge	U/S Reference
	Little Smoky	LSR1	18/09 to 22/09	REF	LSR	Near Highway 744 crossing	Tributary Reference
		LSR2	18/12	REF	LSR	D/S (3 km) LSR1	
	Wabasca	WB	10/10 to 12/10		WB	Near highway 67 crossing	Tributary Reference
Peace-Athabasca	Delta	JV1	19/10		JV	Near Jackfish Lake Village	
		JV2	20/10		JV	Near Big Eddy	
Slave	Delta	SRD1	15/10	FAR	SD	U/S of Nagle Channel	D/S town of Fort Smith
		SRD2	15/10 to 17/10	FAR	SD	At mouth of Nagle Channel	D/S town of Fort Smith

Appendix C

Fish Data

Appendix C. Sample identification (UniqID), date sampled (Datesmp), year day of sample (Day), Northern River Basins Code (NRBS#), sample collection site (Site), species, fish number (Fish), and metallothionein concentrations (µg/g) in fish liver, kidney, gill and intestine.

							MT µg/g			
UniqID	Datesmp	Day	NRBS#	Site	Species	Fish	Liver	Kidney	Gill	Intestine
90287	09/13/94	255	A1-BURB-1	A1	BURB	1	391	5.07	11.0	3.39
90288	09/13/94	255	A1-BURB-2	A1	BURB	2	210	11.0	9.97	2.31
90289	09/13/94	255	A1-BURB-6	A1	BURB	6	476	NA	6.87	3.19
90290	09/13/94	255	A1-BURB-7	A1	BURB	7	377	18.5	7.53	4.77
90291	09/14/94	256	A1-BURB-8	A1	BURB	8	290	NA	7.18	3.93
90292	09/14/94	256	A1-BURB-9	A1	BURB	9	340	4.12	5.06	3.09
90293	09/14/94	256	A1-BURB-10	A1	BURB	10	287	NA	4.37	4.05
90294	09/15/94	257	A1-BURB-11	A1	BURB	11	164	10.2	9.54	3.17
90315	09/21/94	263	A2-BURB-1	A2	BURB	1	303	3.02	4.96	3.81
90316	09/21/94	263	A2-BURB-2	A2	BURB	2	NA	2.37	12.7	2.89
90317	09/21/94	263	A2-BURB-3	A2	BURB	3	113	NA	13.6	3.14
90318	09/22/94	264	A2-BURB-4	A2	BURB	4	237	0.920	5.61	1.64
90319	09/23/94	265	A2-BURB-5	A2	BURB	5	419	7.67	8.49	2.99
90321	09/23/94	265	A2-BURB-7	A2	BURB	7	67.7	2.12	3.16	2.38
90322	09/24/94	266	A2-BURB-8	A2	BURB	8	102	1.70	8.62	3.02
90350	09/27/94	269	A3-BURB-1	A3	BURB	1	472	12.2	16.6	1.68
90351	09/27/94	269	A3-BURB-2	A3	BURB	2	1066	NA	46.9	2.50
90352	09/27/94	269	A3-BURB-3	A3	BURB	3	236	11.0	18.5	2.53
90353	09/27/94	269	A3-BURB-4	A3	BURB	4	463	8.82	13.1	2.32
90354	09/27/94	269	A3-BURB-5	A3	BURB	5	220	32.2	8.02	2.08
90355	09/27/94	269	A3-BURB-6	A3	BURB	6	306	NA	12.0	2.41
90356	09/27/94	269	A3-BURB-7	A3	BURB	7	937	9.44	20.1	2.65
90357	09/27/94	269	A3-BURB-8	A3	BURB	8	535	15.0	11.0	2.76
90358	09/27/94	269	A3-BURB-9	A3	BURB	9	124	9.54	6.49	2.42
90359	09/27/94	269	A3-BURB-10	A3	BURB	10	1021	4.39	11.0	2.12
90360	09/27/94	269	A3-BURB-11	A3	BURB	11	201	8.20	3.02	2.51
90361	09/27/94	269	A3-BURB-12	A3	BURB	12	423	3.49	8.04	3.89
90362	09/27/94	269	A3-BURB-13	A3	BURB	13	246	6.48	21.7	2.14
90363	09/27/94	269	A3-BURB-14	A3	BURB	14	426	4.85	3.92	1.89
90364	09/27/94	269	A3-BURB-15	A3	BURB	15	370	5.77	3.62	2.23
90365	09/27/94	269	A3-BURB-16	A3	BURB	16	375	25.0	17.6	3.38
90366	09/27/94	269	A3-BURB-17	A3	BURB	17	316	NA	28.0	2.82
90367	09/27/94	269	A3-BURB-18	A3	BURB	18	555	11.2	14.1	3.06
90368	09/27/94	269	A3-BURB-19	A3	BURB	19	432	NA	8.36	2.32
90369	09/27/94	269	A3-BURB-20	A3	BURB	20	449	2.76	17.4	4.10
90370	09/27/94	269	A3-BURB-21	A3	BURB	21	351	0.920	32.2	2.53
90371	09/27/94	269	A3-BURB-22	A3	BURB	22	1077	NA	8.77	3.62
90406	10/08/94	280	A4-BURB-1	A4	BURB	1	454	NA	28.8	4.84
90405	10/08/94	280	A4-BURB-2	A4	BURB	2	203	22.5	31.8	3.14
90407	10/08/94	280	A4-BURB-3	A4	BURB	3	231	5.78	22.3	3.95
90408	10/08/94	280	A4-BURB-4	A4	BURB	4	302	6.92	11.9	3.32
90409	10/08/94	280	A4-BURB-5	A4	BURB	5	266	NA	13.8	3.76
90410	10/08/94	280	A4-BURB-6	A4	BURB	6	252	9.96	18.6	3.37
90411	10/08/94	280	A4-BURB-7	A4	BURB	7	240	NA	15.7	4.54
90412	10/08/94	280	A4-BURB-8	A4	BURB	8	158	NA	26.1	5.13
90413	10/08/94	280	A4-BURB-9	A4	BURB	9	680	NA	8.05	5.25
90414	10/08/94	280	A4-BURB-10	A4	BURB	10	869	NA	5.54	5.12
90418	10/09/94	281	A4-BURB-14	A4	BURB	14	862	7.43	10.5	4.82
90419	10/09/94	281	A4-BURB-15	A4	BURB	15	409	5.88	21.4	5.08

1 of 7

Appendix C. Sample identification (UniqID), date sampled (Datesmp), year day of sample (Day), Northern River Basins Code (NRBS#), sample collection site (Site), species, fish number (Fish), and metallothionein concentrations (μg/g) in fish liver, kidney, gill and intestine.

							MT μg/g			
UniqID	Datesmp	Day	NRBS#	Site	Species	Fish	Liver	Kidney	Gill	Intestine
90442	10/14/94	286	A5-BURB-1	A5	BURB	1	480	4.24	31.2	3.81
90443	10/14/94	286	A5-BURB-2	A5	BURB	2	151	3.06	17.1	3.37
90444	10/14/94	286	A5-BURB-3	A5	BURB	3	652	12.5	24.5	5.55
90445	10/14/94	286	A5-BURB-4	A5	BURB	4	509	18.1	52.5	2.55
90446	10/15/94	287	A5-BURB-5	A5	BURB	5	602	10.2	33.7	3.92
90447	10/15/94	287	A5-BURB-6	A5	BURB	6	967	NA	15.8	5.50
90448	10/15/94	287	A5-BURB-7	A5	BURB	7	531	3.59	4.57	4.09
90449	10/15/94	287	A5-BURB-8	A5	BURB	8	182	4.19	96.1	9.73
90450	10/15/94	287	A5-BURB-9	A5	BURB	9	810	NA	34.7	3.37
90451	10/15/94	287	A5-BURB-10	A5	BURB	10	209	NA	32.1	4.44
90452	10/15/94	287	A5-BURB-11	A5	BURB	11	137	NA	30.0	2.95
90453	10/15/94	287	A5-BURB-12	A5	BURB	12	241	NA	22.1	3.13
90454	10/15/94	287	A5-BURB-13	A5	BURB	13	393	NA	16.3	4.08
90455	10/15/94	287	A5-BURB-14	A5	BURB	14	139	NA	27.7	2.83
90456	10/15/94	287	A5-BURB-15	A5	BURB	15	173	NA	34.1	4.15
90457	10/15/94	287	A5-BURB-16	A5	BURB	16	143	NA	25.7	3.95
90458	10/15/94	287	A5-BURB-17	A5	BURB	17	86.6	NA	21.2	3.34
90429	10/11/94	283	CW-BURB-1	CW	BURB	1	156	NA	18.3	3.70
90430	10/11/94	283	CW-BURB-2	CW	BURB	2	195	NA	17.0	6.96
90431	10/11/94	283	CW-BURB-3	CW	BURB	3	144	NA	19.1	5.94
90432	10/11/94	283	CW-BURB-4	CW	BURB	4	235	NA	17.5	5.37
90433	10/13/94	285	CW-BURB-5	CW	BURB	5	175	NA	5.70	3.69
90072	09/20/94	262	LSR2-BURB-1	LSR2	BURB	1	786	3.63	8.16	4.51
90511	12/18/94	351	LSR3-BURB-1	LSR3	BURB	1	227	6.06	12.5	3.61
90383	10/03/94	275	LSV-BURB-1	LSV	BURB	1	268	NA	16.0	3.70
90384	10/03/94	275	LSV-BURB-2	LSV	BURB	2	280	22.3	20.2	3.45
90385	10/03/94	275	LSV-BURB-3	LSV	BURB	3	374	5.95	10.5	3.36
90386	10/03/94	275	LSV-BURB-4	LSV	BURB	4	225	16.8	37.8	6.13
90387	10/03/94	275	LSV-BURB-5	LSV	BURB	5	175	NA	22.6	4.31
90389	10/04/94	276	LSV-BURB-7	LSV	BURB	7	344	5.41	8.18	3.14
90390	10/04/94	276	LSV-BURB-8	LSV	BURB	8	117	11.6	12.4	3.93
90391	10/04/94	276	LSV-BURB-9	LSV	BURB	9	237	4.58	24.6	4.83
90392	10/04/94	276	LSV-BURB-10	LSV	BURB	10	221	7.60	17.8	4.10
90393	10/04/94	276	LSV-BURB-11	LSV	BURB	11	205	2.61	14.3	4.04
90394	10/04/94	276	LSV-BURB-12	LSV	BURB	12	235	6.31	28.0	4.40
90395	10/04/94	276	LSV-BURB-13	LSV	BURB	13	331	3.22	10.2	3.77
90396	10/04/94	276	LSV-BURB-14	LSV	BURB	14	278	5.79	13.5	3.84
90397	10/04/94	276	LSV-BURB-15	LSV	BURB	15	475	NA	12.0	3.90
90398	10/04/94	276	LSV-BURB-16	LSV	BURB	16	227	4.50	13.0	5.06
90399	10/04/94	276	LSV-BURB-17	LSV	BURB	17	876	NA	7.55	4.67
90400	10/04/94	276	LSV-BURB-18	LSV	BURB	18	358	NA	46.4	3.60
90401	10/04/94	276	LSV-BURB-19	LSV	BURB	19	225	4.40	6.88	4.36
90504	12/15/94	348	MCR2-BURB-1	MCR2	BURB	1	202	8.01	50.1	4.78
90505	12/15/94	348	MCR2-BURB-2	MCR2	BURB	2	342	10.0	15.1	4.94

Appendix C. Sample identification (UniqID), date sampled (Datesmp), year day of sample (Day), Northern River Basins Code (NRBS#), sample collection site (Site), species, fish number (Fish), and metallothionein concentrations (μg/g) in fish liver, kidney, gill and intestine.

							MT μg/g			
UniqID	Datesmp	Day	NRBS#	Site	Species	Fish	Liver	Kidney	Gill	Intestine
90506	12/15/94	348	MCR2-BURB-3	MCR2	BURB	3	207	3.39	39.3	5.13
90507	12/15/94	348	MCR2-BURB-4	MCR2	BURB	4	208	10.0	12.2	3.94
90508	12/15/94	348	MCR2-BURB-5	MCR2	BURB	5	357	4.70	6.80	3.68
90509	12/15/94	348	MCR2-BURB-6	MCR2	BURB	6	146	5.52	11.0	3.75
90510	12/16/94	349	MCR2-BURB-7	MCR2	BURB	7	336	10.5	11.3	4.83
90308	09/17/94	259	MR-BURB-1	MR	BURB	1	1452	3.24	10.7	6.14
90373	09/29/94	271	P-BURB-1	P	BURB	1	518	NA	18.7	2.31
90374	09/29/94	271	P-BURB-2	P	BURB	2	403	7.68	47.2	5.57
90375	09/30/94	272	P-BURB-3	P	BURB	3	1358	5.22	6.47	5.18
90376	09/30/94	272	P-BURB-4	P	BURB	4	622	16.7	75.5	4.43
90377	10/01/94	273	P-BURB-5	P	BURB	5	839	6.33	22.7	6.09
90145	09/29/94	271	PR1-BURB-2	PR1	BURB	2	513	13.5	10.4	4.26
90148	09/30/94	272	PR1-BURB-3	PR1	BURB	3	308	NA	6.77	3.75
90158	09/30/94	272	PR1-BURB-4	PR1	BURB	4	610	NA	3.95	4.68
90157	09/30/94	272	PR1-BURB-5	PR1	BURB	5	408	NA	15.4	3.91
90161	10/01/94	273	PR1-BURB-6	PR1	BURB	6	1072	13.2	8.78	5.60
90162	10/01/94	273	PR1-BURB-7	PR1	BURB	7	306	5.53	12.8	4.26
90163	10/01/94	273	PR1-BURB-8	PR1	BURB	8	1048	4.35	13.1	4.73
90171	10/03/94	275	PR2-BURB-1	PR2	BURB	1	1134	4.79	5.76	4.92
90176	10/03/94	275	PR2-BURB-2	PR2	BURB	2	1581	NA	37.7	6.08
90173	10/03/94	275	PR2-BURB-4	PR2	BURB	4	361	1.99	23.3	4.26
90175	10/03/94	275	PR2-BURB-4	PR2	BURB	5	952	NA	8.60	5.23
90172	10/03/94	275	PR2-BURB-6	PR2	BURB	6	342	3.13	6.64	3.60
90183	10/04/94	276	PR2-BURB-7	PR2	BURB	7	333	3.51	13.9	4.21
90181	10/04/94	276	PR2-BURB-8	PR2	BURB	8	789	4.56	10.5	4.69
90182	10/04/94	276	PR2-BURB-9	PR2	BURB	9	726	2.37	3.92	3.71
90194	10/05/94	277	PR2-BURB-10	PR2	BURB	10	982	3.58	14.1	4.62
90208	10/07/94	279	PR3-BURB-2	PR3	BURB	2	922	6.87	12.2	10.2
90214	10/07/94	279	PR3-BURB-4	PR3	BURB	4	1025	NA	5.25	5.23
90213	10/07/94	279	PR3-BURB-5	PR3	BURB	5	633	5.79	36.4	7.43
90209	10/07/94	279	PR3-BURB-6	PR3	BURB	6	1156	NA	13.2	9.85
90231	10/09/94	281	PR3-BURB-8	PR3	BURB	8	1073	NA	31.0	8.70
90230	10/09/94	281	PR3-BURB-9	PR3	BURB	9	496	3.56	13.9	3.31
90479	10/15/94	287	SR-BURB-1	SR	BURB	1	358	54.9	19.0	6.18
90478	10/15/94	287	SR-BURB-2	SR	BURB	2	171	51.4	33.3	3.66
90477	10/15/94	287	SR-BURB-3	SR	BURB	3	166	22.9	6.12	0.780
90476	10/15/94	287	SR-BURB-4	SR	BURB	4	279	NA	41.8	4.37
90483	10/16/94	288	SR-BURB-6	SR	BURB	6	176	149	67.4	9.30
90481	10/16/94	288	SR-BURB-7	SR	BURB	7	104	149	76.5	11.5
90493	10/16/94	288	SR-BURB-8	SR	BURB	8	415	46.3	91.5	3.28
90486	10/16/94	288	SR-BURB-9	SR	BURB	9	165	127	52.5	4.64
90484	10/16/94	288	SR-BURB-10	SR	BURB	10	111	55.9	50.3	1.23
90485	10/16/94	288	SR-BURB-11	SR	BURB	11	134	77.4	30.1	6.1
90494	10/16/94	288	SR-BURB-12	SR	BURB	12	273	78.7	9.19	6.83
90482	10/16/94	288	SR-BURB-13	SR	BURB	13	182	64.4	88.4	5.2
90487	10/16/94	288	SR-BURB-14	SR	BURB	14	155	10.3	23.0	6.18
90497	10/16/94	288	SR-BURB-15	SR	BURB	15	203	18.7	127	5.11
90498	10/16/94	288	SR-BURB-16	SR	BURB	16	416	26.6	1.29	11.5
90495	10/16/94	288	SR-BURB-17	SR	BURB	17	235	74.8	52.0	2.75
90496	10/16/94	288	SR-BURB-18	SR	BURB	18	119	101	70.5	7.51

3 of 7

Appendix C. Sample identification (UniqID), date sampled (Datesmp), year day of sample (Day), Northern River Basins Code (NRBS#), sample collection site (Site), species, fish number (Fish), and metallothionein concentrations (μg/g) in fish liver, kidney, gill and intestine.

							MT μg/g			
UniqID	**Datesmp**	**Day**	**NRBS#**	**Site**	**Species**	**Fish**	**Liver**	**Kidney**	**Gill**	**Intestine**
90490	10/17/94	289	SR-BURB-20	SR	BURB	20	111	119	18.2	3.61
90489	10/17/94	289	SR-BURB-21	SR	BURB	21	207	149	55.3	3.71
90007	09/13/94	255	SR1-BURB-1	SR1	BURB	1	728	NA	44.3	2.41
90008	09/13/94	255	SR1-BURB-2	SR1	BURB	2	526	0.700	7.48	3.25
90001	09/14/94	256	SR1-BURB-4	SR1	BURB	4	288	NA	5.00	2.31
90012	09/14/94	256	SR1-BURB-5	SR1	BURB	5	643	17.8	56.4	4.35
90011	09/14/94	256	SR1-BURB-6	SR1	BURB	6	373	NA	12.0	2.06
90002	09/14/94	256	SR1-BURB-7	SR1	BURB	7	1070	NA	51.0	1.68
90003	09/14/94	256	SR1-BURB-8	SR1	BURB	8	350	NA	15.1	2.61
90005	09/14/94	256	SR1-BURB-9	SR1	BURB	9	225	NA	14.2	2.04
90013	09/14/94	256	SR1-BURB-10	SR1	BURB	10	601	NA	11.1	2.62
90014	09/14/94	256	SR1-BURB-11	SR1	BURB	11	151	NA	10.9	1.81
90006	09/14/94	256	SR1-BURB-12	SR1	BURB	12	553	NA	10.0	1.09
90017	09/14/94	256	SR1-BURB-13	SR1	BURB	13	755	NA	6.70	2.29
90016	09/14/94	256	SR1-BURB-14	SR1	BURB	14	536	NA	8.95	2.08
90018	09/14/94	256	SR1-BURB-15	SR1	BURB	15	582	NA	9.81	2.35
90020	09/15/94	257	SR1-BURB-16	SR1	BURB	16	257	NA	10.4	1.65
90021	09/15/94	257	SR-BURB-17	SR1	BURB	17	292	NA	30.0	4.68
90019	09/15/94	257	SR1-BURB-18	SR1	BURB	18	273	NA	5.58	2.02
90031	09/17/94	259	SR1-BURB-19	SR1	BURB	19	535	14.3	19.8	3.03
90512	12/21/94	354	SR2-BURB-1	SR2	BURB	1	243	5.41	21.8	4.33
90235	10/10/94	282	WAB1-BURB-1	WAB1	BURB	1	1222	NA	5.10	2.27
90236	10/11/94	283	WAB1-BURB-2	WAB1	BURB	2	371	NA	5.82	4.00
90239	10/11/94	283	WAB1-BURB-3	WAB1	BURB	3	655	NA	11.8	3.40
90237	10/11/94	283	WAB1-BURB-4	WAB1	BURB	4	473	NA	8.50	4.19
90241	10/11/94	283	WAB1-BURB-5	WAB1	BURB	5	475	NA	9.53	3.25
90238	10/11/94	283	WAB1-BURB-6	WAB1	BURB	6	323	NA	14.6	3.06
90243	10/12/94	284	WAB1-BURB-8	WAB1	BURB	8	681	NA	10.6	2.20
90244	10/12/94	284	WAB1-BURB-9	WAB1	BURB	9	165	NA	7.37	2.37
90093	09/24/94	266	WR1-BURB-1	WR1	BURB	1	238	4.88	6.83	5.63
90092	09/24/94	266	WR1-BURB-2	WR1	BURB	2	308	4.27	19.4	4.43
90106	09/26/94	268	WR1-BURB-3	WR1	BURB	3	648	15.3	17.9	6.25
90107	09/26/94	268	WR1-BURB-4	WR1	BURB	4	838	8.32	16.7	12.3
90278	10/19/94	291	WR2-BURB-1	WR2	BURB	1	349	NA	46.3	4.56
90280	10/19/94	291	WR2-BURB-2	WR2	BURB	2	96.6	3.63	29.1	5.17
90279	10/19/94	291	WR2-BURB-3	WR2	BURB	3	213	NA	19.7	8.51
90275	10/19/94	291	WR2-BURB-4	WR2	BURB	4	200	NA	30.8	4.85
90281	10/19/94	291	WR2-BURB-5	WR2	BURB	5	48.8	2.20	15.8	3.58
90284	10/19/94	291	WR2-BURB-6	WR2	BURB	6	173	NA	29.1	5.31
90274	10/19/94	291	WR2-BURB-7	WR2	BURB	7	249	NA	27.4	2.94
90276	10/19/94	291	WR2-BURB-8	WR2	BURB	8	99.4	NA	20.8	3.97
90277	10/19/94	291	WR2-BURB-9	WR2	BURB	9	271	NA	7.07	5.52
90282	10/19/94	291	WR2-BURB-10	WR2	BURB	10	258	NA	14.7	3.46
90273	10/19/94	291	WR2-BURB-12	WR2	BURB	12	208	NA	17.8	3.82
90283	10/19/94	291	WR2-BURB-13	WR2	BURB	13	54.9	NA	18.3	3.54
90073	09/21/94	263	LSR2-FLCH-1	LSR2	FLCH	1	NA	44.3	16.5	21.7
90160	10/01/94	273	PR1-FLCH-1	PR1	FLCH	1	NA	79.7	NA	32.2
90184	10/04/94	276	PR2-FLCH-2	PR2	FLCH	2	NA	73.0	10.1	52.6
90193	10/05/94	277	PR2-FLCH-3	PR2	FLCH	3	NA	71.0	12.7	39.2
90015	09/14/94	256	SR1-FLCH-1	SR1	FLCH	1	NA	56.8	5.32	7.49

4 of 7

Appendix C. Sample identification (UniqID), date sampled (Datesmp), year day of sample (Day), Northern River Basins Code (NRBS#), sample collection site (Site), species, fish number (Fish), and metallothionein concentrations (µg/g) in fish liver, kidney, gill and intestine.

							MT µg/g			
UniqID	Datesmp	Day	NRBS#	Site	Species	Fish	Liver	Kidney	Gill	Intestine
90022	09/15/94	257	SR1-FLCH-2	SR1	FLCH	2	NA	37.9	2.12	8.05
90023	09/15/94	257	SR1-FLCH-3	SR1	FLCH	3	NA	70.7	14.7	33.6
90028	09/17/94	259	SR1-FLCH-4	SR1	FLCH	4	NA	72.1	18.5	42.2
90026	09/17/94	259	SR1-FLCH-5	SR1	FLCH	5	NA	65.9	16.2	20.5
90030	09/17/94	259	SR1-FLCH-6	SR1	FLCH	6	NA	NA	15.2	3.67
90040	09/19/94	261	SR1-FLCH-8	SR1	FLCH	8	NA	92.8	11.6	20.4
90039	09/19/94	261	SR1-FLCH-9	SR1	FLCH	9	NA	NA	NA	12.5
90045	09/19/94	261	SR1-FLCH-10	SR1	FLCH	10	NA	34.0	14.0	9.02
90046	09/19/94	261	SR1-FLCH-11	SR1	FLCH	11	NA	89.8	8.26	22.7
90044	09/19/94	261	SR1-FLCH-12	SR1	FLCH	12	NA	70.4	8.87	82.5
90242	10/12/94	284	WAB1-FLCH-1	WAB1	FLCH	1	NA	150	15.8	35.9
90082	09/23/94	265	WR1-FLCH-1	WR1	FLCH	1	NA	30.1	14.6	21.2
90109	09/26/94	268	WR1-FLCH-2	WR1	FLCH	2	NA	63.0	NA	NA
90108	09/26/94	268	WR1-FLCH-3	WR1	FLCH	3	NA	100	NA	NA
90111	09/26/94	268	WR1-FLCH-4	WR1	FLCH	4	NA	56.5	NA	NA
90299	09/12/94	254	A1-LNSC-1	A1	LNSC	1	256	67.6	19.6	39.5
90323	09/21/94	263	A2-LNSC-1	A2	LNSC	1	NA	38.4	21.6	37.2
90324	09/22/94	264	A2-LNSC-2	A2	LNSC	2	NA	31.7	30.9	10.3
90325	09/24/94	266	A2-LNSC-3	A2	LNSC	3	139	41.6	28.6	12.6
90326	09/24/94	266	A2-LNSC-4	A2	LNSC	4	158	101	8.37	15.2
90327	09/24/94	266	A2-LNSC-5	A2	LNSC	5	NA	56.9	20.8	NA
90071	09/20/94	262	LSR2-LNSC-1	LSR2	LNSC	1	86.8	92.2	15.3	25.8
90074	09/21/94	263	LSR2-LNSC-2	LSR2	LNSC	2	149	119	48.1	44.8
90075	09/21/94	263	LSR2-LNSC-3	LSR2	LNSC	3	78.1	41.3	14.0	41.2
90078	09/21/94	263	LSR2-LNSC-4	LSR2	LNSC	4	208	79.3	16.8	36.8
90077	09/21/94	263	LSR2-LNSC-5	LSR2	LNSC	5	86.2	63.6	17.2	35.1
90079	09/21/94	263	LSR2-LNSC-6	LSR2	LNSC	6	NA	72.7	19.8	40.5
90314	09/16/94	258	MR-LNSC-1	MR	LNSC	1	92.9	55.7	27.8	27.1
90139	09/28/94	270	PR1-LNSC-1	PR1	LNSC	1	256	283	64.2	16.0
90135	09/28/94	270	PR1-LNSC-2	PR1	LNSC	2	92.1	65.2	57.2	28.5
90130	09/28/94	270	PR1-LNSC-3	PR1	LNSC	3	125	133	105	22.7
90133	09/28/94	270	PR1-LNSC-4	PR1	LNSC	4	134	110	80.3	18.1
90138	09/28/94	270	PR1-LNSC-5	PR1	LNSC	5	123	42.3	48.8	24.2
90142	09/28/94	270	PR1-LNSC-6	PR1	LNSC	6	154	20.3	84.8	25.0
90136	09/28/94	270	PR1-LNSC-7	PR1	LNSC	7	161	60.1	34.4	13.1
90131	09/28/94	270	PR1-LNSC-8	PR1	LNSC	8	320	160	14.6	18.9
90132	09/28/94	270	PR1-LNSC-9	PR1	LNSC	9	152	199	108	33.8
90141	09/28/94	270	PR1-LNSC-10	PR1	LNSC	10	33.9	70.6	50.6	22.8
90137	09/28/94	270	PR1-LNSC-12	PR1	LNSC	12	184	153	74.1	20.0
90143	09/28/94	270	PR1-LNSC-13	PR1	LNSC	13	182	126	48.6	29.2
90129	09/28/94	270	PR1-LNSC-14	PR1	LNSC	14	86.3	117	38.0	25.5
90147	09/30/94	272	PR1-LNSC-15	PR1	LNSC	15	113	111	49.8	25.8
90156	09/30/94	272	PR1-LNSC-16	PR1	LNSC	16	242	114	58.5	34.1
90146	09/30/94	272	PR1-LNSC-17	PR1	LNSC	17	176	1.76	39.1	14.8
90174	10/03/94	275	PR2-LNSC-1	PR2	LNSC	1	218	8.99	21.4	38.7
90167	10/03/94	275	PR2-LNSC-2	PR2	LNSC	2	63.4	148	14.8	33.2
90168	10/03/94	275	PR2-LNSC-3	PR2	LNSC	3	45.1	28.4	39.9	66.7
90166	10/03/94	275	PR2-LNSC-4	PR2	LNSC	4	291	66.5	29.4	80.2
90177	10/03/94	275	PR2-LNSC-5	PR2	LNSC	5	164	31.4	32.3	34.9
90191	10/05/94	277	PR2-LNSC-7	PR2	LNSC	7	171	142	24.8	24.5

Appendix C. Sample identification (UniqID), date sampled (Datesmp), year day of sample (Day), Northern River Basins Code (NRBS#), sample collection site (Site), species, fish number (Fish), and metallothionein concentrations (μg/g) in fish liver, kidney, gill and intestine.

							MT μg/g			
UniqID	Datesmp	Day	NRBS#	Site	Species	Fish	Liver	Kidney	Gill	Intestine
90192	10/05/94	277	PR2-LNSC-8	PR2	LNSC	8	296	53.2	22.5	28.1
90200	10/07/94	279	PR3-LNSC-1	PR3	LNSC	1	220	247	22.1	51.7
90205	10/07/94	279	PR3-LNSC-2	PR3	LNSC	2	488	514	17.8	43.9
90201	10/07/94	279	PR3-LNSC-3	PR3	LNSC	3	175	162	5.20	31.9
90202	10/07/94	279	PR3-LNSC-4	PR3	LNSC	4	198	361	9.82	98.1
90198	10/07/94	279	PR3-LNSC-5	PR3	LNSC	5	169	465	19.3	92.5
90206	10/07/94	279	PR3-LNSC-6	PR3	LNSC	6	173	353	25.4	96.7
90210	10/07/94	279	PR3-LNSC-7	PR3	LNSC	7	156	65.7	26.6	38.4
90199	10/07/94	279	PR3-LNSC-8	PR3	LNSC	8	112	210	22.9	22.2
90207	10/07/94	279	PR3-LNSC-9	PR3	LNSC	9	77.7	166	13.9	59.6
90203	10/07/94	279	PR3-LNSC-10	PR3	LNSC	10	95.9	76.2	11.7	39.7
90204	10/07/94	279	PR3-LNSC-11	PR3	LNSC	11	235	130	14.6	39.2
90218	10/08/94	280	PR3-LNSC-12	PR3	LNSC	12	220	392	7.27	32.0
90217	10/08/94	280	PR3-LNSC-13	PR3	LNSC	13	138	154	16.1	24.3
90224	10/08/94	280	PR3-LNSC-14	PR3	LNSC	14	162	288	14.6	32.2
90225	10/08/94	280	PR3-LNSC-15	PR3	LNSC	15	124	142	18.7	27.1
90219	10/08/94	280	PR3-LNSC-16	PR3	LNSC	16	122	197	23.3	97.6
90223	10/08/94	280	PR3-LNSC-17	PR3	LNSC	17	193	440	14.7	55.3
90221	10/08/94	280	PR3-LNSC-18	PR3	LNSC	18	132	188	11.9	16.8
90220	10/08/94	280	PR3-LNSC-19	PR3	LNSC	19	128	190	18.5	110
90222	10/08/94	280	PR3-LNSC-20	PR3	LNSC	20	103	143	16.3	84.2
90227	10/08/94	280	PR3-LNSC-21	PR3	LNSC	21	118	414	17.0	101
90226	10/08/94	280	PR3-LNSC-23	PR3	LNSC	23	172	228	31.0	23.9
90027	09/17/94	259	SR1-LNSC-1	SR1	LNSC	1	185	44.6	24.2	25.1
90025	09/17/94	259	SR1-LNSC-2	SR1	LNSC	2	187	73.6	23.8	10.5
90029	09/17/94	259	SR1-LNSC-3	SR1	LNSC	3	NA	36.9	31.6	23.1
90036	09/19/94	261	SR1-LNSC-4	SR1	LNSC	4	295	105	31.5	13.9
90032	09/18/94	260	SR1-LNSC-5	SR1	LNSC	5	364	3.48	48.1	22.6
90035	09/19/94	261	SR1-LNSC-6	SR1	LNSC	6	195	85.2	10.6	10.6
90033	09/18/94	260	SR1-LNSC-7	SR1	LNSC	7	109	20.1	30.9	13.1
90037	09/19/94	261	SR1-LNSC-8	SR1	LNSC	8	270	248	6.21	24.3
90043	09/19/94	261	SR1-LNSC-9	SR1	LNSC	9	206	15.9	16.4	11.4
90041	09/19/94	261	SR1-LNSC-10	SR1	LNSC	10	148	44.8	25.0	23.9
90042	09/19/94	261	SR1-LNSC-11	SR1	LNSC	11	329	74.5	21.7	21.2
90048	09/19/94	261	SR1-LNSC-12	SR1	LNSC	12	297	71.1	23.2	21.5
90245	10/16/94	288	SR2-LNSC-1	SR2	LNSC	1	151	116	18.5	23.3
90089	09/23/94	265	WR1-LNSC-1	WR1	LNSC	1	176	156	14.5	29.2
90088	09/23/94	265	WR1-LNSC-2	WR1	LNSC	2	193	35.7	31.6	33.0
90085	09/23/94	265	WR1-LNSC-3	WR1	LNSC	3	89.3	67.9	8.23	20.2
90086	09/23/94	265	WR1-LNSC-4	WR1	LNSC	4	488	79.6	28.9	50.8
90083	09/23/94	265	WR1-LNSC-5	WR1	LNSC	5	356	81.9	33.5	44.5
90087	09/23/94	265	WR1-LNSC-6	WR1	LNSC	6	71.8	88.1	9.47	15.3
90084	09/23/94	265	WR1-LNSC-7	WR1	LNSC	7	51.2	16.6	15.3	8.26
90091	09/23/94	265	WR1-LNSC-8	WR1	LNSC	8	83.7	94.1	5.80	11.2
90095	09/24/94	266	WR1-LNSC-9	WR1	LNSC	9	93.6	86.6	7.96	21.3
90094	09/24/94	266	WR1-LNSC-10	WR1	LNSC	10	66.1	66.1	10.5	7.39
90096	09/24/94	266	WR1-LNSC-11	WR1	LNSC	11	90.1	78.7	10.7	26.2
90097	09/24/94	266	WR1-LNSC-12	WR1	LNSC	12	72.4	55.8	11.6	36.7
90103	09/25/94	267	WR1-LNSC-13	WR1	LNSC	13	432	511	25.8	21.6
90295	09/13/94	255	A1-NRPK-1	A1	NRPK	1	165	49.9	0.960	9.56

Appendix C. Sample identification (UniqID), date sampled (Datesmp), year day of sample (Day), Northern River Basins Code (NRBS#), sample collection site (Site), species, fish number (Fish), and metallothionein concentrations (µg/g) in fish liver, kidney, gill and intestine.

							MT µg/g			
UniqID	**Datesmp**	**Day**	**NRBS#**	**Site**	**Species**	**Fish**	**Liver**	**Kidney**	**Gill**	**Intestine**
90296	09/13/94	255	A1-NRPK-2	A1	NRPK	2	328	33.9	0.85	6.19
90297	09/15/94	257	A1-NRPK-3	A1	NRPK	3	NA	23.1	0.81	6.59
90298	09/15/94	257	A1-NRPK-4	A1	NRPK	4	79.6	31.1	1.09	5.36
90473	10/15/94	287	A5-NRPK-1	A5	NRPK	1	NA	91.2	6.36	8.16
90434	10/11/94	283	CW-NRPK-1	CW	NRPK	1	NA	245	1.69	28.4
90499	10/19/94	291	JV2-NRPK-1	JV2	NRPK	1	199	208	7.01	10.9
90500	10/19/94	291	JV2-NRPK-2	JV2	NRPK	2	105	32.8	3.65	8.01
90501	10/19/94	291	JV2-NRPK-3	JV2	NRPK	3	227	155	13.9	71.8
90502	10/19/94	291	JV2-NRPK-4	JV2	NRPK	4	95.1	168	7.83	19.3
90503	10/19/94	291	JV2-NRPK-5	JV2	NRPK	5	198	101	7.35	15.8
90309	09/18/94	260	MR-NRPK-1	MR	NRPK	1	338	43.5	4.3	11.3
90310	09/18/94	260	MR-NRPK-2	MR	NRPK	2	62.9	63.7	2.93	8.86
90311	09/19/94	261	MR-NRPK-3	MR	NRPK	3	243	52.6	7.49	8.41
90312	09/19/94	261	MR-NRPK-4	MR	NRPK	4	313	31.9	3.13	12.3
90379	09/29/94	271	P-NRPK-1	P	NRPK	1	NA	159	25.0	16.8
90380	09/30/94	272	P-NRPK-2	P	NRPK	2	NA	152	9.94	13.6
90127	09/28/94	270	PR1-NRPK-1	PR1	NRPK	1	209	152	2.72	9.10
90128	09/28/94	270	PR1-NRPK-2	PR1	NRPK	2	95.1	116	2.82	10.2
90140	09/28/94	270	PR1-NRPK-3	PR1	NRPK	3	103	69.8	2.60	9.27
90159	09/30/94	272	PR1-NRPK-4	PR1	NRPK	4	NA	114	3.30	11.2
90169	10/03/94	275	PR2-NRPK-1	PR2	NRPK	1	126	36.5	3.73	9.96
90170	10/03/94	275	PR2-NRPK-2	PR2	NRPK	2	273	59.0	3.54	11.3
90178	10/03/94	275	PR2-NRPK-3	PR2	NRPK	3	NA	63.2	4.28	26.6
90491	10/17/94	289	SR-NRPK-2	SR	NRPK	2	404	35.7	3.23	3.01
90492	10/17/94	289	SR-NRPK-3	SR	NRPK	3	75.8	68.6	2.17	5.55
90010	09/13/94	255	SR1-NRPK-1	SR1	NRPK	1	379	22.0	1.07	9.86
90004	09/14/94	256	SR1-NRPK-2	SR1	NRPK	2	539	59.5	2.18	9.66
90034	09/19/94	261	SR1-NRPK-3	SR1	NRPK	3	276	56.7	1.03	5.09
90080	09/22/94	264	WR1-NRPK-1	WR1	NRPK	1	78.2	57.0	1.21	10.3
90081	09/22/94	264	WR1-NRPK-2	WR1	NRPK	2	297	39.1	1.15	7.23
90090	09/23/94	265	WR1-NRPK-3	WR1	NRPK	3	378	50.5	1.38	9.77
90098	09/25/94	267	WR1-NRPK-4	WR1	NRPK	4	193	38.3	1.18	7.59
90100	09/25/94	267	WR1-NRPK-5	WR1	NRPK	5	97.3	72.4	1.84	14.7
90099	09/25/94	267	WR1-NRPK-6	WR1	NRPK	6	109	122	1.42	17.0
90102	09/25/94	267	WR1-NRPK-7	WR1	NRPK	7	131	101	2.15	11.5
90101	09/25/94	267	WR1-NRPK-8	WR1	NRPK	8	215	72.7	2.37	7.74
90105	09/26/94	268	WR1-NRPK-9	WR1	NRPK	9	582	63.6	1.23	9.21
90104	09/26/94	268	WR1-NRPK-10	WR1	NRPK	10	159	45.5	1.81	7.77
90112	09/26/94	268	WR1-NRPK-11	WR1	NRPK	11	87.9	69.5	2.03	7.77
90113	09/26/94	268	WR1-NRPK-12	WR1	NRPK	12	NA	52.8	1.81	6.00
90286	10/20/94	292	WR2-NRPK-1	WR2	NRPK	1	47.0	48.7	5.07	6.22

7 of 7