

AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM

**1995 Field evaluation of aquatic effects
monitoring methods - Pilot study**

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**1995 FIELD EVALUATION OF AQUATIC EFFECTS MONITORING METHODS
PILOT STUDY
Volume 1 - Report**

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EXECUTIVE SUMMARY

This study forms part of the Aquatic Effects Technology Evaluation Program (AETE) which is a cooperative government-industry program to review and evaluate environmental monitoring technologies for the assessment of mining-related impacts on the aquatic environment. The intention is to apply sound scientific principles to environmental effects monitoring in a cost-effective manner.

The program will ultimately involve a number of studies to evaluate environmental effects monitoring technologies at a number of mine sites across Canada. Beak Consultants Limited (BEAK) was selected to undertake a pilot study in the Val-d'Or region of Quebec to guide the design of the programs that will be implemented at selected mine sites across Canada. The following environmental monitoring methods were selected by the AETE Technical Committee for evaluation at the Val-d'Or pilot site:

- . a comparison of surficial sediment mapping techniques (sub-bottom acoustical profiling system versus sonar and grab sampling);
- . a comparison of the effectiveness of coring devices (Hornbrook, Kajak-Brinkhurst, and alpine corers) to quantify pre-operational metal levels;
- . an evaluation of the cost-effectiveness of using the lowest method detection limits achievable for water and sediment chemistry analyses;
- . a comparison of analytical methods for assessing sediment quality to predict biological effects by measuring either total metals in sediments by full extraction or those readily extractable (assumed to be more biologically available) by partial digestion;
- . an assessment of three sediment toxicity methods (Microtox™, *Hyalella azteca*, and *Tubifex tubifex*) to assess their ability to accurately predict adverse biological effects from metal contamination of sediments;
- . a comparison of the ability of artificial substrates versus grab samples to measure mining-related effects on benthic invertebrate communities in depositional habitats;
- . an evaluation of the cost-effectiveness and sensitivity of benthic invertebrate processing methods (sieve sizes, level of taxonomy, number of replicates) to delineate and monitor mining-related impacts;
- . an assessment of the effects of mining discharges on fish communities by evaluating the four main response characteristics; age structure, growth, energy storage and reproduction;
- . an evaluation of metal accumulations in various fish tissues (gill filament, kidney, liver, flesh, viscera); and
- . a comparison of metal levels in tissues to metallothionein levels and histopathology.

The sensitivity, cost effectiveness and appropriateness of each method was assessed during the pilot study.

The study area was situated in the headwaters of the Kinojévis River, immediately west of the Town of Cadillac and approximately 40 km east of the Town of Rouyn and involved assessments in the receiving environments of Mine Doyon, Complexe Bousquet and Mine Dumagami. All three mines are primarily gold mining operations. The mine effluent receiving waters were rivière Noire, rivière Bousquet, lac Chassignolle and lac Preissac.

There were three main phases in the 1995 Pilot Study as follows:

- . Phase I was an assessment of the adequacy of the site selected by the AETE Technical Committee by undertaking a field reconnaissance survey and preparing a field study design;
- . Phase II was the implementation of the study design; and
- . Phase III was the preparation of a final report assessing and comparing the methods and recommending modifications to the design that would allow the AETE Technical Committee to reliably compare methods in subsequent years.

COMPARISON OF SURFICIAL SEDIMENT MAPPING TECHNIQUES

This component of the pilot study included a comparative evaluation of subbottom acoustical profiling methods and more conventional bottom grab sampling combined with the use of a standard sonar unit.

Sediment characteristics were identified in more detail by subbottom profiling than by sonar/grab sampling methods. However, results of the subbottom profiling work in this study were far less cost effective for simple mapping of habitat types for an environmental effects monitoring study. Confirmatory cores with up to almost 1 meter penetration in some cases suggested that the sub-bottom profiling was prone to misinterpretation of sediment types. In terms of bathymetry mapping, both the subbottom profiler and conventional sonar yielded comparable results.

It was concluded that bottom characterization using conventional sonar techniques, supplemented with grab sampling, is generally the more cost-effective approach for identifying depositional areas and determining where sediment geochemistry and bioassessment studies should be carried out.

EVALUATION OF SEDIMENT CORING METHODS

Three coring devices were compared: Hornbrook, Alpine and K-B corers.

The study concluded that either the Hornbrook sampler or other core samplers could be used in environmental effects monitoring, however, the gravity type corers allowed for more detailed measurement of metal profiles, including knowing the exact depths at which sediment contamination extends. They also had less risk of contamination of the deeper sediments during collection when compared to the Hornbrook sampler.

Water Quality

Water quality conditions were evaluated in the rivière Bousquet/lac Chassignolle and in the rivière Noire system principally to define conditions which may influence biological communities (i.e., fish and benthic parameters). Information is also presented on costs associated with achieving relatively routine and lower analytical detection limits (1/10th CCME water quality guidelines).

It was found on the rivière Bousquet receiving environment that concentrations of potentially toxic heavy metals (Pb, Zn, Cu, Cd) did not change appreciably between the upstream reference station and the river mouth. Concentrations of heavy metals were low in mine effluents, and were at most only about four times greater than found at the reference station. Trace element analyses of rivière Noire and ruisseau Dormenan showed several parameters elevated above upstream reference levels with arsenic being the most evident.

In terms of parameters and detection limits for trace elements the ICP-MS provided for analysis of more elements than ICP, and typically provided for detection limits which are 1,000 fold lower than provided by ICP for similar costs. Thus, ICP-MS solves detection limit problems with ICP, which typically requires supplementary analyses to achieve detection limits equal to or lower than some water quality criteria.

Sediment Quality

Sediment quality conditions were evaluated for the rivière Bousquet/lac Chassignolle system and more so in the rivière Noire system. Results showed no obvious increases in metal concentrations in lac Chassignolle relative to lac Bousquet (reference). Arsenic concentrations were highly elevated in rivière Noire and the river mouth area. The data indicated that the sources were from inactive mines. Other metals appearing to be slightly enriched downstream of the mine sites included nickel, copper and zinc.

Partial extraction results were compared to total extraction results for selective stations. Partial extraction generally extracted most of the cadmium, little of the copper, about half of the arsenic and zinc, and about 30% of the iron and nickel.

Principal Components Analysis demonstrated that station groupings based on full and partial extraction were similar and both were similar to station groupings based on benthic communities.

The study concluded that full extraction of metals coupled with TOC and grain size analyses was better able to detect significant differences with greater power among reference, near-field and far-field areas.

SEDIMENT TOXICITY TESTING

Sediment toxicity was evaluated by exposing organisms to sediment and measuring survival and growth in the amphipod *Hyaella azteca*, survival and reproduction in the tubificid oligochaete *Tubifex tubifex* and inhibition of photoluminescence in the Microtox® bacterium *Vibrio fischeri*.

Survival of amphipods at the reference sites ranged from 66% to 92% while the sediment toxicity downstream of the mine discharge ranged from 0 to 16%.

Survival of *Tubifex tubifex* at all stations was comparable to the reference sites with the exception of one station in lac Preissac. The number of hatched young produced by oligochaetes exposed to sediment at all stations was comparable to that at reference sites except at one station downstream of an idle mine site. At this location, the number of young counted per adult was also lower than reference site production by about 99%. Oligochaetes exposed to sediment downstream of the mine discharge exhibited an 83% reduction in young produced per adult and showed toxic effects approximately 1 km downstream (32% to 42% reduction in young production).

Sediment from all stations on rivière Noire showed some level of inhibition with the Microtox test when compared to IC50 values for reference stations.

Only *Tubifex tubifex* results showed significant differences between the reference area and the near-field. Percent *Hyaella* survival was the only test which showed a significant difference between reference and far-field areas.

Hyalella survival appeared to provide the most sensitive response. The *Tubifex* production of young per adult provided slightly less sensitivity than *Hyalella* survival but did provide the best graded response to environmental stress and showed less variability among reference stations. The Microtox® test identified the most toxic sediment samples and sediments of intermediate toxicity but revealed no significant difference between reference, near-field and far-field areas.

BENTHIC INVERTEBRATE COMMUNITY ASSESSMENT

The study compared the effectiveness and sensitivity of the following benthic invertebrate processing methods:

- . family, genus and species level identifications;
- . sieve sizes, 200 μm , 500 μm , and 1000 μm ;
- . testing with pooled and unpooled replicate data;
- . artificial substrates versus grab samples in depositional habitats; and
- . effect of the number of replicate samples.

The study also examined the relationships among sediment chemistry, toxicity and benthic invertebrate community structure.

The following are the major conclusions of this study:

- . Unpooled replicate samples allow for characterization of variation of stations within groups, but had little additional value;
- . Taxonomic identifications were influenced by sieve size;
- . 200 μm mesh sieves distinguished area differences most reliably;
- . Species or genus-level taxonomy distinguished area differences most reliably;
- . Ponar-grab samples appeared more likely to detect differences between reference and exposed benthic communities than were suspended artificial substrates;
- . Full extraction of sediment metals showed more reliable separation of reference and impacted areas but was more poorly related to benthic indices than was the case for partial sediment metal extraction.
- . Detailed benthic invertebrate data (e.g., species level taxonomy and 200 μm mesh sieves) showed stronger relationships with sediment chemistry than coarse benthic data (e.g., family level taxonomy and 1000 μm mesh sieves).
- . Agreement between benthic indices and toxicity endpoints was most notable for *Hyalella* mortality and *Tubifex* reproduction tests, with either detailed or coarse benthic data sets.

FISH SURVEY

The fish survey was carried out to evaluate various methods for measuring mining-related fish community impacts. The survey included the collection of information on the general health, condition and reproductive status of fish from various environments, and the collection of information on metal bioaccumulation in various tissues (muscle, gills, liver, kidney) and metallothionein (MT) responses in those tissues. Also, samples of various tissues were collected for histopathological analysis. The fish examined for this study included walleye, white sucker, silver redhorse, northern pike, yellow perch, stickleback, and brown bullhead.

The results of the study showed that on the rivière Bousquet system clear differences in concentrations of heavy metals did not exist between exposure and reference areas for individual fish species and tissue types and MT concentrations showed no near clear upstream-downstream differences in the tissues sampled that might be attributed to mining-related metal exposure or to increased metal concentration downstream of the mines. Several metals varied with age of fish, depending on tissue type, although all of these relationships were weak and metals were significantly elevated in some tissues of some exposed fish, and in other cases in some tissues of some reference fish.

Muscle was rarely the tissue where the greatest level of accumulation occurred; kidney, gill or liver were usually dominant among tissue types in terms of metal concentration.

As observed with adult fish, metal data for small fish were highly variable in terms of differences within and among metals, species and locations.

Regression analysis of the metal and MT data showed significant and positive relationships between metals and MT in liver of adult white sucker and northern pike, although none was found in other tissue types

Histological analyses of gills demonstrated no serious impairments in any of the fish examined. No serious impairments to kidney function were observed in white sucker and northern pike from the Bousquet-reference and Bousquet-exposure sites. Histological analyses of liver from white sucker and walleye from rivière Noire mouth demonstrated some apparently adaptive responses. These included larger hepatocyte nuclear diameter and greater hepatocyte cell areas. Northern pike from the rivière Bousquet exposure site had considerably less glycogen than liver from rivière Bousquet reference pike. Information was also obtained on vitellogenesis (egg development) in female white sucker, northern pike and walleye. No differences were observed between rivière Bousquet reference and exposure sites in egg development of white sucker and northern pike as measured by gonadosomatic indices (GSIs), relative fecundities (eggs/gram of fish), egg diameters and weights, and percent of clutch oocytes.

The major conclusions of the fisheries component of the study are as follows:

- . metals may be readily measured at detectable levels in all tissue types sampled; there is probably some inherent redundancy in the analysis of four tissue types in adult fish;
- . hepatic MT levels covaried with the sum of hepatic cadmium, zinc and copper; monitoring of MT in liver may be more effective than monitoring of MT in gill or kidney;
- . histological work indicated some possible differences in fish tissues among sites, suggesting that such measurements may be of some value as measures of biological response; and
- . young fish are easier to collect and maintain in viable condition than adult fish; field costs could be substantially reduced if small fish viscera could be monitored rather than organs of large fish.

The study has also made a number of recommendations for future AETE programs at selected mine sites across Canada.

RÉSUMÉ

La présente étude a été réalisée dans le cadre du Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ETIMA), initiative conjointe du gouvernement et de l'industrie qui vise à examiner et à évaluer les techniques de surveillance environnementale permettant de déterminer l'impact des activités minières sur les environnements aquatiques. L'objectif visé consiste à appliquer des principes scientifiques rigoureux à la surveillance des effets environnementaux, selon des critères de coût-efficacité.

Le programme prévoit la réalisation d'une série d'évaluations des techniques de surveillance des effets environnementaux dans un certain nombre de sites miniers répartis dans diverses régions du Canada. La société Beak Consultants Limited (BEAK) a été choisie pour réaliser, dans la région de Val-d'Or (Québec), une étude pilote destinée à orienter la conception des programmes qui seront mis en oeuvre dans des sites miniers choisis en divers endroits du pays. Les méthodes de surveillance environnementale suivantes ont été sélectionnées par le Comité technique du Programme en vue de cette évaluation-pilote :

- comparaison de techniques de cartographie des sédiments de surface (relevé acoustique des couches sédimentaires subsuperficielles, relevé au sonar et échantillonnage ponctuel à l'aide d'une benne);
- comparaison de l'efficacité relative des dispositifs d'échantillonnage (carottiers Hornbrook, Kajak-Brinkhurst et Alpine), en vue de quantifier les concentrations pré-opérationnelles de métaux;
- évaluation visant à déterminer s'il est rentable de recourir aux méthodes utilisant les plus faibles seuils de détection pour les analyses chimiques de l'eau et des sédiments;
- comparaison des méthodes analytiques utilisées pour évaluer la qualité des sédiments, en vue de prévoir les effets biologiques. Cette comparaison reposera sur la mesure soit de la totalité des métaux présents dans les sédiments par extraction complète, soit des métaux faciles à extraire par digestion partielle (métaux présentant vraisemblablement une plus grande biodisponibilité);
- évaluation de trois méthodes de détermination de la toxicité des sédiments (Microtox^{MD}, *Hyalella azteca* et *Tubifex tubifex*), d'après leur capacité de prévoir avec précision les effets biologiques indésirables résultant de la contamination des sédiments par les métaux;

- évaluation de la capacité relative des supports artificiels et des échantillonnages ponctuels de mesurer l'incidence des activités minières sur les communautés d'invertébrés benthiques dans les zones de déposition;
- évaluation du rapport coût-efficacité et de la sensibilité des méthodes de traitement des invertébrés benthiques (taille des mailles, niveau d'identification taxinomique, nombre de répétitions) utilisées pour délimiter et surveiller l'impact des activités minières;
- évaluation des effets des effluents miniers sur les communautés de poissons, fondée sur l'examen des quatre principaux indicateurs de la réponse de ces organismes : structure d'âge, croissance, stockage d'énergie et reproduction;
- évaluation des concentrations de métaux accumulées dans divers tissus des poissons (branchies, reins, foie, muscles et viscères);
- comparaison des concentrations de métaux accumulées dans les tissus avec les concentrations de métallothionéine et les données histopathologiques.

La sensibilité, l'efficacité par rapport au coût et l'utilité de chaque méthode ont été évaluées au cours de l'étude pilote.

La zone d'étude était située dans le cours supérieur de la Kinojévis, immédiatement à l'ouest de la ville de Cadillac et à environ 40 km à l'est de la ville de Rouyn. Les évaluations ont été réalisées dans les eaux recevant les effluents de la mine Doyon, du complexe Bousquet et de la mine Dumagami. Ces trois mines, exploitées essentiellement pour l'or, rejettent leurs effluents dans la rivière Noire, la rivière Bousquet, le lac Chassignolle et le lac Preissac.

L'étude pilote de 1995 s'est déroulée en trois étapes :

- Étape I Évaluation du caractère adéquat du site choisi par le Comité technique du Programme ETIMA, d'après une étude de reconnaissance sur le terrain, et préparation du plan de l'étude sur le terrain;
- Étape II Mise en oeuvre du plan de l'étude;

Étape III Préparation d'un rapport final comportant une évaluation et une comparaison des méthodes, ainsi qu'une série de recommandations concernant les modifications à apporter au plan de l'étude en vue de permettre au Comité technique du Programme ETIMA d'effectuer une comparaison fiable des méthodes au cours des années subséquentes.

COMPARAISON DES TECHNIQUES DE CARTOGRAPHIE DES SÉDIMENTS DE SURFACE

Ce volet de l'étude pilote avait pour objet de comparer l'efficacité relative du relevé acoustique des couches sédimentaires subsuperficielles à celle d'une méthode plus conventionnelle, l'échantillonnage ponctuel à l'aide d'une benne jumelée à l'utilisation d'un sonar standard.

Le relevé acoustique des couches sédimentaires subsuperficielles a permis une caractérisation plus fine des sédiments que les méthodes d'échantillonnage ponctuel avec sonar. Toutefois, les résultats des relevés acoustiques des couches sédimentaires subsuperficielles effectués dans le cadre de la présente étude se sont révélés nettement moins utiles, compte tenu des sommes investies pour les recueillir, aux seules fins de la cartographie des types d'habitats pour la surveillance des effets environnementaux. Des carottes prélevées à des fins de confirmation, dans certains cas à une profondeur atteignant presque 1 mètre, ont indiqué que le relevé acoustique des couches sédimentaires subsuperficielles pouvait conduire à des erreurs d'interprétation en ce qui a trait aux types de sédiments. Pour ce qui est de la cartographie bathymétrique, les deux méthodes comparées ont donné des résultats similaires.

L'étude pilote a révélé que la caractérisation des sédiments à l'aide de techniques conventionnelles faisant appel au sonar jumelées à l'échantillonnage ponctuel à l'aide de bennes est généralement la méthode la plus économique pour délimiter les zones de sédimentation et les endroits où les études géochimiques et les évaluations biologiques des sédiments devraient être effectuées.

ÉVALUATION DES MÉTHODES D'ÉCHANTILLONNAGE DES SÉDIMENTS

Trois dispositifs d'échantillonnage (carottiers) ont été comparés : Hornbrook, Alpine et Kajak-Brinkhurst.

L'étude a révélé que l'échantillonneur Hornbrook ou d'autres dispositifs de carottage peuvent être utilisés à profit pour la surveillance des effets environnementaux, mais que les dispositifs faisant appel à la gravité permettent d'obtenir des mesures plus détaillées des profils des métaux et, notamment, de déterminer avec précision jusqu'à quelle profondeur s'étend la contamination des sédiments. Ces dispositifs comportent également un plus faible risque de contamination des sédiments plus profonds durant le prélèvement que l'échantillonneur Hornbrook.

Qualité de l'eau

La qualité de l'eau a été évaluée dans le bassin de la rivière Bousquet/lac Chassignolle et dans le bassin de la rivière Noire. Cet exercice visait essentiellement à déterminer les conditions qui peuvent avoir une incidence sur les communautés biologiques (p. ex., paramètres liés aux poissons et aux invertébrés benthiques). Des données concernant les coûts associés à l'utilisation des seuils de détection analytique plus faibles courants (dix fois plus faibles que les valeurs prescrites dans les normes sur la qualité de l'eau du CCME) sont également présentées.

On a constaté que les concentrations de métaux lourds potentiellement toxiques (Pb, Zn, Cu, Cd) dans les eaux réceptrices de la rivière Bousquet ne variaient pas de manière appréciable entre la station de référence, située en amont, et l'embouchure de la rivière. Les concentrations de métaux lourds dans les effluents miniers étaient faibles, tout au plus quatre fois plus élevées que celles enregistrées dans la station de référence. L'analyse des éléments traces présents dans la rivière Noire et le ruisseau Dormenan a révélé, pour plusieurs métaux, des concentrations plus élevées que les valeurs de référence enregistrées en amont, l'arsenic étant le cas le plus évident.

Pour ce qui est des paramètres et des seuils de détection applicables aux éléments traces, l'ICP-SM a permis d'analyser un plus grand nombre d'éléments que l'ICP. En outre, l'ICP-SM utilise typiquement des seuils de détection 1 000 fois plus faibles que ceux de l'ICP, et ce à un coût comparable. Ainsi, l'ICP-SM permet d'éviter les problèmes découlant des seuils de détection utilisés par l'ICP qui, typiquement, nécessite des analyses additionnelles pour atteindre les seuils de détection prescrits pour certains critères de qualité de l'eau.

Qualité des sédiments

La qualité des sédiments a été évaluée dans les bassins de la rivière Bousquet/lac Chassignolle et de la rivière Noire. Les résultats n'ont révélé aucune augmentation évidente des concentrations des métaux lourds dans le lac Chassignolle par rapport aux valeurs de référence enregistrées dans le lac Bousquet. Les concentrations d'arsenic étaient très élevées dans la rivière Noire et son embouchure. Les données ont indiqué

que les sources étaient des mines désaffectées. Les concentrations de certains métaux, dont le nickel, le cuivre et le zinc, semblaient aussi légèrement plus élevées en aval des sites miniers.

Les résultats de l'extraction partielle ont été comparés à ceux de l'extraction complète pour certaines stations choisies. De façon générale, l'extraction partielle a permis de récupérer la majeure partie du cadmium, une faible proportion du cuivre, environ la moitié de l'arsenic et du zinc et environ 30 % du fer et du nickel.

L'analyse en composantes principales a démontré que les regroupements de stations fondés sur les résultats de l'extraction complète et de l'extraction partielle étaient semblables et comparables aux regroupement fondés sur l'analyse des communautés benthiques.

L'étude a également révélé que l'extraction complète des métaux, couplée au dosage du COT et à l'analyse granulométrique, permet de déceler plus efficacement des différences significatives, avec une plus grande puissance, entre les secteurs de référence et les zones avoisinantes et éloignées du site de déversement.

ÉVALUATION DE LA TOXICITÉ DES SÉDIMENTS

Pour évaluer la toxicité des sédiments, on a exposé des organismes choisis aux sédiments, puis mesuré les paramètres suivants : survie et croissance chez l'amphipode *Hyalella azteca*, survie et reproduction chez l'oligochète tubificidé *Tubifex tubifex*, inhibition de la photoluminescence chez la bactérie *Vibrio fischeri* (épreuve Microtox^{MD}).

La survie des amphipodes a oscillé entre 66 et 92 % dans les sites de référence, tandis que la toxicité des sédiments a varié de 0 à 16 % dans les stations situées en aval des effluents miniers.

Les taux de survie de *Tubifex tubifex* dans toutes les stations étaient comparables aux valeurs enregistrées dans les sites de référence, sauf dans une station du lac Preissac. Le nombre de descendants éclos produits par les oligochètes exposés aux sédiments dans toutes les stations était comparable à celui observé dans les sites de référence, sauf dans une station située en aval d'une mine désaffectée. À cet endroit, le nombre de descendants par adulte était également plus faible que dans le site de référence, dans une proportion d'environ 99 %. En aval des effluents miniers, la réduction du nombre de descendants par adulte atteignait 83 %, et les effets liés à la toxicité chez les oligochètes exposés étaient perceptibles jusqu'à environ 1 km en aval du point de rejet des effluents (réduction du nombre de descendants par adulte oscillant entre 32 à 42 %).

Une comparaison des valeurs de CI_{50} avec celles enregistrées dans les stations de référence a révélé que les sédiments de toutes les stations de la rivière Noire exerçaient une certaine activité inhibitrice (épreuve Microtox^{MD}).

Les seules différences significatives relevées entre la station de référence et les sites avoisinants ont été observées chez *Tubifex tubifex*. Le taux de survie de *Hyaella* (en pourcentage) est le seul paramètre qui différait de façon significative entre les stations de référence et les sites éloignés du site de déversement.

C'est le taux de survie chez *Hyaella* qui s'est révélé l'indicateur de la toxicité le plus sensible. Toutefois, malgré une sensibilité légèrement inférieure, c'est la production de descendants par adulte chez *Tubifex* qui a présenté la réponse la mieux graduée au stress environnemental et présenté la plus faible variabilité d'une station de référence à l'autre. Bien qu'elle ait permis de détecter les échantillons de sédiments de toxicité maximale et intermédiaire, l'épreuve Microtox^{MD} n'a pas révélé de différences significatives entre les stations de référence, les sites avoisinants et les sites éloignés.

ÉTUDE DE LA COMMUNAUTÉ DES INVERTÉBRÉS BENTHIQUES

L'étude a permis de comparer l'efficacité et la sensibilité des méthodes de traitement des invertébrés benthiques suivantes :

- niveau d'identification privilégié (famille, genre et espèce);
- taille des mailles (200, 500 et 1 000 μm);
- type de données répétées (regroupées et non regroupées);
- supports artificiels par opposition à des échantillons ponctuels prélevés à l'aide d'une benne dans les zones de sédimentation;
- nombre d'échantillons répétés.

L'étude a également permis d'examiner les relations entre la chimie et la toxicité des sédiments et la structure de la communauté des invertébrés benthiques.

Les principales conclusions de l'étude sont les suivantes :

- les échantillons répétés non regroupés ont permis de faire ressortir la variation entre les stations au sein des groupes, mais ont procuré peu d'avantages additionnels;
- la taille des mailles a eu une incidence sur l'identification taxonomique;
- les tamis à mailles de 200 μm ont permis de distinguer d'une manière plus fiable les différences entre les zones;
- l'identification jusqu'à l'espèce ou jusqu'au genre a fourni le meilleur potentiel discriminatoire entre les zones étudiées;

- chez les invertébrés benthiques, l'échantillonnage ponctuel à l'aide d'une benne Ponar a semblé plus efficace que les supports artificiels suspendus pour faire ressortir les différences entre les communautés de référence et les communautés exposées;
- l'extraction complète des métaux présents dans les sédiments a permis de discriminer plus efficacement les zones de référence des zones exposées, même si les liens avec les indicateurs benthiques étaient plus faibles que dans le cas de l'extraction partielle;
- les données détaillées sur les invertébrés benthiques (p. ex., identification à l'espèce et utilisation de tamis à mailles de 200 μm) étaient plus étroitement corrélées à la chimie des sédiments que les données grossières sur ces mêmes organismes (identification à la famille et utilisation de tamis à mailles de 1 000 μm);
- la concordance la plus étroite entre les indicateurs benthiques et les effets toxiques étudiés a été révélée par la mortalité chez *Hyalella* et la reproduction chez *Tubifex*, et ce aussi bien avec les données détaillées qu'avec les données grossières sur la communauté des invertébrés benthiques.

ÉTUDE DE LA COMMUNAUTÉ DES POISSONS

Le relevé de poissons avait pour objectif d'évaluer différentes méthodes de mesure des effets de l'activité minière sur la communauté de poissons. Dans le cadre de ce relevé, on a recueilli des données sur l'état de santé général, le coefficient de condition et sur la reproduction des poissons dans divers environnements, ainsi que des informations sur la bioaccumulation des métaux et les concentrations de métallothionéine (MT) dans leurs tissus (muscles, branchies, foie et reins). D'autres échantillons ont été prélevés aux fins des analyses histopathologiques. Les espèces étudiées ont été le doré jaune, le meunier noir, le suceur blanc, le grand brochet, la perchaude, l'épinoche et la barbotte.

L'étude a révélé que dans le bassin de la rivière Bousquet, il n'existait pas de différences marquées entre les zones de référence et les zones exposées pour ce qui est des concentrations de métaux lourds accumulées chez les différentes espèces de poissons et dans les divers types de tissus. De plus, aucune différence claire n'a été relevée entre les zones d'aval et les zones d'amont pour ce qui est des concentrations de MT dans les tissus potentiellement attribuables à une exposition à des métaux produits par les activités minières ou à une augmentation de la concentration des métaux en aval des mines. Les concentrations de plusieurs métaux variaient en fonction de l'âge des poissons, pour un type de tissu donné.

Il convient toutefois de noter que toutes les corrélations observées étaient faibles et que des concentrations de métaux significativement plus élevées ont été enregistrées dans certains tissus de certains poissons provenant tantôt de la zone exposée, tantôt de la zone de référence.

De façon générale, les plus fortes concentrations de métaux accumulés dans les tissus ont été observées dans les reins, les branchies et le foie, beaucoup plus rarement dans le tissu musculaire.

Comme chez les poissons adultes, les concentrations de métaux ont varié considérablement chez les jeunes poissons, tant pour un métal donné que d'un métal à l'autre, ainsi que d'une espèce de poisson à une autre ou d'une station à l'autre.

Une analyse de régression a fait ressortir des corrélations positives significatives entre les concentrations de métaux et les concentrations de MT dans le foie des meuniers noirs et des grands brochets adultes. Ces corrélations n'ont cependant pas été observées avec les autres types de tissus.

Les analyses histologiques des branchies n'ont révélé aucune atteinte grave chez aucun des poissons examinés. Aucune altération sérieuse de la fonction rénale n'a été constatée ni chez les meuniers noirs ni chez les grands brochets provenant des stations de référence et des stations exposées de la rivière Bousquet. Les analyses du foie des meuniers noirs et des dorés jaunes provenant de l'embouchure de la rivière Noire ont toutefois mis en évidence certaines réponses qui semblaient de nature adaptative, à savoir une augmentation du diamètre des noyaux des hépatocytes ainsi que de la taille des hépatocytes. Le foie des grands brochets provenant de la station exposée de la rivière Bousquet contenait beaucoup moins de glycogène que celui des grands brochets issus du site de référence de cette même rivière. La vitellogenèse (développement des oeufs) a également été étudiée chez le meunier noir, le grand brochet et le doré jaune. À ce chapitre, l'analyse des indices gonadosomatiques ainsi que des données sur la fertilité relative (nombre d'oeufs par gramme de poisson), le diamètre et le poids des oeufs et le pourcentage d'oocytes par ponte n'a révélé chez le meunier noir et le grand brochet aucune différence entre les stations de référence et les stations exposées de la rivière Bousquet.

Les principales conclusions du volet de l'étude portant sur les poissons sont les suivantes :

- les concentrations de métaux à des valeurs décelables sont faciles à mesurer dans tous les types de tissus échantillonnés; l'analyse des quatre types de tissus chez les poissons adultes comporte probablement une certaine redondance inhérente;

- les concentrations de MT dans le foie ont varié conjointement avec les concentrations totales de cadmium, de zinc et de cuivre dans le foie; il pourrait être plus efficace d'effectuer le dosage de la MT dans le foie que dans les branchies ou les reins;
- les analyses histologiques ont fait ressortir entre les sites certaines différences possibles dans les tissus des poissons; les résultats donnent à croire que ces mesures pourraient être d'une certaine utilité comme indicateurs de la réponse biologique;
- les jeunes poissons sont plus faciles à capturer et à maintenir en vie que les poissons adultes; on pourrait donc réduire considérablement les coûts de l'étude en examinant les viscères des petits poissons plutôt que les organes des poissons plus âgés.

L'étude a également débouché sur un certain nombre de recommandations concernant les projets qui seront entrepris dans le cadre du Programme dans certains sites miniers choisis répartis dans diverses régions du pays.

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1.0 INTRODUCTION

1.1 Background

Canada's Metal Mining Liquid Effluent Regulations (MMLERs) stipulate "end-of-pipe" final effluent quality limits for metal mines. These limits are under review in order to assess their adequacy with respect to the protection of aquatic resources. This review is being undertaken jointly by government and mining industry representatives, under the AQUAMIN program, which is described by Dumaresq and Prairie (1995) in the Sudbury '95 Conference on Mining and the Environment.

In parallel, the Canadian Centre for Mineral and Energy Technology (CANMET) is coordinating a cooperative government-industry program to review and evaluate technologies for the assessment of impacts in the aquatic environment, with the intention of applying sound scientific principles to environmental effects monitoring in a cost-effective manner. This program is called the Aquatic Effects Technology Evaluation program, or AETE. The focus of the AETE is on the evaluation of environmental monitoring tools that may be used for a mining EEM program, baseline assessments or impact studies. The three principal components of AETE are lethal and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment quality assessments.

The AETE program will eventually evaluate selected monitoring methods in a number of pilot studies across Canada. The program includes both literature-based technical evaluations and comparative field programs at candidate sites. In 1995, the Val-d'Or region of western Quebec was selected for the first pilot study. Additional extensive field evaluations are currently planned for additional mine sites in 1996 and 1997.

Beak Consultants Limited (BEAK) was selected to undertake the first pilot study in the Val-d'Or region of Quebec. The following environmental monitoring methods were selected by the AETE committee for evaluation at the Val-d'Or pilot site:

- a comparison of surficial sediment mapping techniques (sub-bottom acoustical profiling system versus sonar and grab sampling);
- a comparison of the effectiveness of coring devices (Hornbrook, Kajak-

- Brinkhurst, and alpine corers) to quantify pre-operational metal levels;
- an evaluation of the cost-effectiveness of using the lowest method detection limits achievable for water and sediment chemistry analyses;
 - a comparison of analytical methods for assessing sediment quality to predict biological effects by measuring either total metals in sediments by full extraction or those readily extractable (assumed to be more biologically available) by partial digestion;
 - an assessment of three sediment toxicity methods (Microtox™, *Hyalella azteca*, and *Tubifex tubifex*) to assess their ability to accurately predict adverse biological effects from metal contamination of sediments;
 - a comparison of the ability of artificial substrates versus grab samples to measure mine-related effects on benthic invertebrate communities in depositional habitats;
 - an evaluation of the cost-effectiveness and sensitivity of benthic invertebrate processing methods (sieve sizes, level of taxonomy, number of replicates) to delineate and monitor mine-related impacts;
 - an assessment of the effects of mining discharges on fish communities by evaluating the four main response characteristics; age structure, growth, energy storage and reproduction. These characteristics can be evaluated on the basis of one or more of the parameters measured during the field program;
 - an evaluation of metal accumulations in various fish tissues (gill filament, kidney, liver, flesh, viscera); and
 - a comparison of metal levels in tissues to metallothionein levels and histopathology (awarded under separate contract to Freshwater Institute, Winnipeg).

1.2 Study Objectives

The main objectives of the Val-d'Or pilot study were to apply each of the aforementioned environmental monitoring methods and prepare a comprehensive document which addresses the following key areas for each method:

- an assessment of the cost-effectiveness and the validity of each major component of the study (e.g., benthic invertebrates, sediment toxicity, sediment chemistry, etc.) to assess mine-related impacts;

- an assessment of the cost-effectiveness and level of effort required for each component to accurately determine the full extent and severity of a mine-related effect (e.g., level of taxonomy, number of replicates); and
- recommendations to further evaluate the methods used or drop them from the program and/or to evaluate other methods in future programs.

When assessing the cost effectiveness and appropriateness of any methods that are applied to the pilot test sites it is important to evaluate the methods based on their ability to detect and quantify adverse effects on biological communities. Methods that may be finally recommended by AETE to monitor mine-related effects should be well-established and readily available to the mining sector with appropriate levels of sensitivity, accuracy and precision and are amenable to consistent scientific evaluation and interpretation with a high degree of confidence (Environment Canada, 1991).

There were three main phases in the 1995 Pilot Study as follows:

- Phase I was an assessment of the adequacy of the site selected by the AETE committee by undertaking a field reconnaissance survey and preparing a field study design;
- Phase II was the implementation of the study design; and
- Phase III was the preparation of a final report assessing and comparing the methods and recommending modifications to the design that would allow the AETE committee to reliably compare methods in subsequent years.

This document fulfils the requirements of Phase III of the program, which incorporates most of the data from Phases I and II..

1.3 Environmental Effects Monitoring

Similar to most of the other major industrial sectors, the mining industry employs a variety of processes to extract minerals and discharges effluents to a wide variety of receiving environments. The MMLERs, which are based on a relatively uniform standard for end-of-pipe effluent quality may not be sufficient to protect all types of receiving environments

from mining effluents produced by different types of processing .

In order for regulatory agencies (Environment Canada, Department of Fisheries and Oceans) to assess the adequacy of the MMLERs to protect fish, aquatic habitat and the beneficial uses of aquatic resources they will likely require the mining industry to undertake standardized Environmental Effects Monitoring (EEM) programs. It will be important that a fairly consistent program be applied to all mines so that the effectiveness of the regulations can be assessed for different receiving environments and different mining activities.

For the pulp and paper industry the objectives of their EEM program are to assess the adequacy of the Pulp and Paper Effluent regulations to protect fish, fish habitat and the uses of fisheries resources and to address the need for site-specific control measures. The pulp and paper EEM program focused almost exclusively on the monitoring of biological responses (fish, benthic invertebrates, toxicity) to assess the magnitude and spatial extent of mill-related effects and to monitor temporal and spatial changes in biological communities exposed to their discharges.

There are certain data that must be gathered before an effective EEM study can be designed. For example, prior to the implementation of an EEM program it is important to document current and historical operations, understand plume dispersion characteristics, map the receiving environment aquatic habitats and review effluent chemistry and toxicity.

Since the purpose of an EEM program is to assess the magnitude and spatial extent of mine-related effects it is important to define what effects will be monitored. For the pulp and paper industry the EEM studies focused on biological effects. The underlying premise was that if there are no discernible biological effects then slight changes in water quality and sediment quality are of little concern. However, the AETE Technical Committee has expanded on the definition of an environmental effect to include significant changes in sediment chemistry.

The AETE Committee, for the purpose of evaluating environmental monitoring tools, has defined an aquatic environmental effect as:

"a statistically and environmentally significant difference measured in an environmental variable between a receiving area and a temporal or spatial reference as a result of chemical, physical or biological alteration of an ecosystem caused by mining activity".

2.0 STUDY AREA CHARACTERISTICS AND STUDY DESIGN

2.1 Watershed Setting

Rivière Bousquet

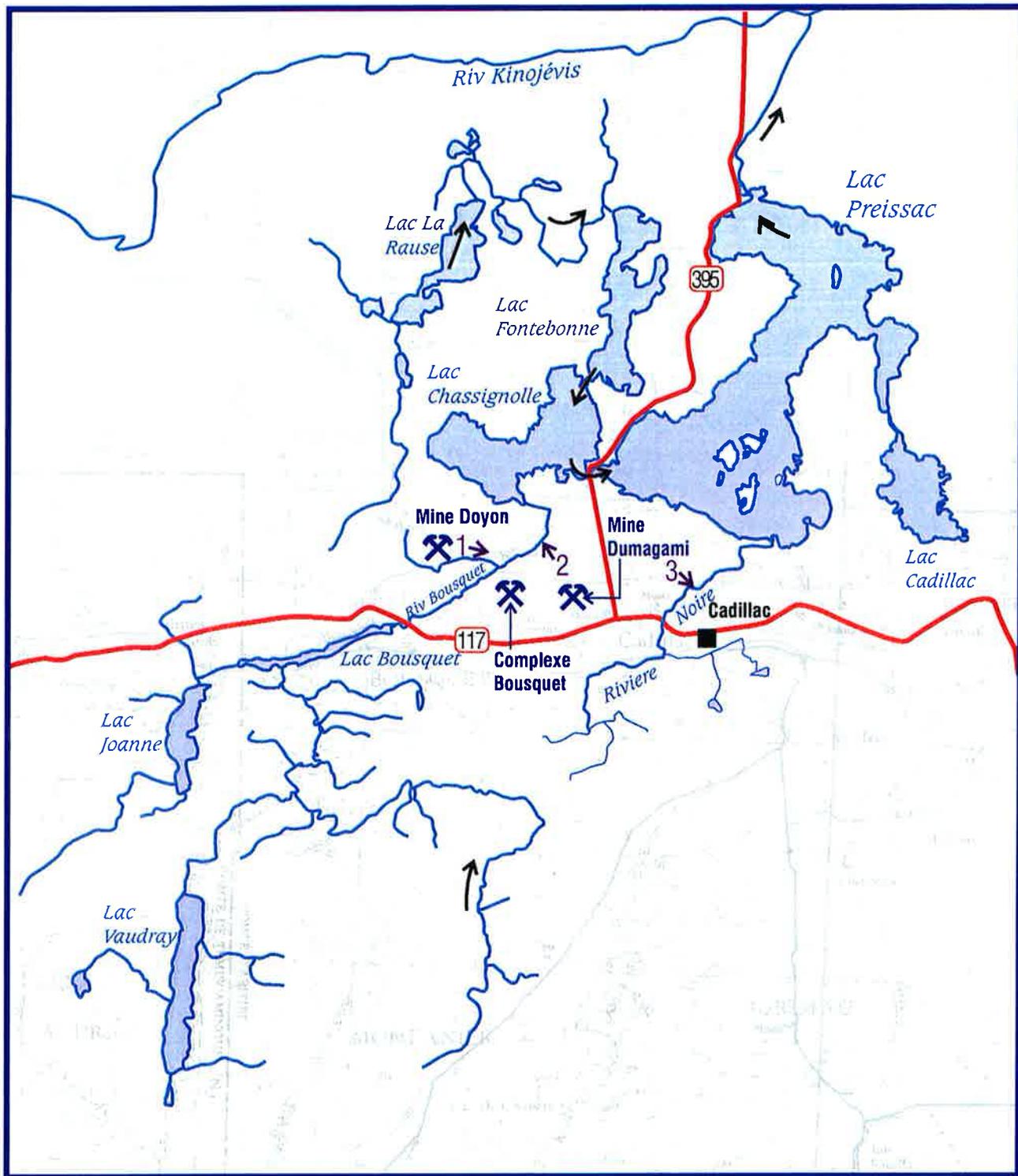
The study area is situated in the headwaters of the Kinojévis River, immediately west of the Town of Cadillac and approximately 40 km east of the Town of Rouyn. Figure 2.1 depicts the general setting along with the locations of Mine Doyon, Complexe Bousquet and Mine Dumagami, which were the three mines selected for the 1995 Pilot Study.

The lac Chassignolle subwatershed, which receives the discharges from Complexe Bousquet and Mine Doyon, consists of two principal sections - a drainage from the south which includes lac Vaudray, lac Joannès, lac Bousquet and the rivière Bousquet, and a northern component which includes lac La Pause and lac Fontbonne. Approximate watershed areas at key locations are as follows:

- Lake Bousquet at outlet 326 km²
- Bousquet River at mouth 370 km²
- Bousquet River at rapids 363 km²
- Lake Fontbonne at outlet 186 km²
- Lake Chassignolle at outlet 604 km²

The drainage basins, including rivière Noire lie in a clay belt (Barlow-Ojibway glacial lake sediments) and are characteristically flat. The stained waters of rivière Bousquet are naturally turbid, and rich in humic acids and lignin (AETE, 1995).

For the purpose of stream flow estimation, the Kinojévis River is gauged at Cléricy, downstream of the study area at a location draining 2,590 km². Based on 18 years of records from Environment Canada (up to 1988), the average annual rainfall is 456 mm, which implies average discharges of 5.46 m³/s at the mouth of the Bousquet River and 8.91 m³/s at the lac Chassignolle outlet. During the September 1995 reconnaissance survey the stream flow, just downstream of Mine Doyon at the rapids was measured as 2.2 m³/sec.



↖ Treated Effluent Discharges

FIGURE 2.1
Regional Watershed Setting

Rivière Bousquet in the section linking lac Bousquet and lac Chassignolle is a low gradient watercourse, with only one minor rapids, located between the Mine Doyon and Complexe Bousquet effluent stream discharge points.

Rivière Noire

The rivière Noire watershed is small relative to the rivière Bousquet watershed, with no significant waterbodies along its course. The watershed is approximately 98 km² and drains into the southeastern end of lac Preissac. Similar to the rivière Bousquet the gradient is low and the waters are stained dark brown with humic acids and lignin, more so than rivière Bousquet. There are numerous beaver dams along the stream. The river is not easily navigated owing to these dams and to a major falls and riffles in the lower reaches. The river is generally 2 m deep with annual flows ranging from 0.14 m³/sec to 8.9 m³/sec.

The Town of Cadillac is situated near the upper reaches of the river, upstream of mining areas. The town has a municipal wastewater discharge which enters a tributary of rivière Noire just upstream of the Hwy 117 crossing (Figure 2.1).

The only active mining facility discharging to rivière Noire is the Agnico-Eagle Dumagami Mine, which discharges to Dormenan Creek, a tributary entering rivière Noire, approximately 750 m upstream of the falls and 4 km upstream of the river mouth. Along the river from Hwy 117 to the confluence with Dormenan Creek there are two inactive mine sites. Conductivity measurements during the field survey suggested that contaminants are entering the river from both sites.

2.2 Overview of Mining Operations/Effluents

In the Bousquet district, Mine Doyon, Complexe Bousquet and Mine Dumagami extract gold from pyritic gold deposits, which have a high sulphide content (AETE, 1995). Pyritic gold deposits are also generally characterized by high arsenic levels. The rivière Noire is contaminated with arsenic throughout most of its length downstream of Hwy 117 and into lac Preissac.

During the reconnaissance and main surveys, interviews were held with environmental coordinators for Mine Doyon, Complexe Bousquet and Mine Dumagami. Both Mine Doyon and Complexe Bousquet are operated by Barrick Gold Corporation, while the Dumagami mine is operated by Agnico-Eagle. A clearer understanding of the mines' contaminant loadings to the environment was obtained, and permission was secured to use the original environmental assessment documents, prepared by Beak Consultants Limited for all three mines, to assist in the final study design.

Mine Doyon is a gold mine and mill operation, located west of rivière Bousquet between lac Bousquet and lac Chassignolle (Figure 2.1). Mill process water is discharged with tailings to the tailings pond, and all water in the tailings pond is recycled. Therefore, there are no discharges of cyanide or heavy metals from the tailings pond to the environment. Some of the mine rock is acid-generating, and contaminated mine water and other site drainage is collected and treated by lime addition using a high density sludge process. Final effluent quality is routinely in compliance with their discharge permit requirements and, under summer/early fall conditions, is discharged to the Bousquet River at a nominal rate of 3 m³/min.

Complexe Bousquet is a gold and silver mine operation located east of the Bousquet River. Like the Doyon Mine, mine water and contaminated site drainage is collected and treated with lime addition. There is no tailings discharge, as Bousquet ore is trucked to Malarctic for milling. The present discharge rate of treated effluent from Complexe Bousquet to the Bousquet River is approximately 0.6 m³/min. Complexe Bousquet generally operates in compliance with its effluent discharge permit.

The Dumagami Mine is a gold and silver mining and milling operation located west of rivière Noire and east of Complexe Bousquet. The mill discharge is generally in compliance, however, the effluent does often produce a toxic response in rainbow trout and *Daphnia magna*. In August 1995, just before the reconnaissance survey, the LC₅₀s for rainbow trout and *Daphnia magna* were 17% and 14% effluent, respectively. The site potentially generates acid-mine drainage and has a variable effluent discharge ranging from 5 to 9 m³/min. The effluent is treated with lime addition, and hydrogen peroxide and ferric treatment is used to remove cyanide. The mine started with a milling operation in 1988 and effluent was first discharged to the watershed in 1990.

A recent report prepared by the Ministère de l'Environnement et de la Faune (MEF) described possible water quality impacts of Mine Doyon, Complexe Bousquet and other mines in the Lake Preissac watershed (Lortie, 1995). This study concluded that pH and zinc levels in Complexe Bousquet effluent have tended to be beyond desirable limits during late winter/spring. Complexe Bousquet's final effluent zinc concentration has averaged about 1 mg/L in recent years. Mine Doyon has historically experienced acid mine drainage problems; however, recent improvements in drainage and water management have apparently greatly reduced any direct metal losses to the environment. At least until recently effluent copper concentrations have been in the 1 mg/L range. Thus, both operations may represent more significant historic contamination sources, with substantially reduced impacts under current conditions.

Mine Dumagami final effluent collected at the point where it enters Dormenan Creek during the main field survey was found to have higher than reference levels of ammonia (14 mg/L) and most metals.

2.3 Effluent Plume Dispersion

The effluent plumes from the Doyon, Dumagami and Bousquet mines were measured in the field by mapping surface and bottom water conductivities. Conductivity served as an adequate tracer because the final treated effluent at all three mines has a conductivity of approximately 2,300 to 3,000 $\mu\text{mhos/cm}$, while the natural background conductivity in both rivière Bousquet and rivière Noire was in the range of 40 to 60 $\mu\text{mhos/cm}$. Thus, a 1% effluent concentration in the receiving water results in an incremental conductivity of about 30 $\mu\text{mhos/cm}$. Measurements were made at various depths upstream and downstream of the mines in the rivière Bousquet and rivière Noire to identify any density related stratification, and at selected locations in lac Chassignolle and lac Preissac.

As a result of the effluent treatment activities at the mines, the effluents were more dense than the receiving waters and consequently, in all cases the plumes moved both upstream and downstream along the river substrates. For both Mine Doyon and Mine Dumagami, the plumes remained on the bottom of the rivers as far downstream as the falls at which point they became fully mixed with the receiving waters.

Mine Doyon discharges high conductivity effluent to the Bousquet River at about 3 m³/min. Higher discharges are experienced in spring. Upstream of Mine Doyon, as measured at the Highway 117 crossing, the conductivity of the water was 40 µmhos/cm. The conductivity of the Mine Doyon effluent was 2,940 µmhos/cm (07 September 1995), which is attributed to the lime treatment. The river-bottom effluent plume was found as far as 1.4 km upstream of the point of discharge, with river bottom conductivities above 2,500 µmhos/cm extending upstream and downstream of the mine discharge for about 1 km in either direction. River surface water conductivities also increase in plume-influenced areas, with river surface conductivities increasing to 277 µmhos/cm (08 September) immediately upstream of the rapids. Through the rapids, both the surface water and deeper, more dense bottom water mix relatively completely. Flow was estimated at 0.9 m³/s on 06 September (cross-sectional method), although flows gradually increased from this value over the course of the week. This relatively low flow condition was consistent with the recent dry weather and obviously low water levels prevalent during the reconnaissance field program. During the main field study on 22 October 1995 the rivière Bousquet flow was measured at 2.2 m³/sec.

The Complexe Bousquet discharge enters the Bousquet River at a lower conductivity level (1,180 µmhos/cm on 07 September) owing to natural dilution between the treatment plant and the river. Minor stratification was observed in the area immediately downstream of the effluent inflow point (e.g., 370 µmhos/cm at surface, 432 µmhos/cm at bottom), but this stratification was found to dissipate within about 250 m of the mouth of the effluent stream.

As the Bousquet River enters lac Chassignolle, conductivity remains relatively high (410 µmhos/cm on 08 September), or about 370 µmhos/cm above the background observed upstream of the mining-affected zone. This implies a total effluent concentration for the two combined mine discharges of about 12% at the mouth of the river, based on a conductivity of about 3,000 µmhos/cm for each effluent at the point of discharge from the treatment systems.

Conductivity in lac Chassignolle was measured near the lake outlet at 116 µmhos/cm, but was lower (67 µmhos/cm) in the northeast arm immediately offshore of the mouth of the lac Fontbonne inlet. This latter reading indicates a low background conductivity in other portions of the lac Chassignolle watershed. If natural background conductivity in lac

Chassignolle is in the 40 to 50 $\mu\text{mhos/cm}$ range (as in the Bousquet River), the implied effluent concentration at the lac Chassignolle outlet is about 2.2% at the time of sampling.

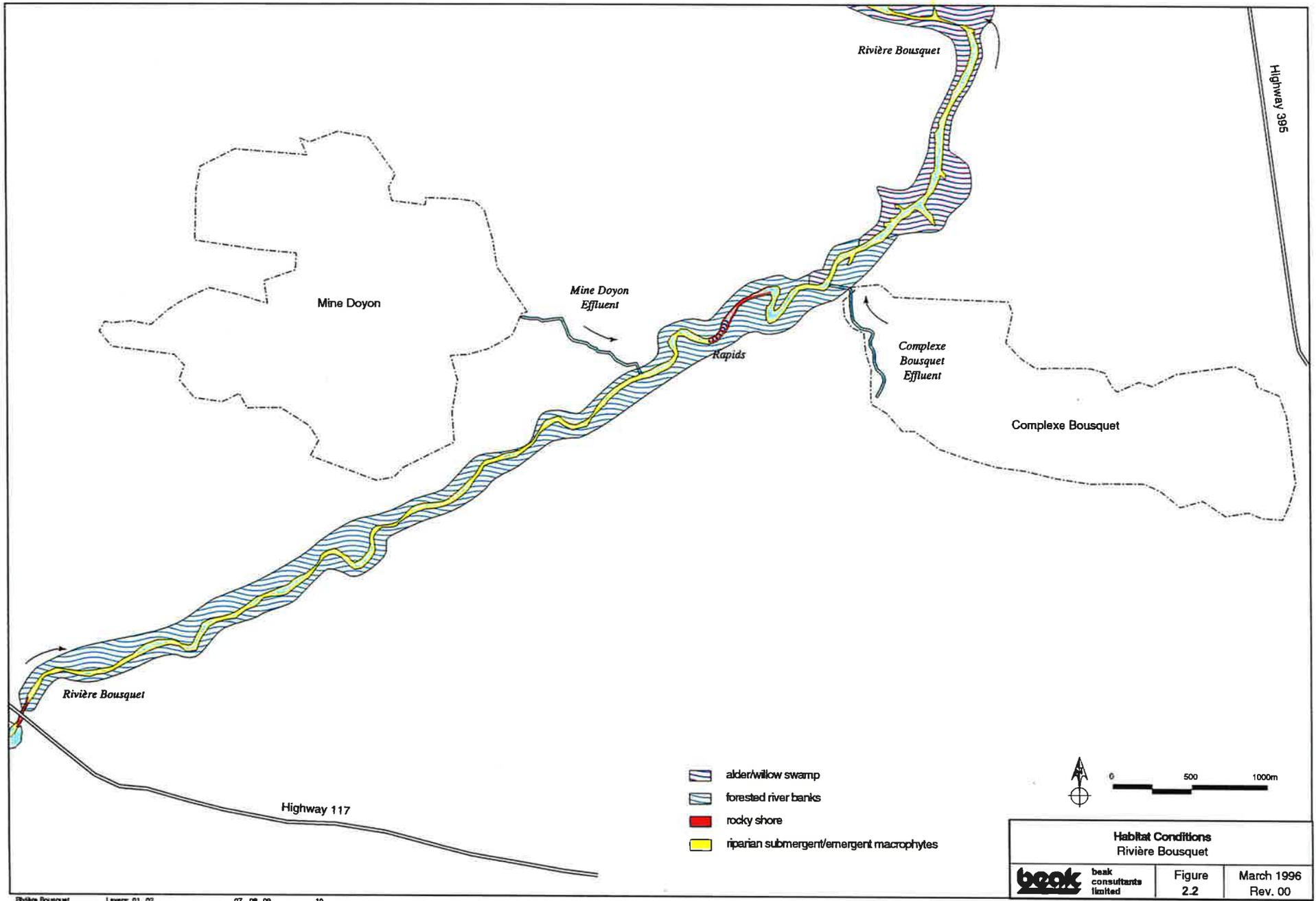
The rivière Noire appears to be more influenced by contaminated runoff and seepages than the Bousquet River, due to the presence of idle mine properties north and northwest of Cadillac (refer to Figure 2.5). Contamination from these abandoned mines was evident in water chemistry results and could be detected in the field by conductivity measurements (discussed further in Section 5.0). There was an increase in conductivity downstream of each mine operation - both active and inactive sites. In these cases, contaminants may enter the watershed from waste rock dumps, road fill, etc., which may have acid-generating potential, as well as, seepage from abandoned polishing and treatment ponds.

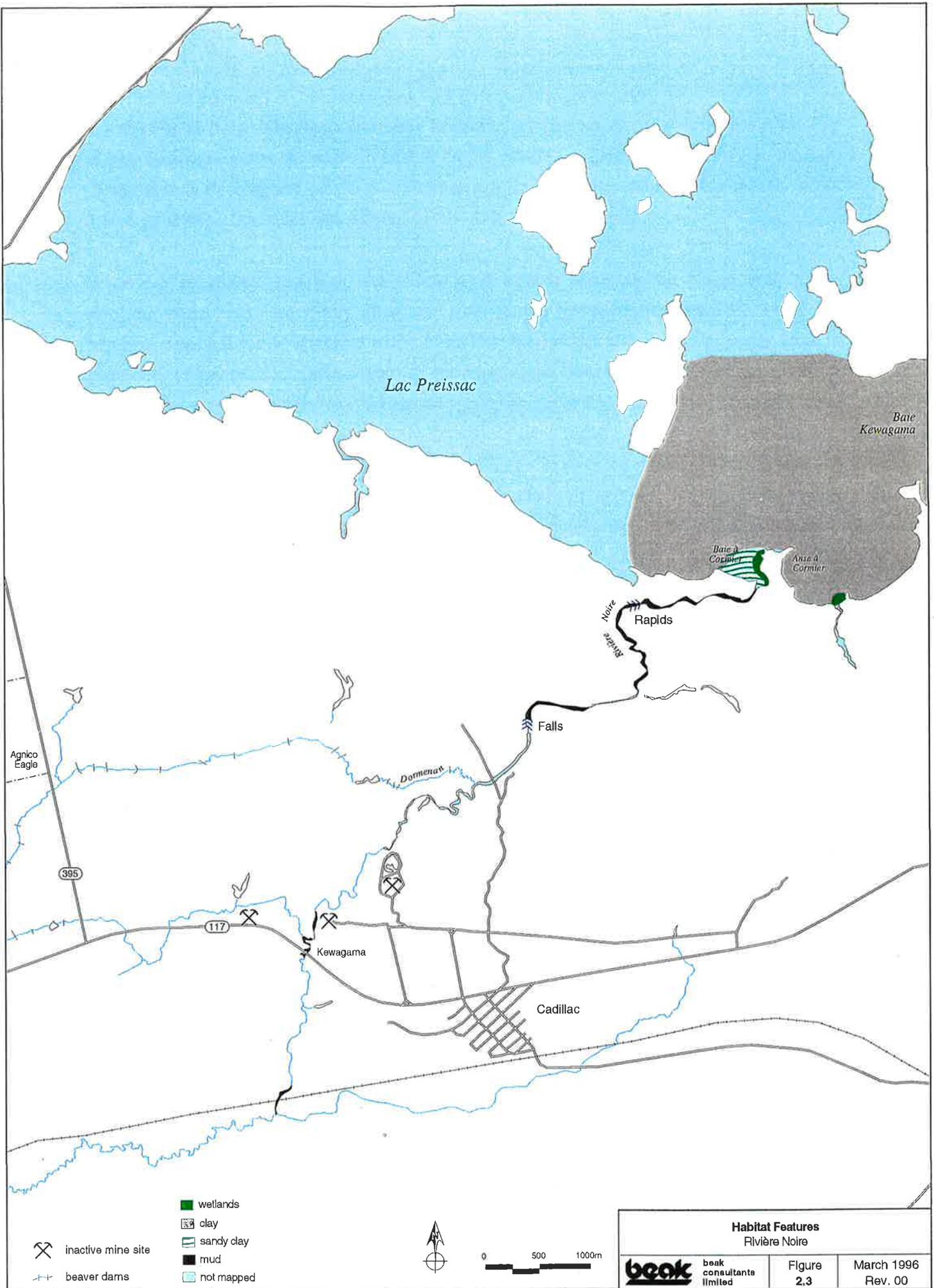
The conductivity of the Dumagami effluent is approximately 2,300 $\mu\text{mhos/cm}$ reflecting the high level of dissolved solids mainly from the treatment processes. Similar to the Doyon and Complexe Bousquet effluents the Mine Dumagami effluent is denser than the receiving water and therefore, remains on the bottom of rivière Noire until the falls (750 m downstream), where it becomes completely mixed. At the time of the survey the effluent reached a maximum dilution of approximately 3:1 (30% effluent) in the river. Up to the falls there was notable chemical stratification of the river with bottom conductivities of 1,700 $\mu\text{mhos/cm}$ compared to surface conductivities of 100 $\mu\text{mhos/cm}$. In the middle of Baie Kewagama, in lac Preissac, conductivity from the surface to the bottom was 100 $\mu\text{mhos/cm}$, indicating that the Dumagami effluent was diluted to less than 1% in this area. Background conductivity in lac Preissac was 100 $\mu\text{mhos/cm}$.

2.4 General Aquatic Habitat Conditions

Rivers

Both the rivière Bousquet and rivière Noire are relatively deep, slow-flowing rivers with a predominantly clay and mud bottom with coarse organic matter from the adjacent forest and from high beaver activity. The typical mid-channel depths vary between 2.5 and 4 m, with deeper sections (up to 9 m) in two or three meanders downstream of the rapids on rivière Bousquet. On rivière Bousquet the rapids occur about 3.5 km upstream of the river mouth (Figure 2.2) while on rivière Noire they occur approximately 4 km upstream from the





- inactive mine site
- beaver dams
- wetlands
- clay
- sandy clay
- mud
- not mapped



Habitat Features Rivière Noire		
beak beak consultants limited	Figure 2.3	March 1996 Rev. 00

mouth (Figure 2.3). The rapids in rivière Bousquet are characterized by substrate consisting of cobble and larger rock, while in rivière Noire, where the falls are much larger, they are characterized by bedrock substrate. The vertical drop through these rapids is only about 0.5 m in rivière Bousquet and approximately 5 m in rivière Noire.

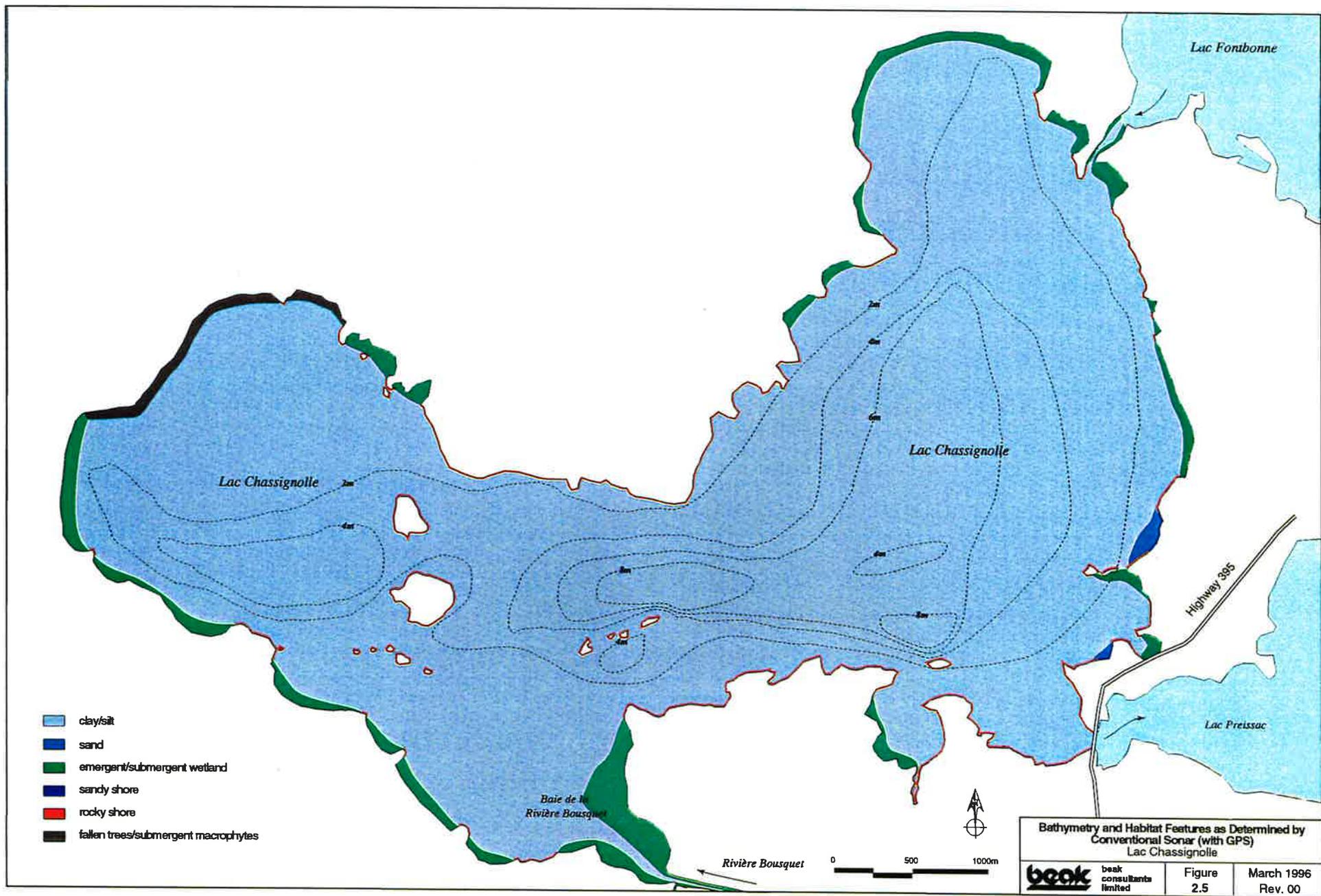
In rivière Bousquet, emergent and submergent aquatic macrophytes fringe both gently sloping river banks for nearly all of the river length (excluding the rapids), with these fringing wetlands more extensive at the Lake Bousquet outlet and along the inside bank of meanders (Figure 2.2). Downstream of the rapids, the river is fringed with an extensive alder/willow wetland while near the rapids and upstream to Lake Bousquet, the river banks are forested to the river's edge.

In contrast, rivière Noire is somewhat more channelized and has only limited aquatic vegetation along the edges. However, like rivière Bousquet, the banks are well forested.

Lakes

Lac Bousquet is relatively long and narrow in shape with a small surface area relative to lac Chassignolle. The lake has a predominantly rocky shoreline, although emergent wetlands are also common in embayments and at the Bousquet River inlet to the lake (Figure 2.4). The water is relatively clear, with little or no suspended sediment. The maximum lake depth is about 19 m. Lake sediments consist of a soft, light brown silt.

Lac Chassignolle has a relatively large surface area and is shallow, with a maximum depth of about 8.5 m. Emergent and submergent wetlands are extensive, occupying about half of the shoreline, and achieve their greatest development at the lac Fontbonne inflow, at the Bousquet River inlet, and at the northern extremities of the western and eastern basins (Figure 2.5). Rocky islands are abundant in the central and western portions of the lake. Lake sediments consist of a thin layer of soft clay-silt (e.g., typically 2 cm thick), underlain with a stiff, grey-brown clay. Sediments near the mouth of rivière Bousquet and into the bay are more gritty indicating a greater proportion of sand. Sandy deposits are also present in open embayments along the exposed eastern shoreline. Organic detritus is sparse except near the lake outlet area where pieces of woody debris are present. Rocky shorelines grade quickly to clay bottom conditions generally within 30 m of shore. The water of lac



Chassignolle is turbid and light brown, reflecting wind-driven resuspension of bottom sediments.

Similar to lac Chassignolle, lac Preissac is a relatively large, shallow lake characterized predominantly by clay substrates. The mouth of the rivière Noire, Baie a Cormier and Baie Kewagama are very shallow with depths ranging from 10 cm to 2.5 m. The substrate is clay with a fine silt layer, which is most noticeable in the mouth of rivière Noire.

2.5 Water Quality

General water quality conditions observed during the reconnaissance survey included surface water temperatures of 13° to 15°C, pH of 6.5 to 7.3, and dissolved oxygen levels close to saturation in surface water. Some degree of oxygen depletion occurs in the deep layer of Bousquet River in sections impacted by poorly diluted Mine Doyon effluent, probably owing to restricted water circulation. The dissolved oxygen level reached a minimum of 4.5 mg/L in bottom water immediately upstream of the rapids (versus 8.0 mg/L at surface).

A similar oxygen sag was also noted downstream of the Dumagami discharge into rivière Noire. The lowest oxygen recorded was 6 mg/L. A further discussion on dissolved oxygen levels in rivière Noire at the benthic invertebrate sampling sites is provided in Section 7.0.

Lac Bousquet was thermally stratified, with the thermocline located at 9 m to 12 m of depth. Dissolved oxygen levels reach zero at the bottom of the hypolimnion (17 m to 18 m). It is interesting to note that lake sediments below this seasonally anoxic layer are strikingly laminated with alternating thin bands of black and brown deposits (several per centimetre), probably reflecting alternating periods of sulphide formation (black layer) and oxidizing conditions resulting from seasonal lake circulation patterns.

Lac Chassignolle and lac Preissac showed high dissolved oxygen levels and relatively uniform temperatures at all depths, reflecting the shallow depth and strong wind influence over the large surface areas.

During the preliminary survey it was found that concentrations of potentially toxic heavy metals (Pb, Zn, Cu, Cd) do not change appreciably between the upstream reference station

and the river mouth; concentrations of heavy metals are low in both the Mine Doyon and Complexe Bousquet effluents, and are at most only about three to four times greater than found at the reference station.

A full description of water quality of the rivière Noire is discussed in Section 5.0.

2.6 Sediment Quality

Sediment samples were collected at selected locations during the preliminary survey to identify potential effects of mining on sediment metal concentrations and to assess and compare the use of the Geological Survey of Canada's Hornbrook sampler to conventional limnological corers (discussed in Section 4.0).

Sediment quality results for lac Bousquet, lac Chassignolle and rivière Bousquet showed no obvious increases in metal concentration in lac Chassignolle (exposure area) relative to lac Bousquet (reference area). Sediments collected from the effluent streams show relatively low metal concentrations relative to pre-mining levels (taken from lower end of cores) and reference levels in lac Bousquet. Consistent with earlier observations in the area, sediment Cd concentrations are relatively high in both lakes.

Surface versus subsurface metal concentrations at the same location in lac Chassignolle indicated some enrichment of the surface layer, although this could be due to atmospheric deposition, as well as, past mining activity in the watershed.

A full description of the sediment quality in rivière Noire and lac Preissac is provided in Section 5.0.

2.7 Benthic Invertebrate Community

Twelve benthic macroinvertebrate samples were collected in rivière Bousquet during the preliminary survey. The benthic communities were dominated by gatherers and collectors which are typical of depositional habitats. Samples were collected from upstream and immediately downstream of the Mine Doyon discharge. There were no obvious effects of the mine effluent on the benthic invertebrate community structure.

A complete description of the benthic communities in rivière Noire and lac Preissac is provided in Section 7.0.

2.8 Fish Communities

Fish were readily captured by experimental gill net (larger fish) and seine (young-of-the-year fish, juvenile yellow perch) both upstream and downstream of the mine effluent discharge points in the Bousquet River, during the preliminary survey. Common species captured by gill net included walleye, sauger, silver redhorse, white sucker, yellow perch, rock bass and northern pike. Species most readily captured in high numbers by seine net were young-of-the-year northern pike and brown bullhead. Juvenile perch were present in lower numbers in seine hauls.

The fish community of lac Preissac was also characterized by warmwater species, primarily walleye, white sucker and northern pike. No fish were found downstream of the Dumagami discharge, where it enters rivière Noire. However, in the upper reaches near Hwy 117, limited numbers of warmwater species were captured including, white sucker, sticklebacks, yellow perch and a few cyprinid species.

2.9 Evaluation of Assessment Tools

Some of the tools to be compared were alternative monitoring parameters, such as different sediment toxicity tests, or different benthic community metrics. Others were different sampling and sample processing techniques such as full or partial extractions of metals from sediments, different sieve sizes for extraction of benthic organisms from sediments, different levels of benthic taxonomy, or different levels of sample replications.

The approach to evaluation of assessment tools was to use those tools in an attempt to detect environmental effects of mine discharge, and to compare alternative tools with respect to their ability to detect meaningful effects. This approach depends for success on the actual existence of mine-related environmental effects.

The determination of whether observed effects were meaningful involved a degree of professional judgement. However, correlations were also examined between benthic

community metrics, sediment chemical parameters and sediment toxicity test results. These relationships form the basis of the triad or weight of evidence approach in ecological impact or risk assessment. A meaningful biological effect is at least potentially related to population success, and is also related to the stressor(s) of interest, in this case to toxic chemicals discharged from the mine.

2.10 Study Design

The initial phase of the pilot program included the preparation of a study design for the main field program which applied different aquatic effects monitoring techniques. The study design was based on:

- examination of literature reviews on field protocols (bathymetry/sediment mapping and benthic sampling by artificial substrates);
- examination of environmental assessment documents and monitoring data available for mines operating in the study area (Mine Doyon, Complexe Bousquet, only); and
- completion of a preliminary field reconnaissance study to assess site conditions (presented above in Sections 2.1 to 2.8) required for the study design and to determine the site's suitability as a pilot test site for a two to three year period.

Prior to this document, two brief reports have been prepared and submitted to the AETE Committee which document the completion of the preliminary reconnaissance survey, including a description of the study area, a documentation of field methods and preliminary findings, and a summary of analytical results on water quality, sediment quality, benthic invertebrate and fish community structures, and metallothionein and metal levels in fish tissues. Most of the data from these previous submissions are summarized throughout this document in the appropriate sections.

The key results from the preliminary survey that affected the study design are highlighted further in this section.

Results of the preliminary survey (September 1995) in terms of water quality, sediment quality, benthic community structure and fish tissue metal and metallothionein levels all

indicated little or no measurable impact gradient from the Mine Doyon and Complexe Bousquet operations. Based on this outcome, BEAK made an initial recommendation to relocate the main (Phase II) study to an alternate site where we could be confident in finding a clear impact gradient, which is required in order to fully evaluate the appropriateness of various methods. After discussion with the AETE Technical Committee, it was agreed that Phase II would be carried out on the Bousquet River watershed (the site of the preliminary survey), with a reallocation of some of the effort to the rivière Noire, where more severe impacts were suspected. This was considered of greatest importance in terms of the bioassessment components of the study and selecting a site which could be studied for a two to three year period.

Optimization of the sampling effort was difficult since the proposed location of the final study (rivière Noire) was different than the location of the reconnaissance survey (Rivière Bousquet). Essentially, there was no prior knowledge of variability, either within or between the proposed reference and exposure areas, for any of the fish population or benthic community parameters to be measured. Thus, there was no statistical basis for design optimization. We could only rely on previous experience at other mine sites as to what has been an adequate level of effort for detection of mine impacts. We could not specify any particular degree of resolution between reference and exposure areas that we expected to achieve in this study. However, this study did provide data to support statistical design of subsequent studies at the same site.

This section describes the study design approved by the AETE Committee for Phase II of the Pilot Study, and provides further detail on preliminary survey results from the rivière Bousquet preliminary study.

Sediment Types

Sub-bottom profiling was carried out in lakes Bousquet and Chassignolle to provide a more detailed description of surficial and subsurface sediment characteristics. Descriptions of the surficial sediment layer, as provided by S. Phaneuf, were to be confirmed through additional grab and core sampling of sediment in representative areas of each of the sediment types, as mapped in the substrate maps provided by Ms. Phaneuf (Section 3.0).

Additional Mapping

Since a new study area (rivière Noire) was selected for the benthic invertebrate, water and sediment chemistry and sediment toxicity assessments, aquatic habitat maps similar to those presented above for rivière Bousquet, had to be developed for the rivière Noire while on site. If the river and creeks had poor access and navigability, descriptions were to be based on observations at key access points, with extrapolation to areas between, where possible, by interpretation of mapping. These descriptions and maps are presented above.

Water Quality

Samples of mine effluent (at the points of entry into the Bousquet River), water from the upstream reference area (outlet of Lake Bousquet) and lower Bousquet River (at the mouth) and from lac Chassignolle (near the lake outlet) were collected in the preliminary survey for analysis of trace metal content to characterize mine source terms and upstream-downstream gradients. As discussed above, water quality results from this initial survey indicated that:

- concentrations of potentially toxic heavy metals (Pb, Zn, Cu, Cd) did not change appreciably between the upstream reference station and the river mouth; concentrations of heavy metals were low in both effluents, and were at most only about three to four times greater than found at the reference station; and
- elements increasing in concentration downstream of the mine discharges included the alkali and alkali earth metals, which were associated with treatment of ARD.

In the Phase II program, BEAK was to collect surface water samples for chemical analysis from various locations, including the benthic invertebrate and sediment toxicity collection stations in rivière Noire and lac Preissac. In addition, any obvious or apparent contaminant sources were to also be sampled for chemical characterization, especially those in the rivière Noire watershed, which was poorly documented. A total of 18 water samples were provided for in the original budget (i.e., total of 22 minus those collected in the preliminary survey). All samples were to be collected in clean, pre-rinsed sample containers, preserved as appropriate and analyzed by RPC Laboratories for the parameters requested, including Al, Cr, Cu, Fe, Mn, Mo, Ni, Sb, Zn, Pb, Ag, As, Hg, Cd, CN, NH₃, SO₄, chloride, NO₃-

N, total P, TSS, TDS, alkalinity, hardness, DOC, DIC and acidity. Samples included two "hidden" field duplicates and a trip blank.

Sediment Quality

Sediment samples had already been collected at selected locations during the preliminary survey to identify potential effects of mining on sediment metal concentrations and to assess and compare the use of the GSC's Hornbrook sampler to conventional limnological corers (Section 4.0).

Surficial sediment samples (top 5 cm) were collected by petite ponar or directly in hand-held sampling bottles at the mouth of the final effluent streams (at the point of entry into the Bousquet River) for each mine, in the central basin of lac Chassignolle (situated between the mouth of the Bousquet River and the Lake Chassignolle outlet) and in the deep basin of lac Bousquet.

Preliminary sediment quality results (provided previously) showed no obvious increases in metal concentration in lac Chassignolle relative to lac Bousquet (reference). Sediments collected from the effluent streams showed relatively low metal concentrations relative to reference areas. Therefore, no further sediment sampling would be conducted in rivière Bousquet watershed.

During the Phase II study, sediment samples were to be collected by Hornbrook sampler and by surface grab from each of the benthic invertebrate and sediment toxicity sites on rivière Noire and lac Preissac and analyzed for metals, as well as water content, bulk density, TOC, LOI, Munsell colour and particle size,. Surficial sediment samples were to be analyzed by both partial digestion and total digestion using an Aqua Regia with nitric acid/hydrochloric acid. BEAK's existing budget and scope provided for a total of 42 sediment analyses for the Phase II study.

Benthic Invertebrate Program

Artificial Substrates and Grab Samples

Artificial substrates consisting of cobble-filled barbecue baskets of the type identified as generally preferable in the CANMET literature review were installed on 09 September in depositional habitats at an upstream reference area and a downstream exposure area in the Bousquet River. Six baskets were installed at each location, and were suspended above the bottom from sturdy overhanging trees. The installations were visually obscure, and were safe from tampering and vandalism. Exposure was confirmed by conductivity measurement. Dissolved oxygen levels were also notably lower at the exposure site compared to the reference site.

Also at each artificial substrate location, six replicate benthic samples were collected by Petite Ponar to assess natural variability, and to compare the communities to those obtained from artificial substrates. Each replicate sample consisted of two pooled grabs of sediment (0.046 m²). All petite Ponar samples were field-sieved with a 200 µm mesh, preserved to a level of 10% buffered formalin and returned to BEAK's Benthic Ecology Laboratory in Brampton for processing.

The installation of artificial substrates in depositional habitats coupled with the collection of endemic benthos at the same sites in the rivière Bousquet allowed for technical and cost-effective evaluations of the artificial substrate method.

Invertebrate Sample Processing Evaluation

As outlined above, the impacts of the Doyon mining operations on the benthic community, downstream of their discharge, were negligible. In order to assess the effectiveness of different invertebrate processing techniques to delineate mining impacts it is necessary to have a gradient in the response of the benthic community to the mine discharge. BEAK recommended to the AETE Committee that the benthic invertebrate component of the Pilot Study be relocated to a different area where impacts on the benthic community were known to be more severe, and where there was a known gradient from severely impacted to slightly impacted communities.

Based on the committee's recommendations and preliminary data that BEAK had received, the area for the benthic invertebrate, sediment chemistry and sediment toxicity assessments was relocated to the ruisseau Dormenan, rivière Noire and lac Preissac waterbodies.

In order to adequately assess the benthic invertebrate community in this new area with no increases in the original budget BEAK proposed to reduce the number of replicates per station from six to five. This allowed for 15 benthic invertebrate sampling stations (75 samples). The actual siting of these stations was to be undertaken while in the field, by a senior benthic ecologist, based on results of *in situ* water quality measurements, confounding factors (e.g., other discharges, abandoned mines), accessibility, habitat types (e.g., erosional and depositional) and any readily apparent structural changes in the benthic community in the vicinity of mining operations.

The level of effort that was proposed for each aquatic system was as follows:

- three sites along ruisseau Dormenan (one reference, two exposure),
- eight sites along rivière Noire (three reference, five exposure), and
- four sites in lac Preissac (two reference, two exposure).

This study design allowed for the comparison of different invertebrate processing methods in both riverine and lacustrine habitats. If there were both erosional and depositional habitats in the rivière Noire then additional samples might have had to be collected, however, this was not the case.

All benthic invertebrate samples were to be processed to three levels of taxonomy (species, genus, family) and three size classes ($>200 \mu\text{m}$, $>500 \mu\text{m}$, $>1000 \mu\text{m}$).

Sediment Toxicity

At fourteen of the fifteen benthic invertebrate collection sites additional sediments were to be collected for sediment toxicity testing. At benthic invertebrate sites that were located in erosional habitats sediments were to be collected from depositional sites immediately upstream of the erosional site for toxicity testing.

Fish

During the preliminary survey, fish were readily captured by experimental gill net (larger fish) and seine (young-of-the-year fish, juvenile yellow perch) both upstream and downstream of the mine effluent discharge points in the Bousquet River. Common species captured by gill net included walleye, sauger, silver redhorse, white sucker, yellow perch, rock bass and northern pike. Species most readily captured in high numbers by seine net were young-of-the-year northern pike and brown bullhead.

Metallothionein (MT) levels were measured in gill, liver and kidney of walleye, white sucker and silver redhorse from reference and exposure areas of the Bousquet River by the Winnipeg Freshwater Institute (Dr. Jack Klaverkamp's laboratory). Samples of whole gut of young-of-the-year brown bullhead and northern pike from the same sites were also analyzed.

Results of the analyses were provided previously, and showed generally comparable levels of MT in exposure and reference site tissues. The highest MT levels were found in liver and kidney. While it may be argued that fish caught in the reference area could have experienced exposure to mine effluent in the past (i.e., may move into downstream areas), distance to impacted areas (about 3 to 4 km) is such that recent exposure to effluent is unlikely. Furthermore, young-of-the-year fish are very small and local in their distribution, so that reference and exposure young-of-the-year fish can be considered distinct with a high degree of confidence. It was concluded from these samples that MT production was not significantly enhanced in mining affected areas in the species and tissues sampled.

Tissue metals were analyzed in samples of the same large fish and tissues that were analyzed for MT, and on replicates of the same species for young-of-the-year brown bullhead and northern pike. Although there were some apparent occasional anomalies in the data, the following conclusions were made:

- clear differences in concentrations of heavy metals did not exist between exposure and reference areas for individual fish species and tissue types;
- muscle tissue was a relatively poor bioaccumulator of most heavy metals - a result which was consistent with literature on the subject; and

- among gill, liver and kidney, concentrations varied substantially among tissues and species, at least for some metals.

The adult and forage fish sampling program originally proposed by BEAK provided for the capture of adult fish of two species to be collected from three locations (reference, near-field impacted area, far-field less impacted area). Forage fish and/or young-of-the-year fish were to be captured at the same locations.

Based on the preliminary data and discussions with the AETE Committee and Dr. Klaverkamp, BEAK proposed to reassign one-third of the adult fishing effort to the rivière Noire, where higher metal exposure levels were expected. This would hopefully result in the demonstration of mine-related responses using the monitoring methods employed. Thus, fish collection areas were to include the Bousquet River (near the lac Bousquet outlet), the Bousquet River mouth area (only slightly impacted by Doyon and Bousquet mines) and the rivière Noire (hopefully, significantly impacted by mining operations). Based on our examination of mapping information, we expected that it could be difficult to capture large adult fish in various areas upstream in the rivière Noire owing to the presence of beaver dams and to the smaller size of the stream which may provide limited habitat. Our fall-back location for capture of metal-exposed fish, if collection efforts in the mouth of the rivière Noire were unsuccessful, was to collect small fish in lac Joannès, based on the evidence of high cadmium levels in that lake.

As discussed with Dr. Klaverkamp, it was preferable (and more scientifically meaningful) to undertake all analyses (MT, histopathology, metals) on the same fish, rather than from separate fish as indicated in the original terms of reference. This would improve our tissue metal/biochemical/histopathology database in the event that it was not possible to collect the requisite number of adult fish at all sites (i.e., 20 males and 20 females of two species at three sites).

It has been our experience that it is sometimes difficult to collect a pre-determined number of fish of both sexes within a reasonable time period. Of the time allocated for the field component of Phase II, about 75% was allocated for the collection and processing of fish (the equivalent of 12 ten-hour days for two crews of two). If after the first few days of fishing effort catches were low we would consider reallocation of the remaining effort (and analytical allotment) to the collection greater numbers of species and/or locations for small

fish collections, particularly in the rivière Noire watershed where multiple impact sources and containment gradients may have yielded valuable results.

Given our previous success in capturing adult pike, walleye, sucker and redhorse in the Bousquet River, we proposed to target one bottom feeding fish and one predator for the Phase II Study, with the final selection of species dependent upon their relative catchabilities in the rivière Noire. The final species selection was to be made in the field after the first two to three days of fishing effort. In terms of small fish, BEAK proposed to collect and process the range of species found in relative abundance at all locations sampled.

Processing of fish was to be carried out as described in the terms of reference and in BEAK's proposal. All MT samples were to be collected from live fish and frozen on dry-ice until delivery to the Winnipeg Freshwater Institute and histological samples were to be collected and preserved according to the protocols specified in the Terms of Reference.

The level of effort provided for analysis of tissue metal residues was for 360 tissue samples, of which 51 were analyzed during the preliminary survey. After consultation with Dr. Klaverkamp, BEAK proposed to undertake the tissue analysis using a tiered approach, so that samples for metal analysis would be based on the results of the MT analysis. For example, fewer metal analyses may be carried out in Bousquet River fish if, as observed in the preliminary survey, MT levels are similar at reference and exposure sites. Also, for example, it may be of less interest to analyze muscle tissue (which have shown little accumulation) than organ tissues, so that the analytical budget can be focused in the areas of greatest value. If additional tissue analyses (i.e., > 360 samples) are considered desirable at that time, it would be discussed with the AETE Committee.

3.0 COMPARISON OF SURFICIAL SEDIMENT MAPPING TECHNIQUES

Characterization of bottom sediments in lakes is important from the standpoint of identifying erosional and depositional areas, which influence aquatic habitat conditions and indicate locations where mining-associated contaminants (i.e., metals) are likely to accumulate. It is also important for locating similar habitat conditions in reference and exposed areas for characterization of biological communities. Biological sampling (e.g., benthic invertebrates) and geochemical investigations may then be designed to more effectively identify and delineate any contaminant impacts. This component of the pilot study included a comparative evaluation of subbottom acoustical profiling methods and more conventional bottom grab sampling combined with the use of a standard sonar unit.

Phaneuf (1995) completed a literature review of sonar profiling techniques for identifying and mapping bottom sediments for the geochemical evaluation of lake sediments. Subbottom profiling records vertical sedimentary sequences in lake sediments, thereby providing both surface and subsurface sediment characteristics. Subbottom profilers may penetrate up to 100 m into the sediments, with greater penetration achieved at the expense of resolution. Phaneuf (1995) suggested that subbottom profiling may be combined with side-scan sonar to provide a more accurate surface and subsurface representation of sediment characteristics, but concluded that this is generally not cost-effective for routine monitoring.

Phaneuf (1995) also concluded that sampling on a square or triangular grid pattern over the sediment surface with a small grab sampler such as a petite Ponar is effective in characterizing surficial sediment characteristics, although it provides little or no information on stratigraphy and may be somewhat ineffective in measuring spatial variability as it is not continuous. Phaneuf (1995) did not specifically consider the combined use of conventional sonar units and grab sampling to determine bathymetry and bottom type. Such approaches are conventionally applied by field biologists in environmental effects monitoring studies to identify surficial sediment characteristics for the purposes of delineating erosional and depositional areas and characterizing aquatic habitat conditions.

For the 1995 field evaluation, both sonar/grab sampling and subbottom profiling were carried out independently during the same period and the results compared. Sonar/grab

sampling was undertaken by BEAK biologists, while subbottom profiling was undertaken by Ms. S. Phaneuf, under subcontract. Two lakes were surveyed in this evaluation: lac Chassignolle and lac Bousquet. Both surveys were undertaken during the preliminary reconnaissance survey from 05 to 10 September 1995.

3.1 Sonar/Grab Sampling

3.1.1 Methods

The BEAK field crew undertook a combined sonar/bottom grab survey using a "Lowrance X-16" chart-recording sonar, a hand-held Global Positional System (GPS) unit and a petite Ponar grab sampler. Multiple sonar transects were run on each lake to determine depth characteristics, with GPS coordinates taken at the ends of each transect and at one or two intermediate locations to allow correction for wind drift and changes in boat speed. Sonar transects were run using a 17-foot inflatable boat or a 16-foot aluminum boat outfitted with a 20 HP motor. Sonar transects are plotted in Figures A3.1a and A3.2a (Appendix A).

Petite ponar grabs of surficial sediment were collected pseudo-randomly from all main lake basins and over the range of depths encountered to identify and describe sediment characteristics. Grab samples were also collected in any areas where there was a change in the intensity of the sonar printout, which reflects a change in the density of the substrate. Rocky substrates were identified along the lac Bousquet shoreline, with the distance of rocky bottom from the shoreline determined by pulling a weighted drag line along short transects perpendicular to shore to facilitate tactile identification of rock bottom-fine sediment boundaries. Confirmatory petite Ponar grabs were taken to confirm the fine sediment boundary. Locations of petite Ponar grabs were determined by GPS, and are shown in Figures A3.1a and A3.2a (Appendix A).

3.1.2 Results

Results of sediment and bathymetry mapping of lac Bousquet and lac Chassignolle, based on sonar and grab sampling, have been previously depicted in Figures 2.4 and 2.5 (Section 2.0), respectively.

In lac Chassignolle, results show a predominantly clay/silt sediment, with some sandy deposits found on the eastern (windward) shoreline. Rocky deposits along shorelines did not extend into the lake beyond about 1 m of water depth. Some sand was present within the silty-clay in the Baie de la rivière Bousquet, as apparent by tactile inspection. However, the sediments were still classified as predominantly clay. Water depths in lac Chassignolle range up to approximately 8 m, with a main eastern basin and a second, shallower western basin.

In lac Bousquet, sediments were predominantly silty (mud) in texture, with localized sandy deposits and silty-sand mixtures. As in lac Chassignolle, rocky shorelines did not extend substantially into the lake, except along submerged cliff faces located in various shoreline sections. Sediments collected from the hypolimnion (below about 16 m of water depth) were laminated or varved, with alternating light and dark laminae of 1 mm or less in thickness, possibly reflecting seasonal anoxia and formation of dark-coloured sulphide minerals. Water depths ranged up to 19 m, with two distinct deep basins (≥ 16 m deep).

3.1.3 Effort and Costs

The field effort required in bottom characterization and bathymetry mapping was approximately 24 person-hours based on a two-person field crew. Synthesis of the data to produce report-ready maps required approximately 24 person-hours including 16 technician-hours to produce draft maps and 8 hours of drafting final maps and verification by field staff. This effort would have cost a total of about \$2,800.00 in professional time for experienced technicians to undertake most of the work, exclusive of mobilization and travel. Project management and interpretive reporting required about eight additional hours of senior staff, or about \$680.00. Associated office expenses (e.g., computer time, graphics costs, etc.) totalled about \$200.00.

Field equipment required includes a bottom grab sampler (petite Ponar, approximate cost \$900.00 including import duty and taxes), a chart-recording sonar (retailing at about \$2,000.00), a GPS unit (approximately \$500.00 to \$1,200.00) and a boat and motor (approximate value \$4,000.00). Equipment of this type is generally available and relatively widely used by the environmental consulting community, and could be typically assigned to a project of this nature for approximately \$750.00 to \$1,000.00 for one week of field use.

Based on the above, routine application of this approach, exclusive of mobilization and travel costs, would be expected to cost in the order of \$4,700.00 to generate aquatic habitat maps and to characterize surficial sediments to allow for selection of sampling sites.

3.2 Subbottom Profiling

3.2.1 Methods

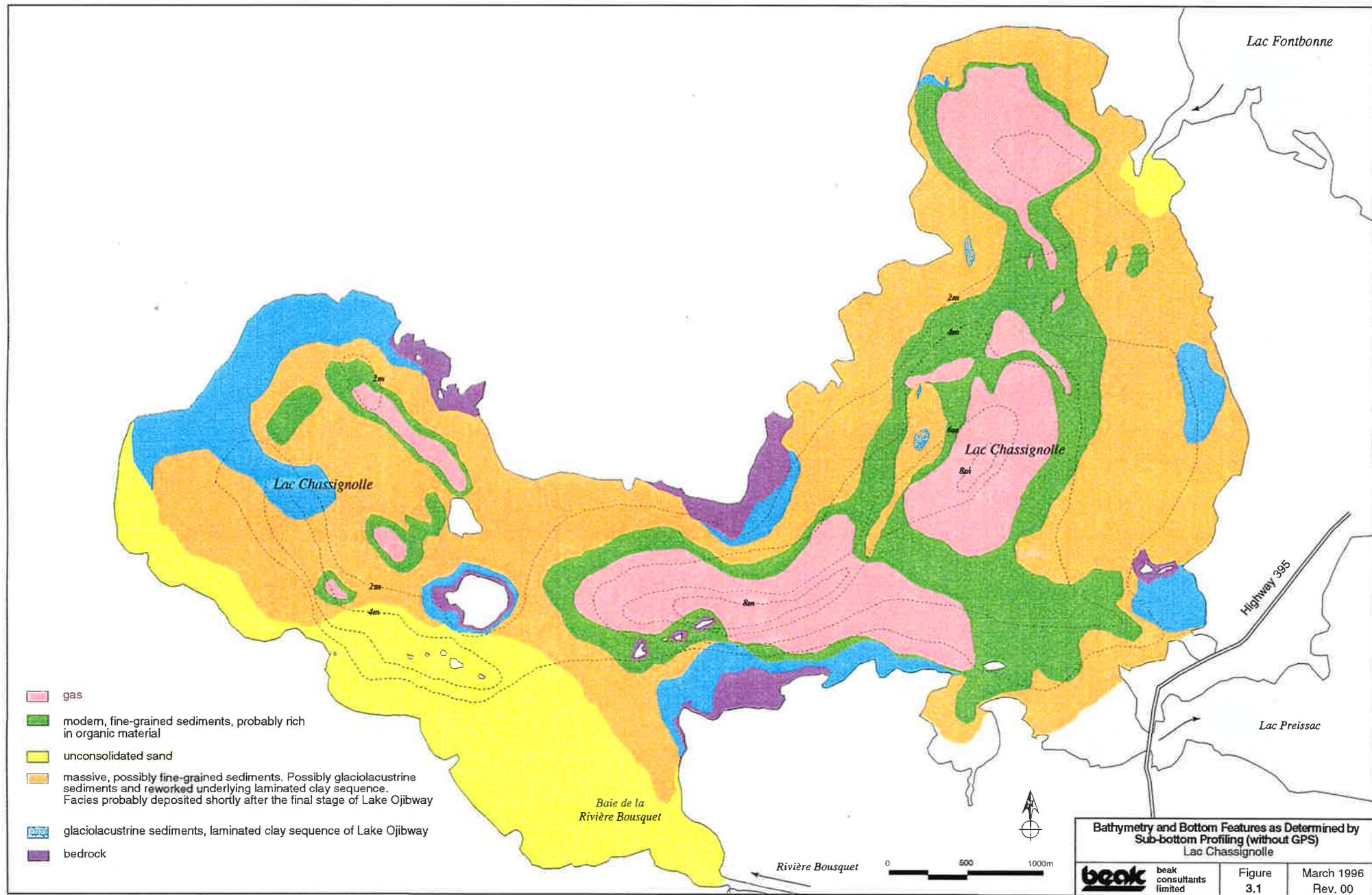
Subbottom profiling was undertaken by S. Phaneuf along multiple transects in each lake using a "Raytheon 1000" subbottom acoustic profiler rented from the Geological Survey of Canada. The instrument was mounted on a 17-foot inflatable boat equipped with a 20 HP outboard motor. Sonar tracings were interpreted by Ms. Phaneuf, and used to produce bathymetry maps and to map surficial characteristics. Results of the mapping and interpretation, and sonar tracings were forwarded to GSC for review to provide further verification of the interpretation. It was somewhat difficult to locate professionals who had enough experience in this field to interpret the sonar tracings. Subbottom profiling transects are illustrated in Figure A3.1b and A3.2b (Appendix A).

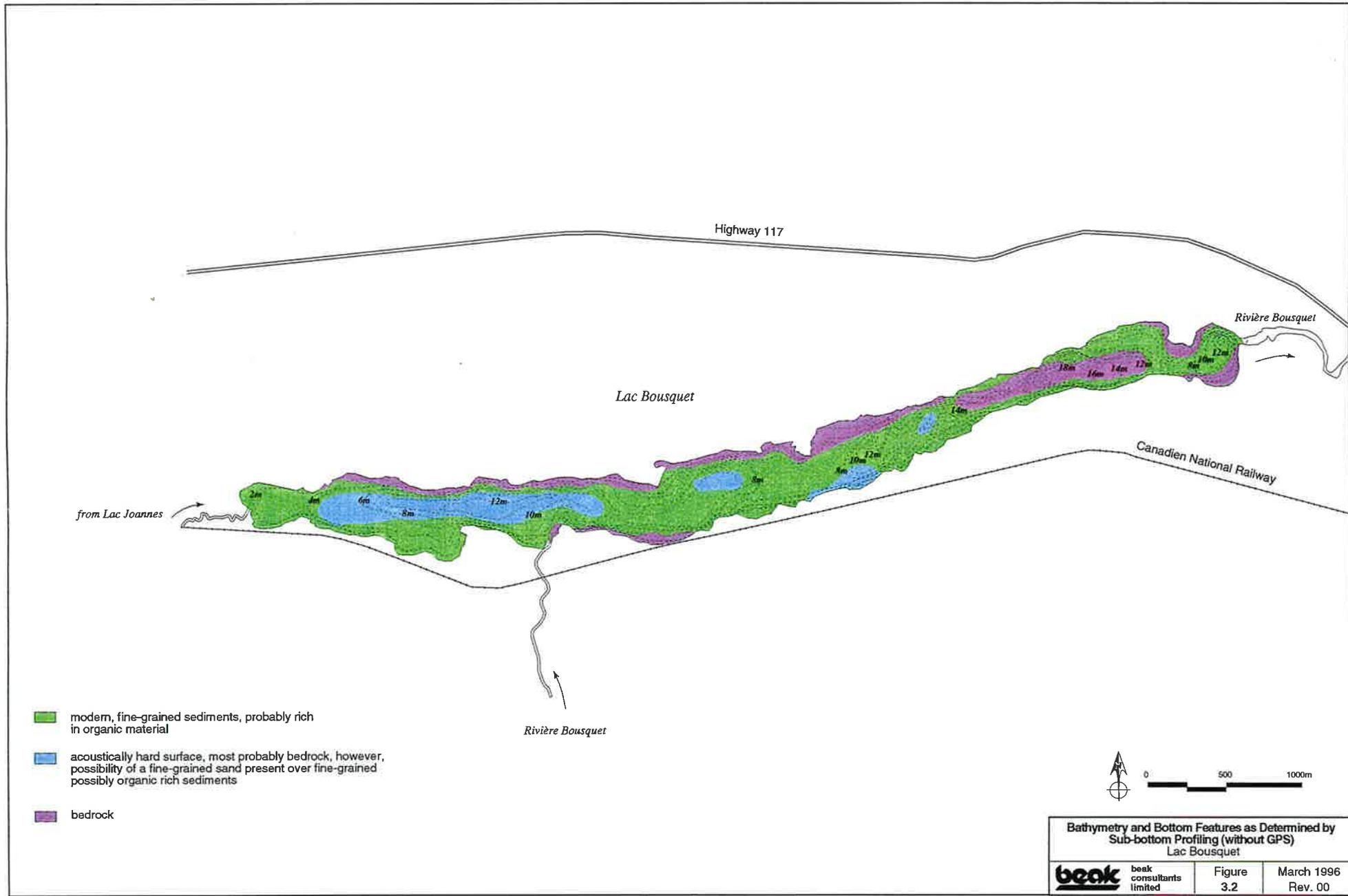
3.2.2 Results

Subbottom profiling produced more complex maps of bottom characteristics than did the sonar/grab sample technique (Figures 3.1 and 3.2).

Subbottom profiling of lac Chassignolle suggested various types of fine-grained sediments, with the deepest basins difficult to interpret owing to apparent subsurface gas accumulations. The southwestern portion of lac Chassignolle was mapped as dominated by sand. As in the sonar/grab sampling, lake bathymetry showed a larger eastern basin and a smaller western basin.

In lac Bousquet, subbottom profiling results were interpreted as showing extensive shoreline and offshore deposits of bedrock or probable bedrock, with fine-grained sediments in other areas. Lake bathymetry mapping identified a deep (18 m) basin in the eastern portion of the lake, and a somewhat shallow (to 12 m deep) basin in the western portion of the lake.





The GSC reviewer of the subbottom profiling results noted that the interpretation appeared reasonable, but required direct bottom sampling (e.g., coring) for confirmation, and that the profiling work should not be considered conclusive. While S. Phaneuf was equipped with a Hornbrook sampler, the degree of bottom verification completed on-site is unclear as it was not specified in her report.

3.2.3 Costs

The field and mapping effort associated with the subbottom profiling work approximately equalled the effort required for sonar/grab sampling. It should be noted, however, that the specific training necessary to undertake this type of work and to interpret results is probably rare within the environmental consulting industry across Canada. Rather, the technique appears to be more commonly used in geophysical/geochemical applications which may be relatively rarely applied to support biological effects monitoring. For example, within Beak Consultants Limited, we employ one specialist trained in undertaking a survey of this type, with this individual based in our Seattle office.

In terms of field expenses, the principal differences between sonar/grab sampling and subbottom profiling are the need for relatively costly subbottom profiling technologies and senior personnel to operate the equipment. As outlined by Phaneuf (1995), these instruments have purchase prices of \$26,200 to \$140,000 U.S., exclusive of duties and taxes. Rental of equipment is possible from some suppliers, at typical rates of 10 to 15% of the purchase price for the first month of rental. Thus, retail rental rates available to undertake even a relatively short field survey such as outlined here might be in the order of \$4,000 to \$20,000, including some allowance for additional insurance and shipping costs. Alternately, a firm specializing in oceanographic or geophysical studies might have such equipment and trained staff available on a subcontract basis, but this would require dedicated specialists for this aspect of an environmental effects monitoring study (in addition to the equipment), and result in costs in excess of those listed above for equipment rental. Note that BEAK's cost for equipment rental was a nominal \$1,200 from the GSC, but this covered relatively old instrumentation and does not reflect a current retail market rental rate which were quoted in the range of \$540 to \$1,000 per day including shipping days.

For this specific study, it was necessary to employ a specialist (S. Phaneuf) familiar with subbottom profiling. Thus, our costs associated with subbottom profiling required substantial additional mobilization, travel and subsistence costs which would not have been incurred if subbottom profiling could be carried out by field biologists/technicians. These additional costs totalled about 32 person-hours plus field expenses (about \$2,500.00). Additional project management requirements (communications, coordination, etc.) totalled about \$800.00.

In summary, subbottom profiling surveys of lac Chassignolle and lac Bousquet would total approximately \$3,700 for field work and mapping, \$4,000 to \geq \$20,000 for equipment rentals plus about \$3,300 of additional costs for travel, subsistence, mobilization, etc., or \geq \$11,000 total.

3.3 Evaluation of the Methods

Sediment characteristics are identified in further detail by subbottom profiling than by sonar/grab sampling methods. However, results of the subbottom profiling work in this case appear unreliable based on two key observations:

- confirmatory cores with up to almost 1 meter penetration in some cases (lac Bousquet) collected at probable bedrock substrates during the October survey (after completion of subbottom profiling) confirmed the silt (mud) and clay bottom interpretations made by sonar/grab sampling. There was no surficial bedrock in any of the areas identified as bedrock in lac Chassignolle and lac Bousquet; and
- confirmation grabs taken in each of the different substrate types identified in lac Chassignolle indicated very little difference between any of the substrates. Substrates were fairly uniform (silt and clay) with no obvious visual or textural differences throughout the lake with the only notable difference in substrate texture being in the mouth of the Bousquet River where the clay was more sandy.

In terms of bathymetry mapping, both the subbottom profiler and conventional sonar should yield comparable results. Discrepancies between results produced by S. Phaneuf and BEAK

can probably be ascribed to any positional uncertainty and the number of transects per lake and their position. More transects across the lake will result in better accuracy in delineating depth contours.

If the purpose of bottom surveys is to delineate aquatic habitat conditions, identify general sediment types and depositional areas, both subbottom profiling and sonar/grab sampling appear to provide adequate results, provided that subbottom profiling incorporates both direct grab or core sampling to confirm all sediment types. Also, because depositional environments in lakes typically accumulate sediments at a slow rate (e.g., up to a few millimetres per year) relative to mining history (e.g., years to decades), characterization of the surficial sediment layer (e.g., < 50 cm) is typically adequate to encompass the zone of altered geochemistry (e.g., metal enrichment) and biological activity. BEAK has observed this to be the case at various sites with decades of mining history. Again, both subbottom profiling and sonar/grab sampling should adequately describe potential mining-impacted sediments.

In unusual cases where sediment stratigraphy may confound the delineation of mining effects on geochemistry, subbottom profiling may be preferable. Such instances may occur where, for example, modern, mine-impacted sediments overlay sediments of a different origin. Given the additional expense, limited availability and technical expertise required to implement subbottom profiling, a decision to undertake this technology should be made based on information gained from previously completed sonar/grab sampling surveys, profiling of sediment (metal) gradients in sediment cores and assessment of biological effects. In this field evaluation, for example, decisions on where sediment coring and biological sampling should be undertaken in either lake would be made more cost-effectively based on the results of sonar/grab sampling, with any additional uncertainties associated with undetermined stratigraphic variation possibly addressed by collecting a few additional sediment cores. The cost differences between the two sediment characterization methods (i.e., >\$5,000 in this case) would typically be significantly greater than the cost of collecting and analysing metal profiles in sediments from two or three additional sampling stations per lake.

In summary, aquatic effects of importance at mine sites include adverse biological effects and chemical effects in sediments (especially where these lead to biological impairment).

Bottom characterization using conventional sonar techniques, supplemented with grab sampling, is generally the more cost-effective approach for identifying depositional areas and determining where sediment geochemistry and bioassessment studies should be carried out, as these provide for characterization of the first ≤ 50 cm of sediment where mining effects are likely to occur. Where natural background levels of mining-related parameters cannot be identified in accompanying sediment core sampling measurements, due to possible subbottom influences, subbottom profiling might be justified in subsequent EEM monitoring studies.

It is our opinion that further field evaluation of subbottom profiling is unnecessary and we recommend that for future pilot studies mapping of bottom substrates be undertaken using conventional sonar and stratified grab sampling, which is the most cost-effective method.

4.0 EVALUATION OF SEDIMENT CORING METHODS

Sediment cores may be collected to measure profiles of metals or other sediment quality parameters potentially influenced by mining activities, and to provide an indication of (pre-mining) baseline concentrations at depth in downstream depositional areas.

The Geological Survey of Canada (GSC) uses a device called a Hornbrook sampler in their extensive geochemical reconnaissance program carried out in support of mineral resource inventories (Photo 1). This device consists of a two-piece lead-weighted cylindrical steel core tube designed to penetrate through the first few centimetres of sediment to recover a single sample of deep (subsurface) sediment that may be indicative of background (Friske and Hornbrook, 1991). The device is equipped with a butterfly valve mounted on a steel stem near the mouth of the core to prevent sample loss upon withdrawal of the sample from the sediment. The device was specifically designed for use by the GSC, and is not generally used in other limnological survey work. The GSC's interest in evaluation of the Hornbrook sampler arises principally from a concern that conventional surface sediment grabs cannot be used to sample deeper, uncontaminated sediments and that background conditions measured by surface sediment grabs in upstream or reference lakes may not represent conditions in downstream lakes owing to potential unique geological influences in each waterbody. The Hornbrook sampler represents a specific example of a gravity corer.

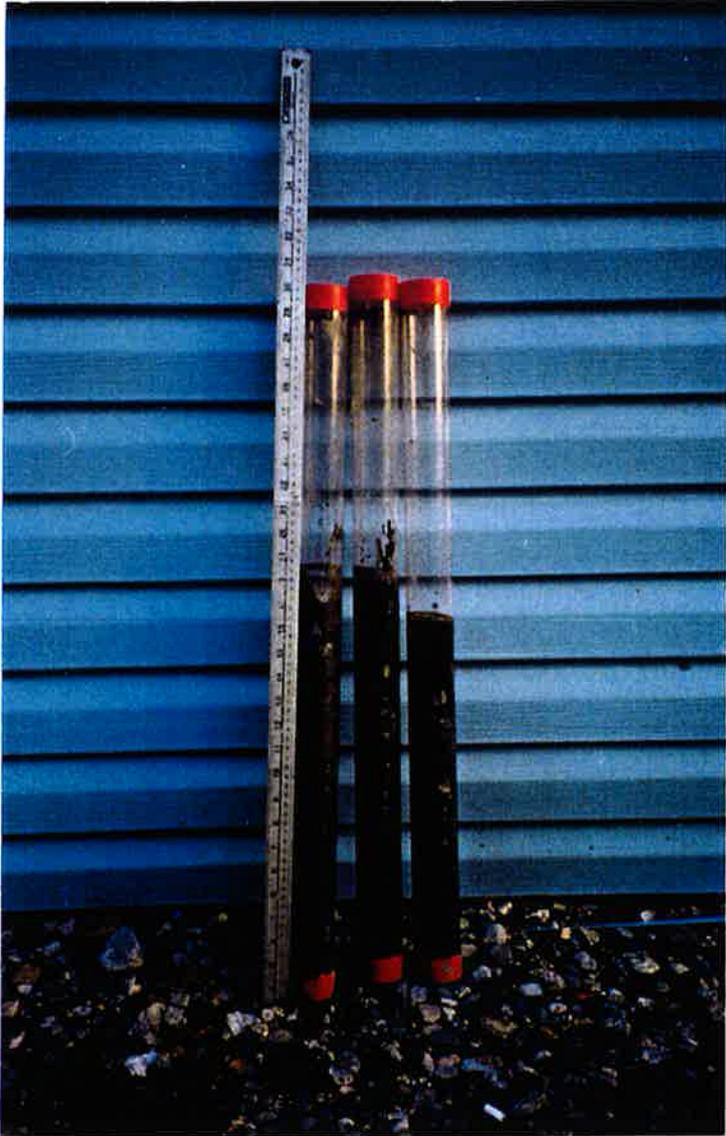
Other gravity corers are more routinely used in limnology to collect not only deeper (subsurface) sediment, but also to more readily evaluate profiles of metals (or other sediment constituents). Such devices are reviewed and discussed by Mudroch and MacKnight (1991). For this program, two such corers, a Kajak-Brinkhurst (KB) corer and an Alpine corer (Photo 1) were selected for comparison with the performance of the Hornbrook sampler. Both corers may be used either with or without core catchers (also known as "egg shells") to assist in prevention of sample loss (analogous to the butterfly valve of the Hornbrook sampler) may be used with different sizes of core tubes (e.g., up to 1 or even 1.5 m long) and employ non-metallic (e.g., polyacrylic) core tubes.

This study evaluates the use of the Hornbrook, K-B and Alpine core samplers at three locations in lac Chassignolle and lac Bousquet.



Photo 1: Hornbrook Sampler - left
Alpine Corer - right

Photo 2: Cores from Alpine Corer



4.1 Methods

Preliminary Survey

Sediment samples were collected at selected locations to identify potential effects of mining on metal concentrations and to assess and compare the use of the GSC's Hornbrook sampler and a conventional limnological corer (K-B corer).

The Hornbrook sampler and K-B corer were deployed in the central basin of lac Chassignolle, with the intent of developing metal profile data (K-B corer) and of identifying background (pre-mining) conditions from subsurface sediment (Hornbrook sampler). Neither sampler functioned as intended owing to the fact that surficial lake sediments in Lac Chassignolle consist of a stiff clay that could not be effectively penetrated. The K-B corer could not retrieve a sample without the aid of an egg shell which was not available at the time of the preliminary survey. The Hornbrook sampler, which is weighted near the core bottom, penetrated to a depth of about 20 to 25 cm, but was unable to pass through and reject the surface layer because of the shallow nature of the lake (8 m) and the hardness of the clay substrate. Based on sediment observations in Lake Bousquet, it was felt that both samplers would function in that waterbody, but no sampling was attempted during the preliminary survey. A subsample of the Hornbrook-collected sediment from lac Chassignolle was collected and submitted for analysis for comparison with the sediment collected by petite Ponar.

Main Survey

During the main survey, an Alpine corer fitted with a 1 m core tube and sleeve was used instead of the K-B corer, since the Alpine corer is weighted near the bottom and heavier than the K-B corer it would better penetrate the clay sediments of lac Chassignolle. However, after initial trials, it was apparent that the Alpine corer, like the K-B corer, was unable to retrieve a core from lac Chassignolle without a stainless steel core catcher which functioned similar to the butterfly valve in the Hornbrook corer. Cores of about 40 cm length could readily be retrieved once the corer was equipped with a core catcher installed (Photo 2). Generally, a core catcher should not be used, unless necessary, as it may disturb sediment stratigraphy during sediment penetration. However, the butterfly valve in the

Hornbrook corer because of its thickness causes much more disturbance than the core catchers.

Alpine and Hornbrook cores were collected at one location each in lac Bousquet (LB-2) and lac Chassignolle (LC-2) (see Figure 5.1 to 5.3 in Section 5). The penetration depth for the Hornbrook was determined by measuring the length of the sediment core. In all cases the sediment core did not reach beyond the window on the device. In each case, the bottom 5 cm of the Hornbrook core was collected. The Alpine cores were held intact, and extruded into 2 cm sections to a 10 cm depth, and subsequent 5 cm sections to the core bottom. Core extrusion and sectioning was carried out onshore.

Selected sections of each Alpine core (three in lac Bousquet, four in lac Chassignolle) were selected for analysis of total metals. These samples, plus the Hornbrook samples, were submitted to RPC Laboratories for analysis.

4.2 Results

Results of metal analyses are tabulated in detail in Appendix B.

The Hornbrook sample collected at LC-1 (lac Chassignolle) demonstrated somewhat lower concentrations of As, Cd, Cu and Pb than in the surface (petite Ponar) grab sample (Table 4.1). This indicates the usefulness of the Hornbrook in collecting a deeper (background) sediment for analysis. Unfortunately, the depth of Hornbrook penetration was variable and would not always be measurable (e.g., it may penetrate deeper in soft sediments). This may be important if the depth of contamination is of interest.

In all cases the Alpine corer, being heavier than the Hornbrook corer, was able to collect deeper sediments. As shown in Figures 4.1 to 4.5, the Hornbrook sampler produced results similar to those found in Alpine core sample sections from the similar sediment depths. Thus, the Hornbrook and Alpine corer were effective in collecting subsurface "background" levels of metals. However, arsenic, cadmium and lead (Figures 4.1, 4.2, and 4.5) levels in the Hornbrook sample were slightly higher than in Alpine core sections from shallower depths. This appears to be a result of contamination of the sediments in bottom section of the Hornbrook from the surface sediments. The butterfly valve in the Hornbrook sampler

TABLE 4.1: COMPARISON OF TRACE ELEMENT CONCENTRATIONS COLLECTED BY PETITE PONAR AND BY HORN BROOK SAMPLER AT STATION LC-1, LAC CHASSIGNOLLE, SEPTEMBER, 1995

Analysis	Petite Ponar	Hornbrook Sampler
Aluminium	71700	74800
Antimony	0.33	0.17
Arsenic	11	2
Barium	583	623
Beryllium	1.6	1.4
Bismuth	9.94	0.87
Cadmium	1.73	0.35
Calcium	17000	16100
Chromium	137	137
Cobalt	32.8	22.7
Copper	74.6	27.7
Iron	48500	44400
Lead	48.9	16.7
Lithium	42	50
Magnesium	14700	15600
Manganese	1470	789
Mercury	0.10	0.06
Molybdenum	16.3	1.8
Nickel	68.0	62.1
Phosphorus	921	814
Potassium	22700	23400
Rubidium	112	111
Selenium	<2	<2
Silver	0.95	0.40
Sodium	19900	19300
Strontium	264	251
Tellurium	2.0	0.3
Thallium	0.6	0.6
Tin	2.8	1.6
Uranium	2.0	1.1
Vanadium	97	93
Zinc	222	113

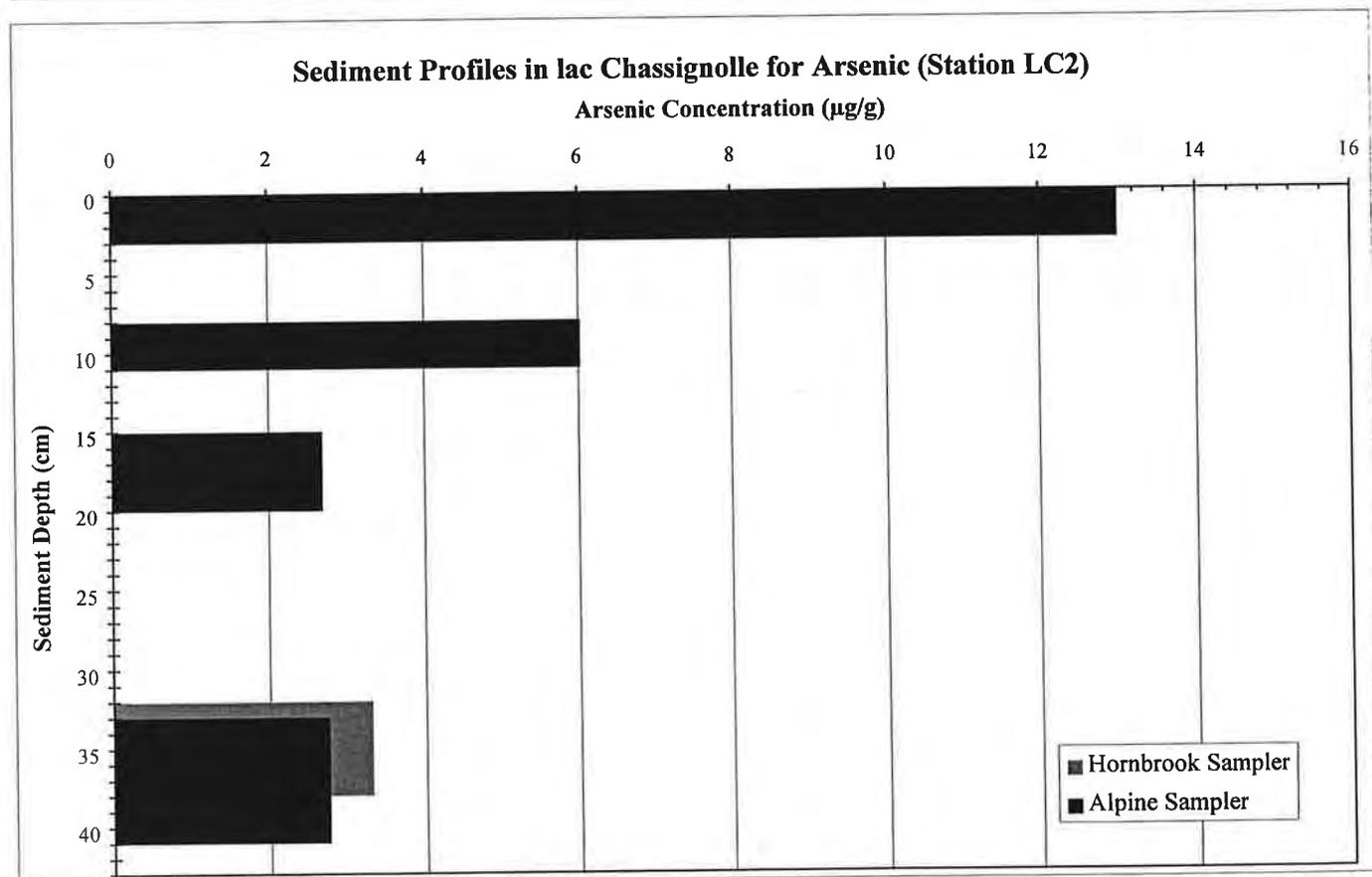
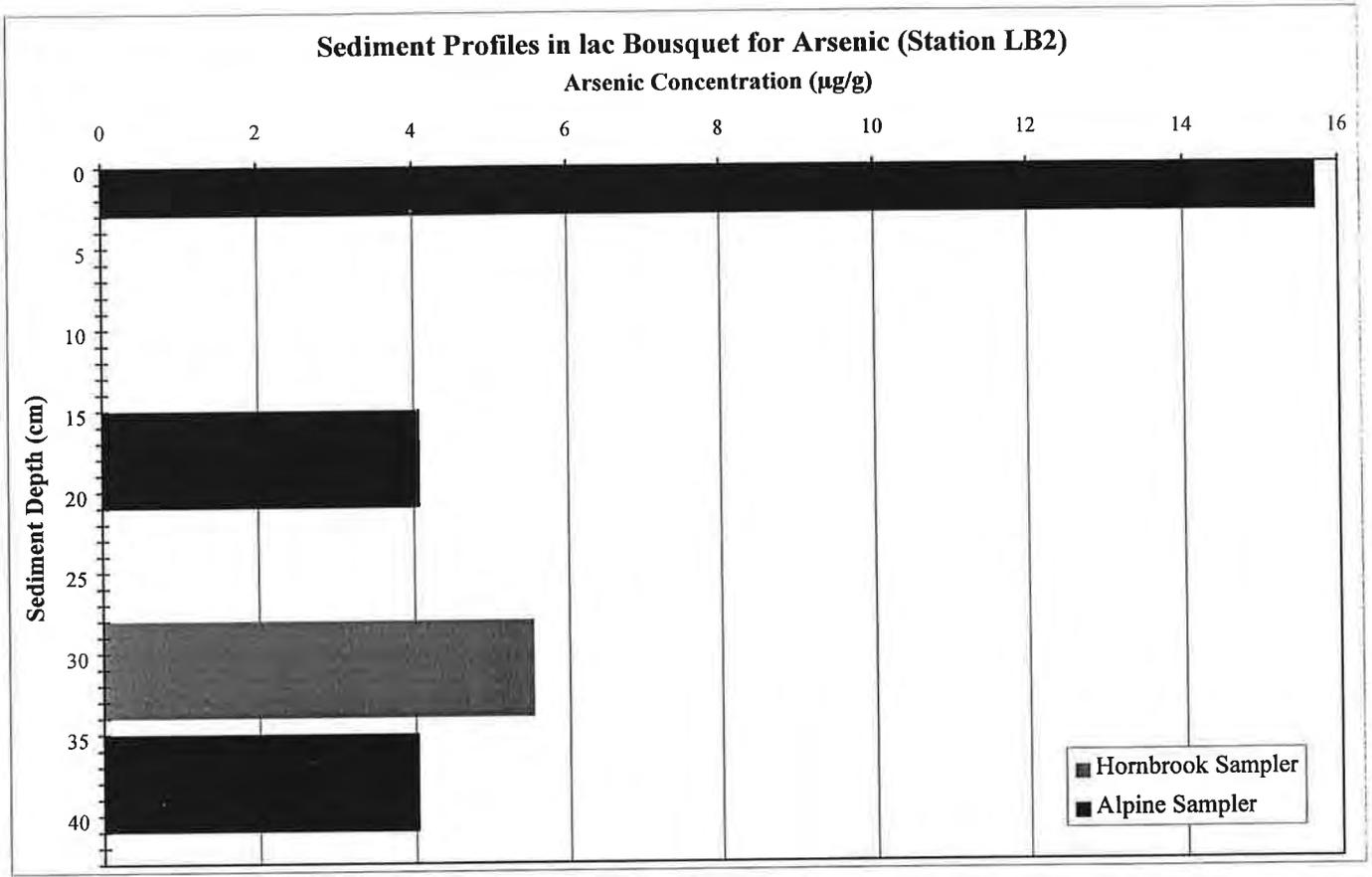


Figure 4.1 Alpine Corer versus Hornbrook Corer Comparison, Arsenic

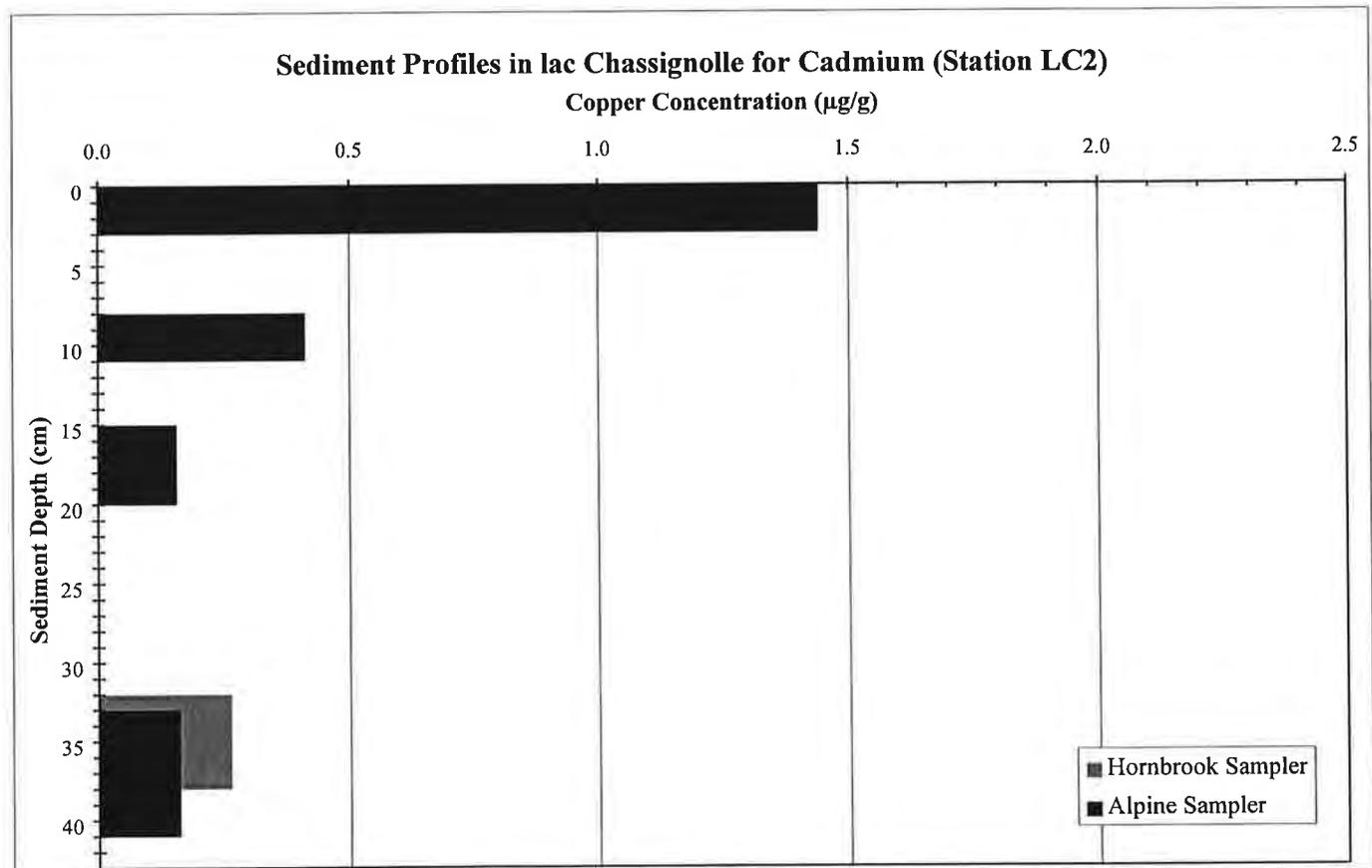
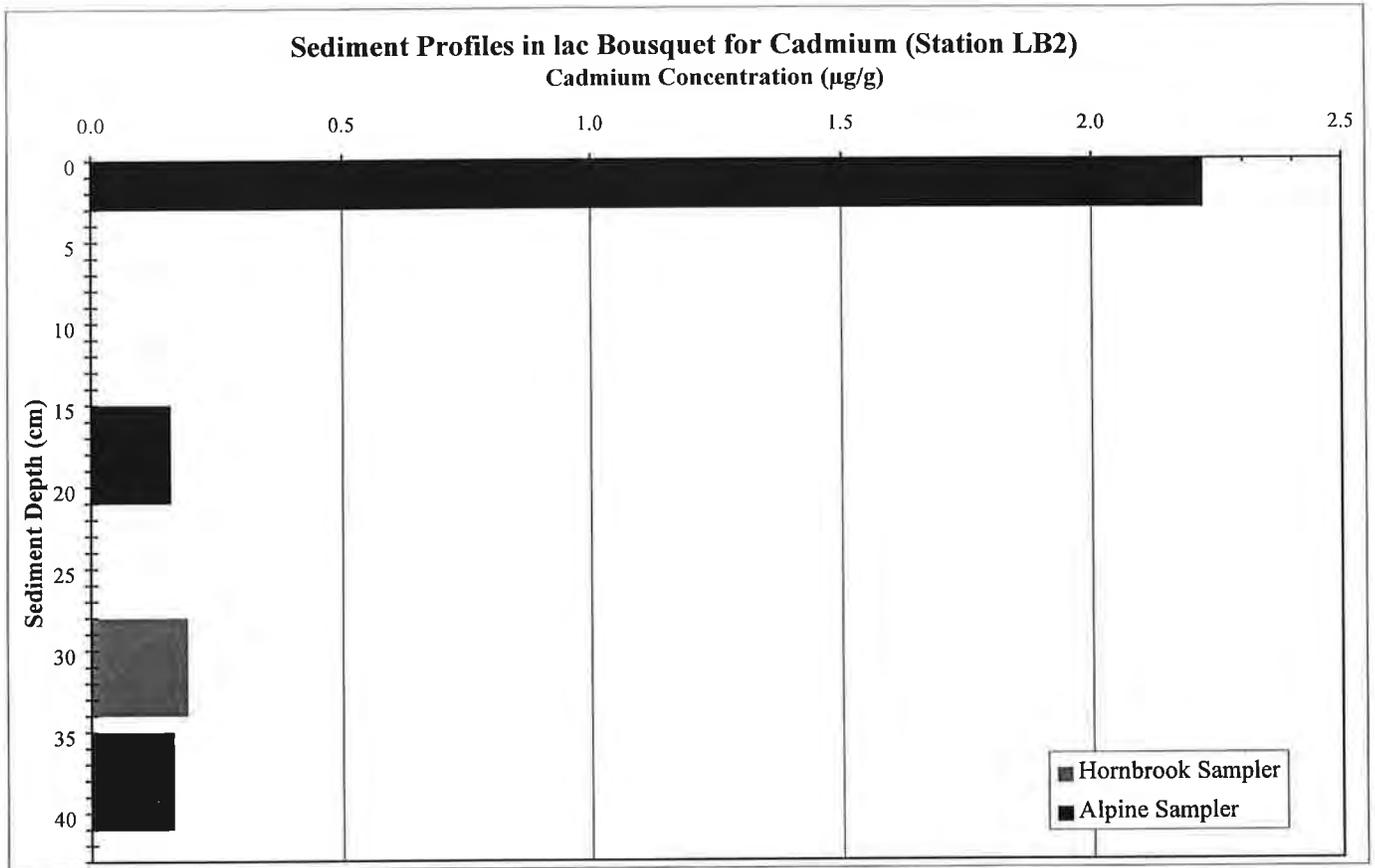


Figure 4.2 Alpine Corer versus Hornbrook Corer Comparison, Cadmium

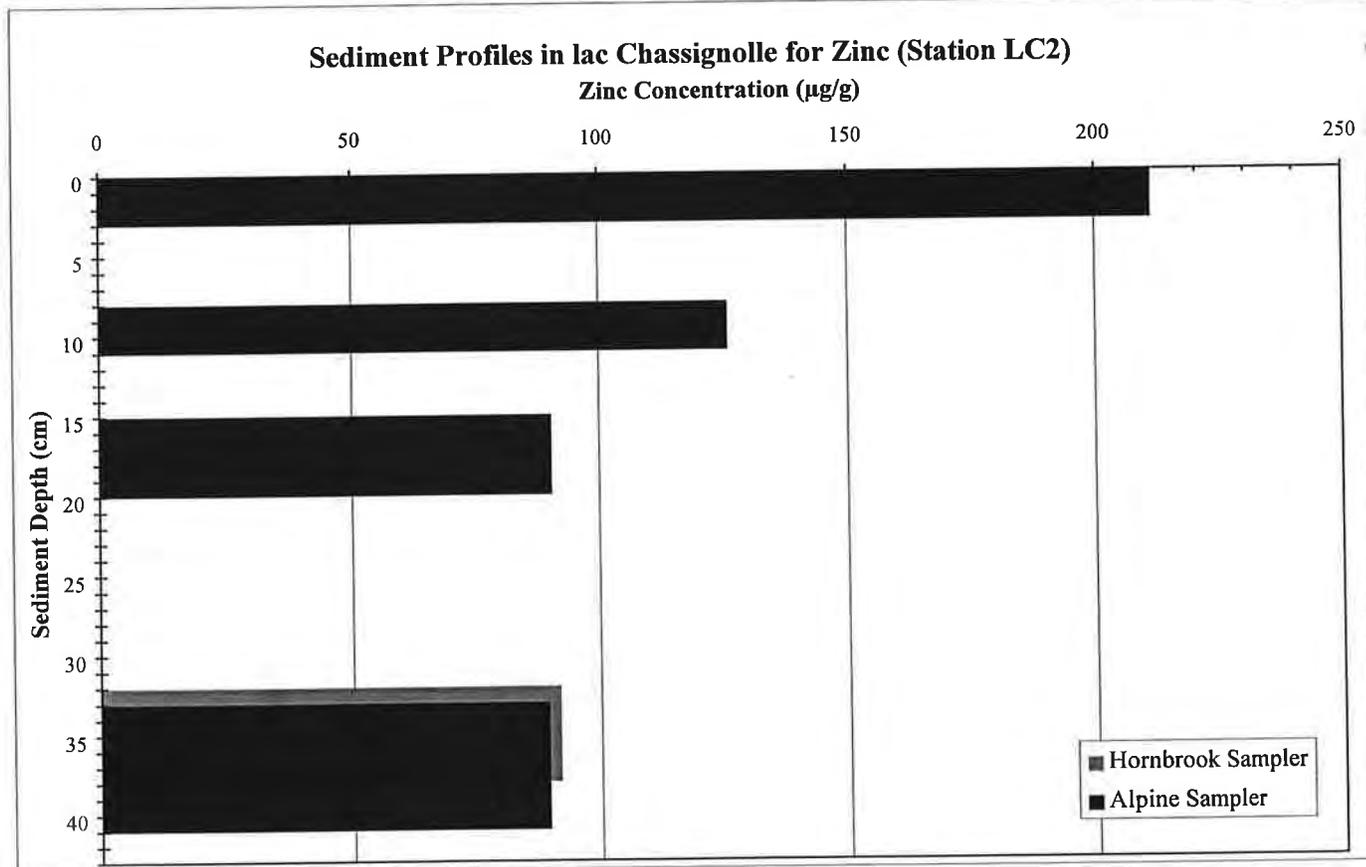
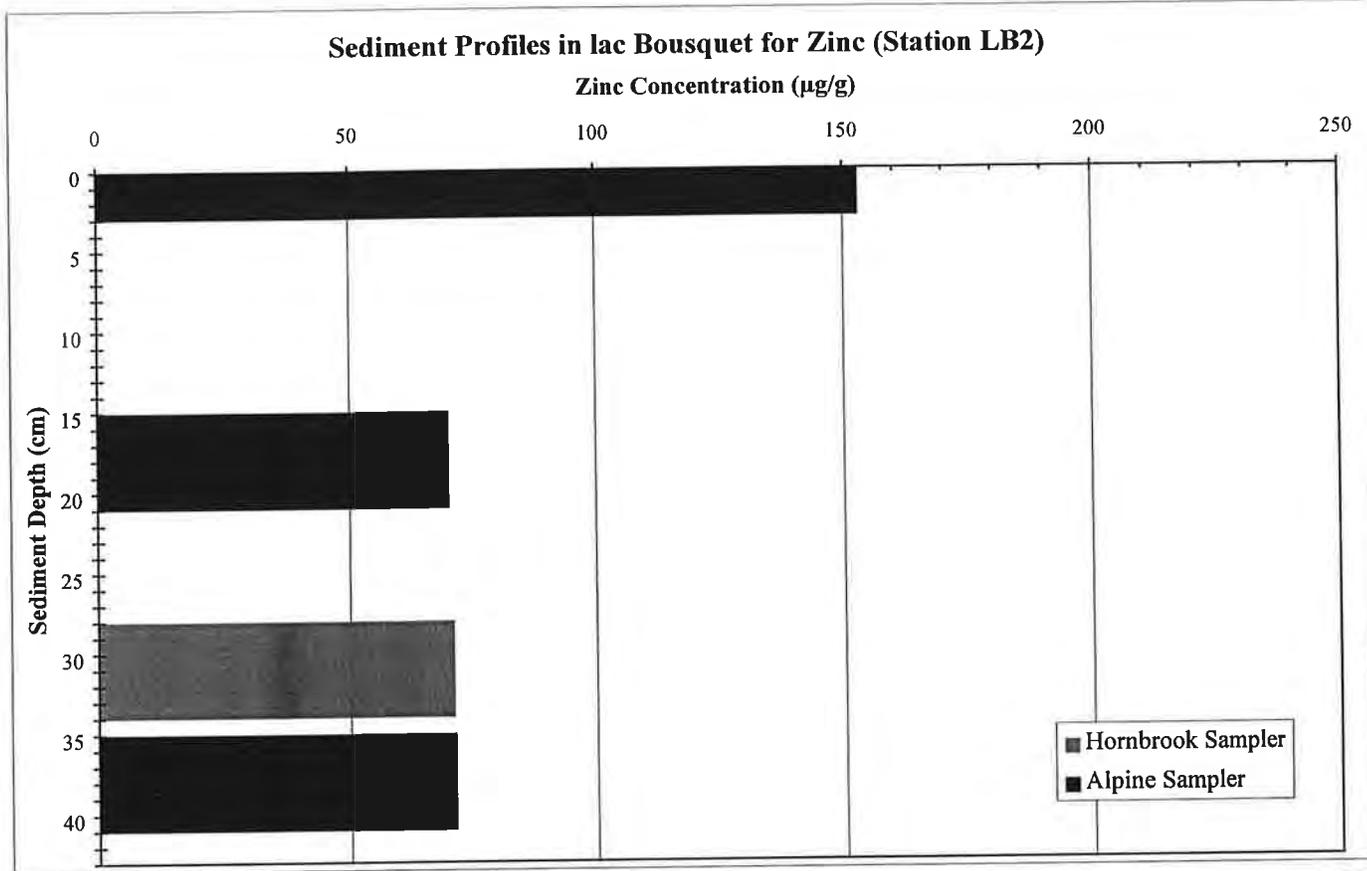


Figure 4.3 Alpine Corer versus Hornbrook Corer Comparison, Zinc

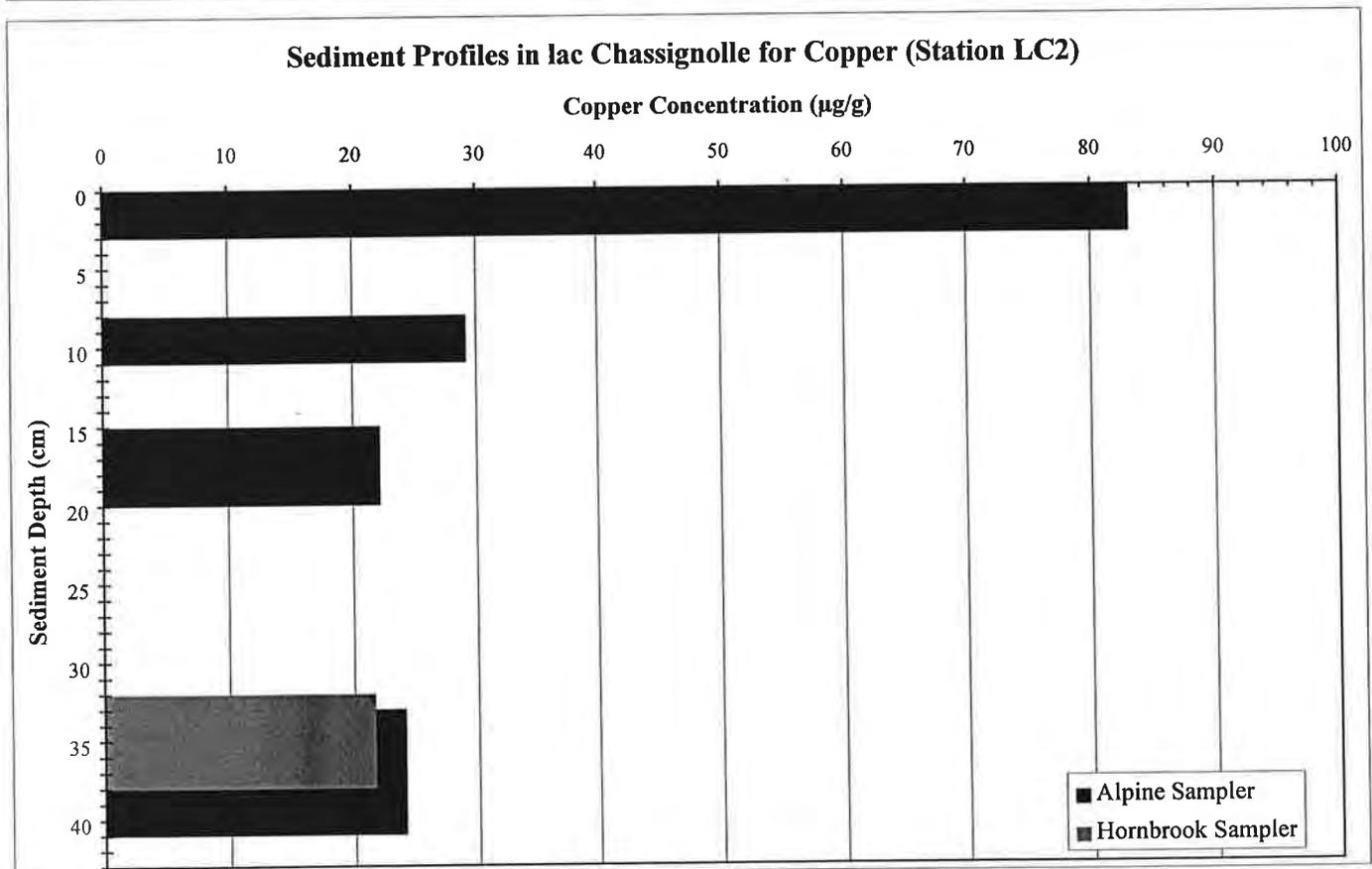
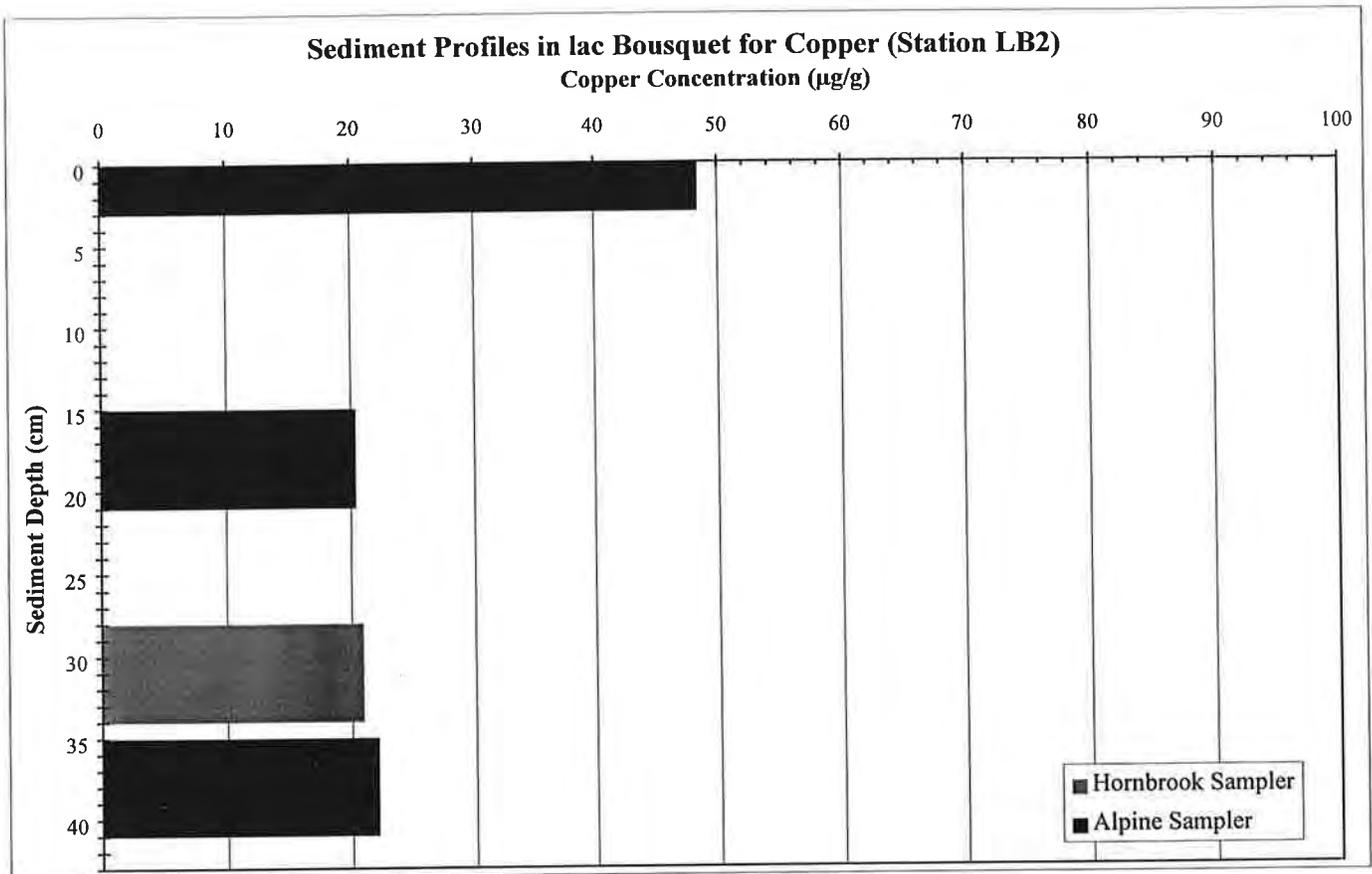


Figure 4.4 Alpine Corer versus Hornbrook Corer Comparison, Copper

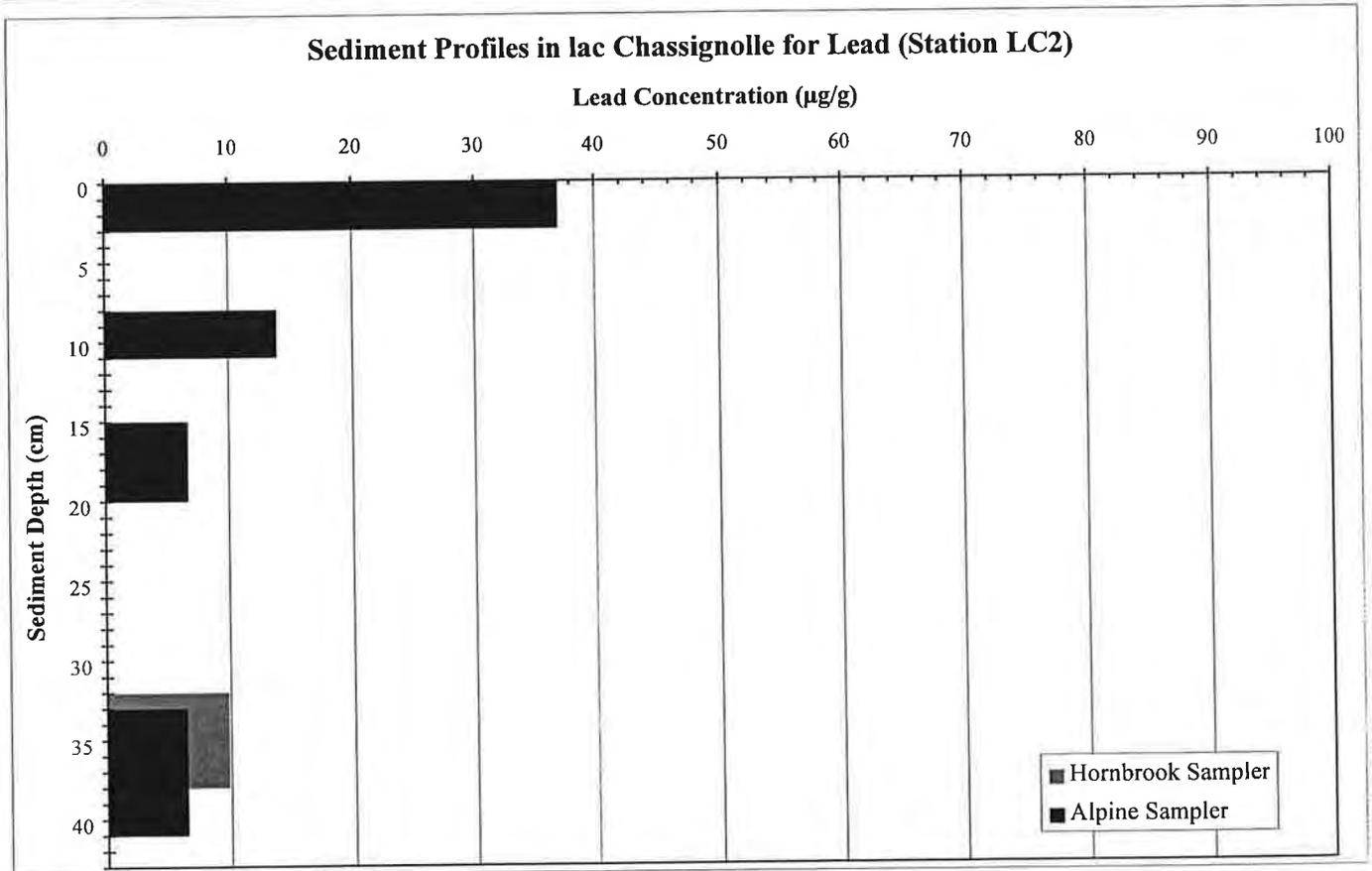
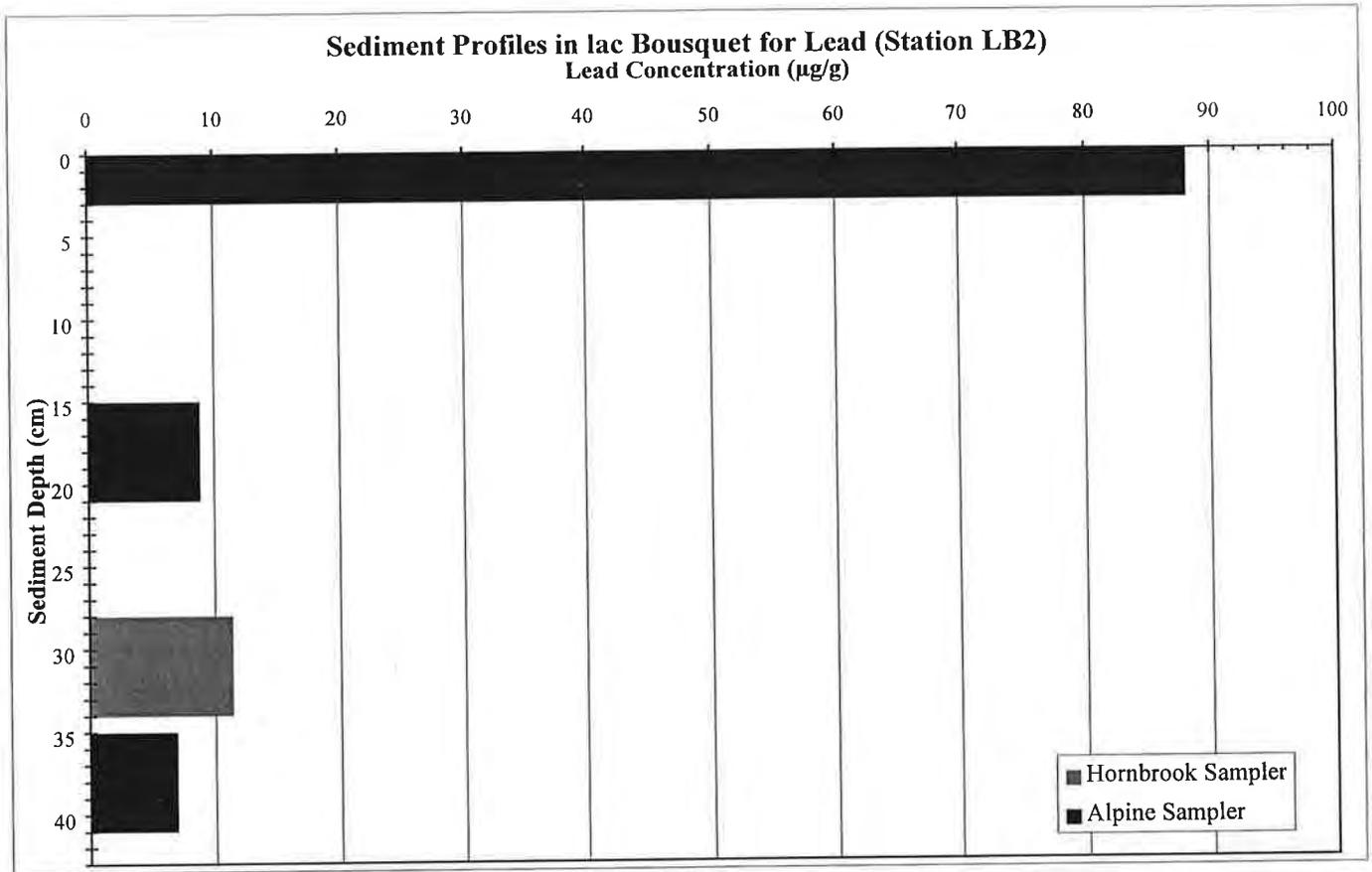


Figure 4.5 Alpine Corer versus Hornbrook Corer Comparison, Lead

is fairly thick and during penetration slices through the centre of the core, often leaving a channel from surface to bottom. We found it virtually impossible to collect the bottom section from the Hornbrook core without surface silt and silty water contaminating the bottom section.

It is interesting to note that all metals shown here are enriched in the surface sediment of each lake, with no consistent evidence that lac Chassignolle (downstream of the mines) is any more contaminated than lac Bousquet (upstream reference). This may imply an unidentified source other than local mines for the surficial sediment enrichment.

4.3 Costs

The costs and effort associated with the use of either the Hornbrook and Alpine corer are similar. The Hornbrook is limited in availability (fabricated to order for the GSC), but is inexpensive (apparently <\$500.00). Gravity corers are available through various suppliers of limnological sampling equipment, and are somewhat more expensive (in the order of \$1,500 to \$2,000 depending on the sampler type and features desired). Both types may be deployed by a technician with minimal training. Once on station, the time required to deploy either corer is generally less than ten minutes, with the Hornbrook requiring somewhat less time than the Alpine due to the need to remove and replace core tubes and core catchers. Cores collected by Alpine corers (and other gravity corers used to sample sediment profiles) require additional time for sectioning and measuring - typically 15 minutes per core for two people, plus in the order of five to ten minutes each for set-up and cleanup activities for each series of cores processed. However, if the objective is to only determine background metal levels then a sample from the bottom end of the Alpine core requires no more effort than collection of the bottom section of the Hornbrook core.

Analytical costs per sample are unaffected by sampler type, although multiple core sections may be collected by Alpine corer, resulting in possibly greater analytical costs per site but at the same time obtaining additional data. This additional cost must be weighed against the benefits of obtaining additional analytical data.

4.4 Evaluation of the Methods

The Hornbrook corer and conventional gravity corers may be qualitatively compared as follows:

	Hornbrook	Alpine/K-B Types
Ease of Use	Simpler.	Slightly more intricate.
Data Obtained	Based on one sample/core, from somewhat indeterminate depth.	Many samples per core possible. Exact penetration depth known.
Integrity of Core	Disturbance by stem of butterfly valve with possible contamination of background levels.	Less disturbance, especially if core catcher not required. Far less contamination of deeper sediments.
Contamination from Sampling Device	Possible due to non-stainless steel construction and sampler contact with sample.	Less possible due to separation of sample from metallic parts (non-metallic core tubes).
Need for Other Equipment	Requirement for surface grab sample use if sampling of mine-impacted layer desired.	None required.

Overall, we conclude that either the Hornbrook sampler or other core samplers should be used in environmental effects monitoring, as it is important to identify metal concentrations at sediment depths underlying any surficial zone affected by mining. The Hornbrook sampler must be accompanied with other samplers (e.g., Ponar grab, petite Ponar grab, Ekman grab, etc.) to collect the surficial layer of interest. Overall, we preferred the gravity corers to the Hornbrook. The gravity corers allowed for more detailed measurement of metal profiles, including knowing the exact depths at which the sediments were collected. In addition, they allowed for visual inspection of the core to identify changes in sediment type with depth and had less risk of contamination of the deeper sediments during collection. Both corers can provide background metal levels however, the gravity corers with core tubes provide much more information for the same sampling effort and are more readily available.

It is our opinion that no further comparative evaluation of coring methods is necessary in future AETE field evaluation programs. However, we do recommend that future pilot

studies undertake limited coring in the main depositional zones upstream and downstream of the mine discharge. This will allow for determination of pre-mining sediment metal levels and provide a relative measure of the degree of mine-related sediment contamination.

5.0 WATER AND SEDIMENT QUALITY

5.1 Water Quality

Water quality may be affected by mining operations through the release of treated effluents, as well as through untreated acid rock drainage (ARD). Downstream waters may be affected in terms of dissolved and total metal concentrations, as well as through other process or water treatment byproducts (e.g., cyanide from gold mill operations, total dissolved solids from treatment of ARD). Water quality conditions were evaluated in the rivière Bousquet/lac Chassignolle and in the rivière Noire system principally to define conditions which may influence biological communities (i.e., fish and benthic parameters). Information is also presented on costs associated with achieving relatively routine and lower analytical detection limits (1/10th CCME water quality guidelines).

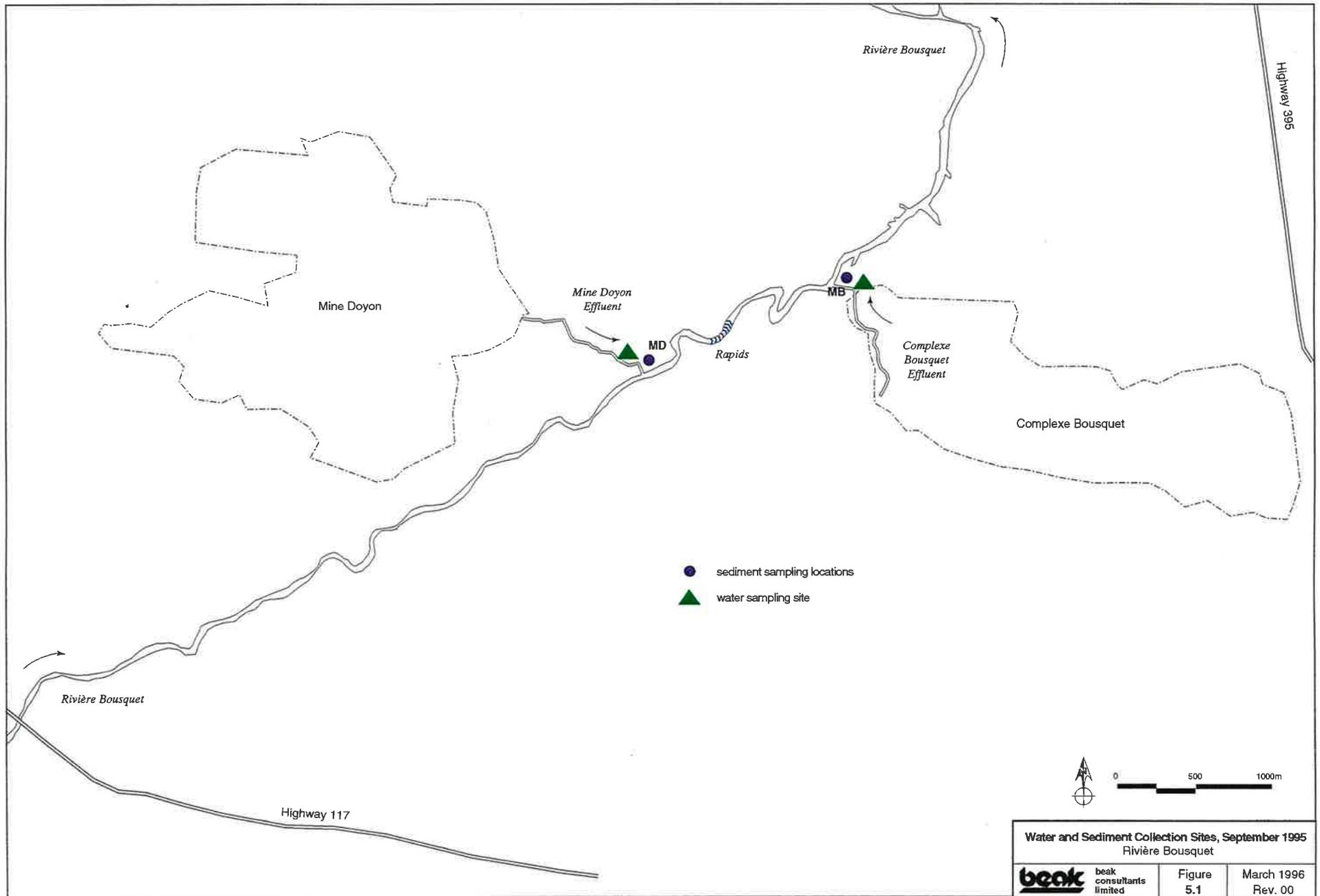
5.1.1 Methods

Stations where water quality samples were collected in September and October 1995 for analysis are shown in Figures 5.1 to 5.4.

During the preliminary (September 1995) survey, water samples were collected from the vicinity of Complexe Bousquet and Mine Doyon to characterize mine sources and upstream-downstream water quality gradients. Field measurements of pH, conductivity, dissolved oxygen and temperature were also collected at various sites. Samples for trace element determination by ICP-MS were collected as unfiltered samples directly in clean, hand-held bottles at four sites - the Complexe Bousquet and Mine Doyon effluent streams, the rivière Bousquet mouth (downstream site) and the lac Bousquet outlet (reference).

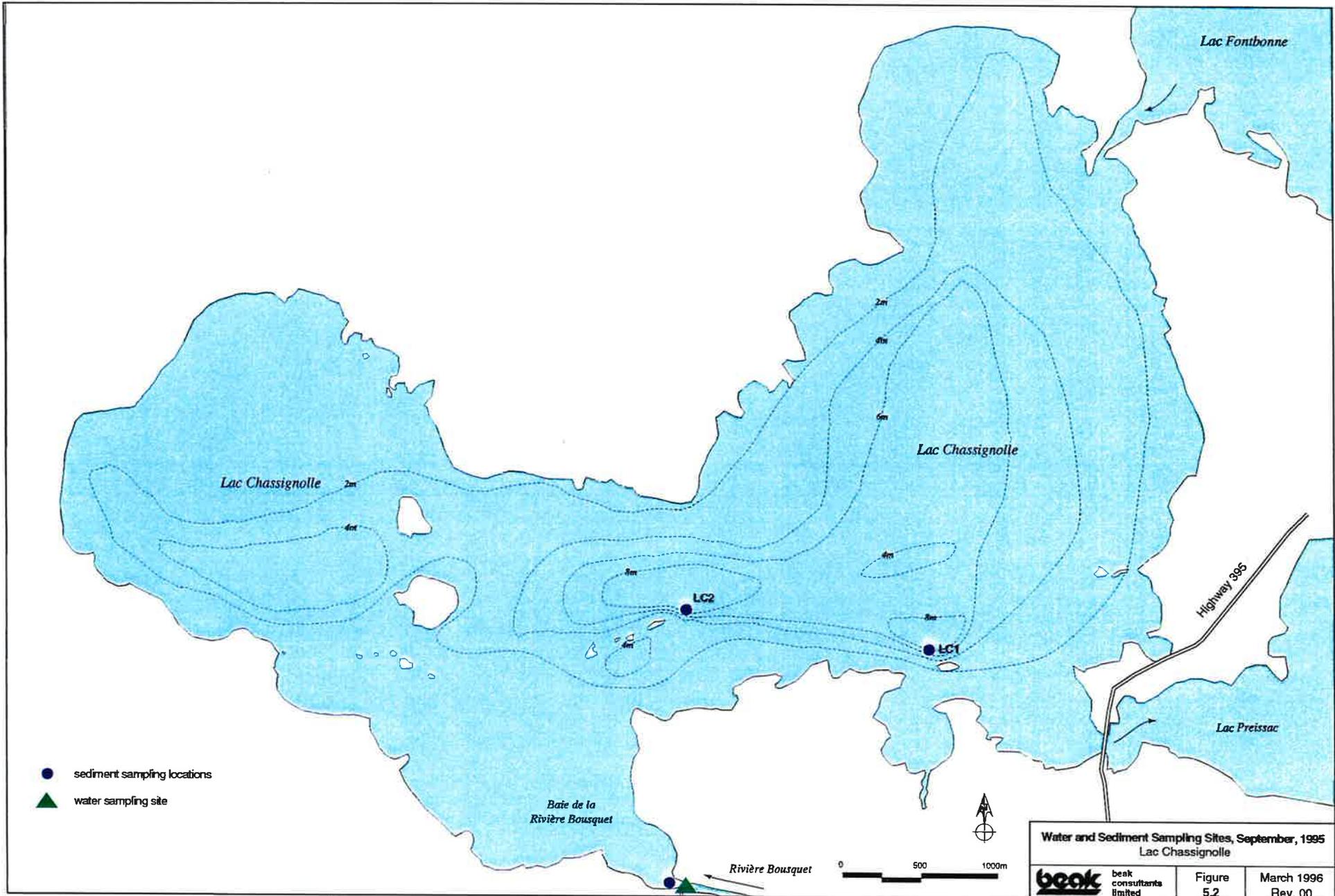
Field measurements focused on conductivity used to detect mine effluent in the environment (Section 2.0) and dissolved oxygen to evaluate deoxygenation arising from thermal stratification (e.g., lac Bousquet) or chemical stratification (e.g., rivière Bousquet, rivière Noire) downstream of mine effluents.

The main (October 1995) survey focused on characterization of water quality at an array of stations along the rivière Noire system to characterize conditions at stations sampled for



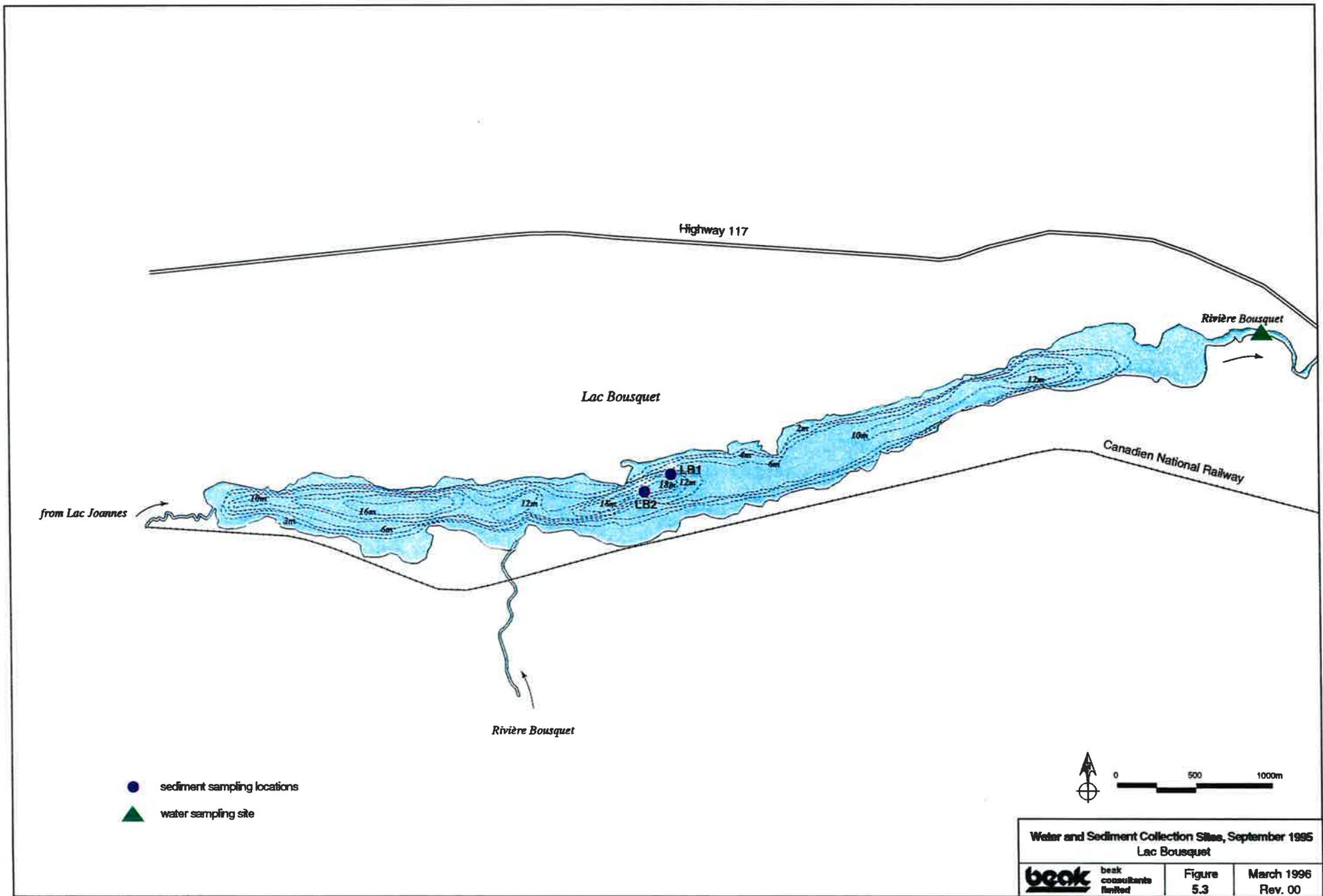
Water and Sediment Collection Sites, September 1995
 Rivière Bousquet

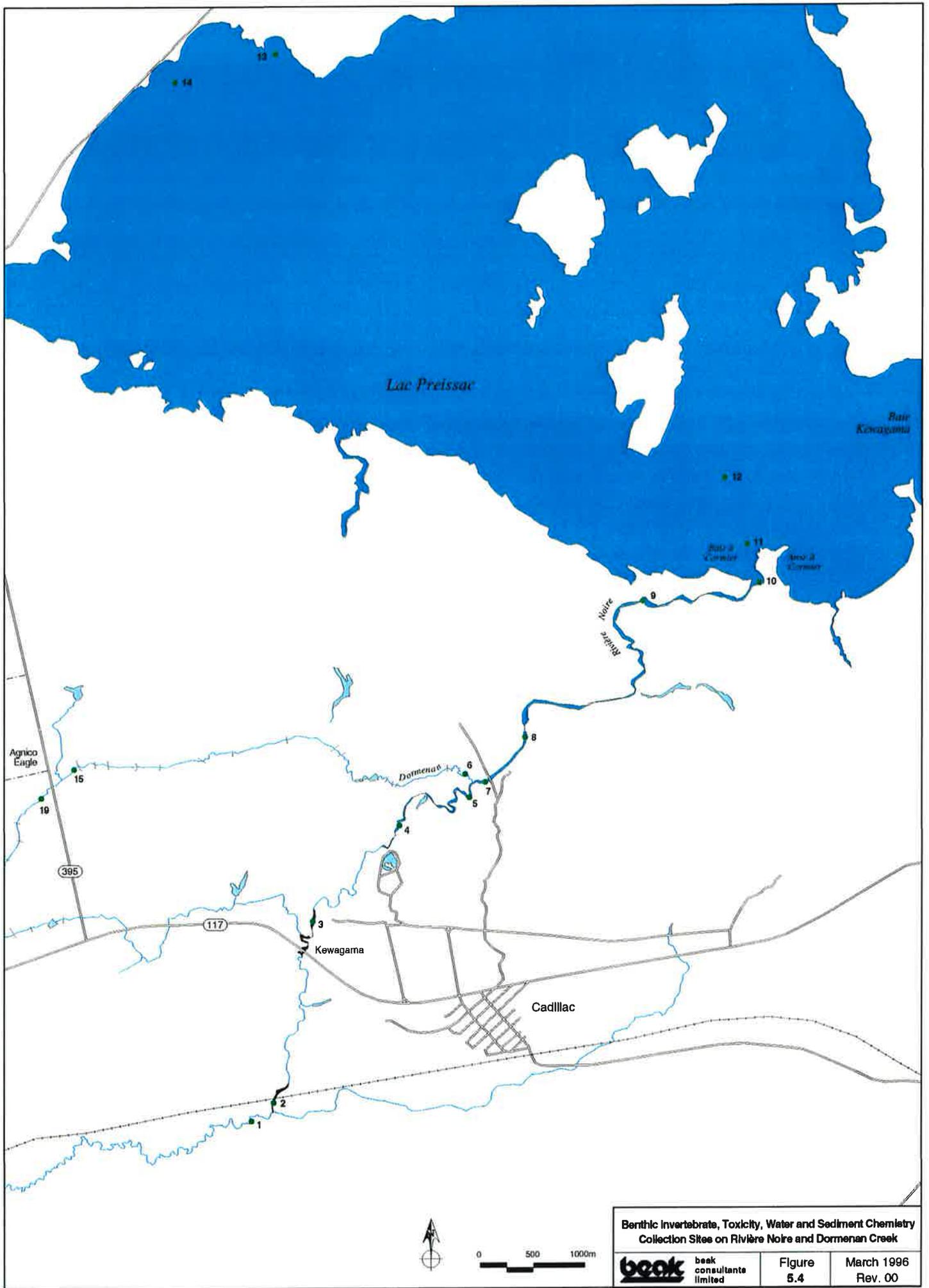
 beak consultants limited	Figure	March 1996
	5.1	Rev. 00



Water and Sediment Sampling Sites, September, 1995
Lac Chassignolle

 beak consultants limited	Figure	March 1996
	5.2	Rev. 00





Lac Preissac

Rivière Kewagama

Rivière Noire

Dornenau

Kewagama

Cadillac

Agnico Eagle

395

117

Point de Curvature

Point de Curvature

13

14

12

11

10

9

8

4

5

6

7

3

2

19

15



**Benthic Invertebrate, Toxicity, Water and Sediment Chemistry
Collection Sites on Rivière Noire and Dornenau Creek**

beak beak consultants limited

Figure 5.4

March 1996 Rev. 00

benthic invertebrates and sediment toxicity. Water samples were collected to identify sources from the Dumagami Mine and from the two inactive properties located upstream of the Dumagami Mine. These locations were similarly sampled for trace elements and field parameters (pH, conductivity, dissolved oxygen, temperature), as well as for other parameters not sampled at rivière Bousquet during the preliminary survey, including nitrogen species, total cyanide, hardness, sulphate, phosphorus, chloride, acidity, alkalinity and dissolved organic carbon.

All water samples for laboratory analysis were collected directly in clean, hand-held 0.5-L bottles. Samples for trace metal determination were acidified to pH <2. All unpreserved samples were refrigerated and delivered to RPC Laboratories, Fredericton, for analysis within four days of collection. Laboratory method detection limits are as described in Appendix B.

Field conductivity was measured with a YSI Model 33 S-C-T meter and probe. An Omega Model PHH-80 Digital pH meter with a gel-filled probe was used to measure pH. Temperature and dissolved oxygen were measured with a YSI Model 57 DO meter and probe. Meters were calibrated daily using standard solutions.

5.1.2 Results

5.1.2.1 Preliminary Reconnaissance

General water quality conditions observed during the September survey are discussed briefly in Section 2.5 and have been summarized in Table 5.1A. Temperature and dissolved oxygen profiles for lac Bousquet and lac Chassignolle are provided in Table 5.1B. There was an anoxic hypolimnion in lac Bousquet but was not present in lac Chassignolle.

Trace metal analysis results from September sampling are shown in Table 5.2, and include an upstream reference station and a downstream station in the Bousquet River, as well as one station in each of the two mine effluent discharge streams. The following key results are highlighted.

TABLE 5.1A: SUPPORTING FIELD MEASUREMENTS, SEPTEMBER, 1995

Station	Latitude North	Longitude West	Depth (m)	Top Temp. (°C)	Bottom Temp. (°C)	Top D.O. (mg/L)	Bottom D.O. (mg/L)	pH	Top Cond. (µmhos/cm)	Bottom Cond. (µmhos/cm)
<u>rivière Bousquet</u>										
Reference ¹	48°14.45'	78°31.67'	2.6	13.0	13.0	8.5	8.8	7.32	63	63
Exposure ²	48°15.35'	78°29.60'	2.7	13.0	13.0	8.0	4.5	6.90	277	1810
Mine Bousquet	48°15.47'	78°28.99'	0.3	8.0	8.0	-	-	7.6	1180	1180
Mine Doyon	48°15.16'	78°30.02'	0.3	11.0	11.0	-	-	6.24	2940	2940

¹ Reference - rivière Bousquet at lac Bousquet outlet

² Exposure - rivière Bousquet at lac Chassignolle inlet

TABLE 5.1B: TEMPERATURE AND DISSOLVED OXYGEN PROFILES OF LAC BOUSQUET AND LAC CHASSIGNOLLE, SEPTEMBER 1995

Depth (m)	lac Bousquet		lac Chassignolle	
	Temperature (°C)	Dissolved Oxygen mg/L	Temperature (°C)	Dissolved Oxygen mg/L
Surface	15.0	8.2	10.8	13
1	15.0	8.1	10.7	13
2	15.0	8.1	10.7	13
3	14.5	8.1	10.7	13
4	14.5	7.7	10.7	13
5	14.5	7.7	10.6	13
6	14.5	7.8	10.6	13
7	14.5	7.8	10.6	13
8	14.5	7.6	10.6	13
9	14.0	6.1	Bottom	Bottom
10	11.0	1.6	-	-
11	8.0	1.7	-	-
12	7.0	1.6	-	-
13	6.0	1.6	-	-
14	5.5	1.6	-	-
15	4.5	1.6	-	-
16	4.0	1	-	-
17	3.5	0.2	-	-
18	3.0	0	-	-
	Bottom	Bottom	-	-

TABLE 5.2: TRACE ELEMENT CONCENTRATIONS ($\mu\text{g/L}$) IN WATER SAMPLES, SEPTEMBER

	lac Bousquet Outlet (reference)	Complexe Bousquet Effluent	Mine Doyon Effluent	rivière Bousquet Near Mouth
Aluminum	244	1130	1230	311
Antimony	0.1	0.1	<0.1	<0.1
Arsenic	2	<1	<1	1
Barium	9.6	32.9	33.5	17.3
Beryllium	<0.1	<0.1	<0.1	<0.1
Bismuth	<0.1	<0.1	<0.1	<0.1
Boron	2	48	56	6
Cadmium	0.44	1.2	0.13	0.1
Calcium	5320	165000	526000	47700
Chromium	2	6	1	2
Cobalt	0.3	1.8	8.5	1.9
Copper	4.5	7.4	8.4	5.5
Iron	530	1380	185	560
Lead	3.7	5.8	1	2.7
Lithium	0.9	8	78	7
Magnesium	1280	27400	70000	7120
Manganese	40	445	515	138
Mercury	<0.2	<0.2	<0.2	<0.2
Molybdenum	0.1	0.3	0.3	0.2
Nickel	3	8	14	5
Potassium	561	6230	11100	1240
Rubidium	1.9	11.3	27.4	3.8
Selenium	<1	<1	6	<1
Silver	<0.1	<0.1	<0.1	<0.1
Sodium	1810	16300	49400	5380
Strontium	23	525	1710	155
Tellurium	<0.1	0.1	0.1	<0.1
Thallium	<0.1	<0.1	<0.1	<0.1
Tin	0.1	0.1	<0.1	<0.1
Uranium	<0.1	0.3	<0.1	<0.1
Vanadium	1	3	<1	<1
Zinc	13	64	11	10

- Concentrations of potentially toxic heavy metals (Pb, Zn, Cu, Cd) do not change appreciably between the upstream reference station and the river mouth. Concentrations of heavy metals are low in both effluents, and are at most only about four times greater than found at the reference station.
- Elements increasing in concentration downstream of the mine discharges include the alkali and alkali earth metals, which are associated with treatment of ARD.
- Arsenic concentrations, which are high in the rivière Noire, are low in the rivière Bousquet system.

5.1.2.2 Main Survey

Water quality conditions in October focused on the rivière Noire system (Table 5.3). Dissolved oxygen conditions were relatively high throughout the river, and were well above levels which would lead to any biological impairment. Likewise, pH conditions were near neutral except in ruisseau Dormenan, which was acidic at the upstream reference site (pH 4.97) and basic downstream of Mine Dumagami sources (pH 8.96). Conductivity illustrated the effects of water treatment, with elevated levels in bottom layers of rivière Noire in areas upstream and downstream of ruisseau Dormenan (as at Mine Doyon, the high conductivity effluent moved upstream in the bottom layer of the river), and became well mixed with depth at Stations 9 and 10 downstream of the rivière Noire falls. Contaminant levels at Station 7 clearly show the difference in water quality between surface and bottom waters before complete mixing of the effluent and receiving water (Tables 5.4 and 5.6).

In terms of general water quality parameters (nutrients, major ions, etc.), a general trend of slightly increasing concentrations of ammonia, sulphate and total dissolved solids (TDS) was apparent between reference Station 1 and Station 5, as rivière Noire is influenced by sewage from Cadillac and by drainage from the two inactive mine properties situated east and west of the river (near Stations 3 and 4) (Table 5.4). Ruisseau Dormenan represents an important source of ammonia (78 mg/L measured at the mouth of Dormenan Creek), sulphate, total dissolved solids and hardness, with the latter three parameters introduced by lime treatment of effluent at Mine Dumagami. The ammonia concentrations observed

TABLE 5.3: SUPPORTING FIELD MEASUREMENTS, OCTOBER 1995

Station	Latitude North	Longitude West	Depth (m)	Top Temp. (°C)	Top D.O. (mg/L)	Bottom D.O. (mg/L)	pH	Top Cond. (µmhos/cm)	Bottom Cond. (µmhos/cm)	Substrate Type
<u>rivière Noire</u>										
1	48° 13' 03"	078° 24' 66"	2.0	10.0	8.0	8.0	6.43	37	37	clay & organics
2	48° 13' 06"	078° 24' 67"	3.0	10.0	7.4	1.8	-	52	180	clay & organics
3	48° 14' 03"	078° 24' 27"	2.5	9.5	7.3	7.2	7.14	70	70	clay & organics
4	48° 14' 58"	078° 23' 61"	2.5	9.5	7.0	6.5	7.08	80	80	clay with some organics
5	48° 14' 62"	078° 23' 05"	2.5	10.0	7.3	7.3	6.90	70	70	clay & organics
7	48° 14' 74"	078° 23' 02"	2.5	9.5	7.3	8.2	7.10	100	1 700	clay & organics
8	48° 14' 91"	078° 22' 65"	2.5	10.0	7.2	6.0	7.19	170	1 700	clay & organics (slightly anoxic)
9	48° 15' 60"	078° 21' 63"	2.5	9.0	9.7	9.6	8.01	650	650	clay & organics
10	48° 15' 69"	078° 20' 80"	2.5	9.5	9.7	9.1	8.02	680	700	clay & organics
<u>Dormenan Cr.</u>										
19	48° 14' 70"	078° 26' 50"	0.4	6.0	8.4	8.4	4.97	45	45	mud, silt, clay
15	48° 14' 77"	078° 26' 21"	1.0	15.0	9.6	9.6	6.65	380	2 300	mud & clay
6	48° 14' 75"	078° 23' 19"	1.0	10.0	9.9	9.9	8.96	2 150	2 150	clay & coarse, black organic material
<u>lac Preissac</u>										
11	48° 15' 95"	078° 20' 75"	2.0	13.0	10.6	10.6	7.20	110	650	clay, silt & sand, coarse organic material
12	48° 16' 31"	078° 20' 82"	2.0	11.0	11.1	11.1	7.15	100	100	clay with brown silt, sparse algae
13	48° 18' 42"	078° 24' 39"	2.0	12.0	11.2	11.2	7.21	100	100	clay with brown silt, sparse algae
14	48° 18' 23"	078° 25' 43"	2.0	11.5	11.0	11.0	7.30	100	100	clay with brown silt, sparse algae

TABLE 5.4: GENERAL WATER CHEMISTRY RESULTS FROM RIVIÈRE NOIRE, OCTOBER 1995

Station I.D.	Guideline	REF-1	2	3	4	5	6 *	7 Top	7 bottom	8	Falls	9	10
General Chemistry (mg/L)													
Acidity (as CaCO ₃)	-	7	13	16	10	9	27	10	23	10	12	14	14
Alkalinity (as CaCO ₃)	-	13	18	19	25	24	147	26	120	29	53	60	60
Ammonia (as N)	2.5	0.02	0.21	0.31	0.37	0.07	78.0	0.63	59.5	3.50	16.0	19.4	22.0
Chloride	-	2.6	5.0	7.4	10.8	6.7	170	7.4	136	10.4	40.1	47.7	46.8
Nitrate + Nitrite (as N)	-	< 0.02	< 0.02	0.10	0.10	0.06	0.39	0.07	0.35	0.14	0.43	0.42	0.43
o-Phosphate (as P)	-	< 0.01	< 0.01	0.04	0.10	0.12	< 0.01	0.18	0.02	0.30	0.24	0.08	0.10
Sulfate	-	9.0	9.4	11.5	15.0	15.8	1250	26.6	825	75.0	260	305	315
Total Dissolved Solids	-	75	78	86	101	101	1280	114	1720	180	492	624	635
Total Suspended Solids	15	5	9	10	8	12	15	14	9	12	9	8	9
Cyanide	0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.100	0.070	< 0.005	0.020	0.070	0.030
Dissolved Organic Carbon	-	27	23	20	28	26	57	30	30	30	13	36	21
pH (units)	6.5-9.0	6.4	6.5	6.9	6.9	7.0	7.6	6.8	7.5	6.8	7.3	7.1	7.2
Calculated Parameters (mg/L)													
Bicarbonate as CaCO ₃	-	13.3	17.8	18.8	24.6	23.6	146.4	25.6	119.6	29.1	52.6	60.0	60.2
Carbonate as CaCO ₃	-	0.003	0.005	0.014	0.018	0.022	0.548	0.015	0.356	0.017	0.099	0.071	0.090
Hardness as CaCO ₃	-	17.5	20.7	24.2	37.0	34.2	657	41.1	166	60.6	585	199	184

 exceeds CCREM (1987) guidelines for the protection of freshwater aquatic life

* Sample collected from the mouth of Dormenan Creek

indicate a potential source of toxicity to aquatic biota in the creek and river and as far as Station 11 in lac Preissac (Table 5.5).

Trace element analyses of rivière Noire and ruisseau Dormenan show several parameters elevated above background (Station 1) conditions downstream of mine facilities, with arsenic being the most evident (Tables 5.6 and 5.7). Arsenic does not appear to be associated with treated effluent from Dumagami, since arsenic levels are relatively low in ruisseau Dormenan samples and since arsenic levels are high as far upstream as Station 4, apparently reflecting effects from the two inactive properties. Although high, arsenic levels in the range observed (up to 827 $\mu\text{g/L}$) are unlikely to be acutely lethal to fish, but may lead to sublethal and possibly acute effects in invertebrate (CCREM, 1987). Other metals showing less substantial increases downstream of the mine sites include zinc, copper and selenium, with these metals apparently associated with Mine Dumagami effluent.

5.1.3 Costs

The typical cost for a single ICP-MS analysis (water) is about \$45 to \$50. The single sample cost for a conventional ICP analysis is about the same, with interlaboratory costs within methods generally varying more than costs between methods. Per sample price reductions are typically provided for sample numbers of five or ten and greater. Also, the cost per scan (ICP-MS or ICP) may be reduced if results for only a few parameters are required (e.g., \$15 for the first metal and \$5 to \$10 for each additional metal up to the full metal scan price). However, if supplementary analyses are required (e.g., for Cd, Cu, As, Pb, Hg) to determine if concentrations are below water quality guidelines, the single sample, per metal costs are in the range of \$15 (Cd, Cu, Pb, As, Hg); such additional costs can readily double the costs of metal analyses if done by conventional ICP supplemented by other methods. Supplementary analysis of mercury (@ about \$15/sample) only is likely to be necessary with ICP-MS.

TABLE 5.5: WATER CHEMISTRY RESULTS FROM LAC PREISSAC AND DORMENAN CREEK, OCTOBER 1995

Station I.D.	Guideline	lac Preissac				Dormenan Creek		
		Ref-13	Ref-14	11	12	Ref-19	15	6
General Chemistry (mg/L)								
Acidity (as CaCO ₃)	-	5	3	4	2	9	10	27
Alkalinity (as CaCO ₃)	-	16	14	19	14	13	84	147
Ammonia (as N)	2.5	0.09	0.07	4.10	0.10	0.19	14.4	78.0
Chloride	-	2.9	2.9	6.6	3	3.1	168	170
Nitrate + Nitrite (as N)	-	0.44	0.48	0.53	0.55	0.16	1.28	0.39
o-Phosphate (as P)	-	< 0.01	< 0.01	< 0.01	< 0.01	0.022	< 0.01	< 0.01
Sulfate	-	43.2	42.4	80	42	10.9	950	1250
Total Dissolved Solids	-	96	87	137	98	67	1950	1280
Total Suspended Solids	10	6	4	9	6	< 2	15	15
Cyanide	0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.008	< 0.005
Dissolved Organic Carbon	-	8	8	8	7	24	31	57
pH (units)	6.5-9.0	7.6	7.5	7.3	7.6	6.8	7.9	7.6
Calculated Parameters (mg/L)								
Bicarbonate as CaCO ₃	-	15.4	14.1	18.7	14.2	13.1	83.3	146.4
Carbonate as CaCO ₃	-	0.058	0.042	0.035	0.053	0.008	0.622	0.548
Hardness as CaCO ₃	-	48.6	49.7	53.5	45.7	22.8	541	657

Exceeds CCREM (1987) guidelines for the protection of freshwater aquatic life

TABLE 5.6: METAL CONCENTRATIONS IN WATER SAMPLES COLLECTED FROM RIVIÈRE NOIRE, OCTOBER 1995

Sample I.D.	Guideline	Ref-1	2	3	4	5	6 *	7 Top	7 Bottom	8	Falls	9	10
Trace Metals (µg/L)													
Aluminium	100	423	429	428	390	398	99	415	317	415	206	271	237
Antimony	-	< 0.1	< 0.1	0.1	0.1	0.1	2.2	0.1	0.6	0.2	1.8	0.8	0.7
Arsenic	50	2	2	16	218	390	7	534	653	827	59	309	372
Barium	-	14.0	15.2	17.1	19.3	17.8	33.0	17.9	21.0	18.3	31.6	23.0	23.0
Beryllium	-	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Bismuth	-	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.09	< 0.05	< 0.05	0.05	< 0.05	< 0.05
Boron	-	< 5	< 5	< 5	5	< 5	34	< 5	11	6	30	13	13
Bromine	-	4	6	7	8	6	93	7	24	10	76	28	28
Cadmium	1.8	0.11	0.08	0.08	0.07	0.09	0.38	0.06	0.13	0.09	0.39	0.14	0.13
Calcium	-	4690	5550	6630	10390	9710	252000	12200	60000	19800	223000	73400	67500
Chromium	2.0	2.4	2.4	2.5	2.5	2.4	2.0	2.3	2.0	2.3	1.8	2.0	1.9
Cobalt	-	0.4	0.5	0.7	0.8	0.7	41.5	1.1	9.4	2.6	34.5	11.7	11.4
Copper	2.0	2.4	2.5	2.7	3.4	2.8	38.0	3.2	10.4	4.7	33.9	10.3	10.2
Iron	300	736	851	873	1070	906	915	920	870	904	953	823	811
Lead	7.0	0.6	0.4	0.7	0.5	0.5	0.5	0.4	0.4	0.5	1.1	0.4	0.3
Lithium	-	1.3	1.3	1.4	1.5	1.5	25.6	1.8	6.6	2.6	22.1	7.8	7.6
Magnesium	-	1400	1660	1860	2690	2410	6840	2580	3840	2700	6920	3810	3830
Manganese	-	23.4	33.6	52.8	76.3	79.0	506	82.2	168	101	480	214	216
Mercury	0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Molybdenum	-	< 0.1	0.1	0.1	0.2	0.2	13.0	0.3	2.7	0.7	10.3	3.2	3.2
Nickel	25	5	4	5	5	5	17	5	8	6	17	9	8
Potassium	-	529	719	783	1200	995	11000	1080	3000	1420	9040	3820	3810
Rubidium	-	2.7	2.9	3.3	4.0	3.5	17.4	3.5	6.2	4.0	15.0	7.1	6.8
Selenium	1.0	< 1	< 1	< 1	< 1	< 1	7	< 1	1	< 1	5	2	2
Silver	0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.89	< 0.05	< 0.05	< 0.05	0.37	< 0.05	< 0.05
Sodium	-	1840	3500	4280	7040	4500	336300	6960	71500	17300	208000	90700	85200
Strontium	-	30.1	32.4	39.9	50.7	51.8	1030	57.7	237	88.5	834	289	289
Tellurium	-	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.4	< 0.2	< 0.2	< 0.2	0.4	0.2	< 0.2
Thallium	-	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Tin	-	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.08	< 0.05	< 0.05
Titanium	-	11	12	13	13	13	7	13	11	14	11	10	9
Thorium	-	0.05	0.06	0.05	0.06	0.05	< 0.05	0.06	< 0.05	0.07	< 0.05	0.06	0.06
Uranium	-	< 0.05	< 0.05	< 0.05	0.05	0.06	0.24	0.06	0.09	0.07	0.20	0.10	0.10
Vanadium	-	< 1	< 1	1	1	1	1	1	< 1	1	1	1	< 1
Zinc	30	12	11	12	10	13	69	10	23	16	58	25	24

exceeds CCREM (1987) guidelines for the protection of freshwater aquatic life

* Sample collected from the mouth of Dorneman Creek

TABLE 5.7: METAL CONCENTRATIONS IN WATER SAMPLES COLLECTED FROM LAC PREISSAC AND DORMENAN CREEK, OCTOBER 1995

Station I.D.	Guideline	lac Preissac				Dormenan Creek		
		Ref-13	Ref-14	11	12	Ref-19	15	6
Trace Metals (µg/L)								
Aluminium	100.0	289	279	213	205	260	246	99
Antimony	-	< 0.1	0.1	0.1	< 0.1	< 0.1	2.6	2.2
Arsenic	50.0	9	10	19	12	2	6	7
Barium	-	14.5	14.4	15.2	14.3	11.8	30.3	33.0
Beryllium	-	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Bismuth	-	0.20	0.20	0.15	0.14	< 0.05	0.08	< 0.05
Boron	-	< 5	< 5	< 5	< 5	< 5	33	34
Bromine	-	5	5	6	5	5	77	93
Cadmium	1.80	< 0.05	< 0.05	< 0.05	< 0.05	0.09	0.43	0.38
Calcium	-	14500	14900	16900	14100	6170	208000	252000
Chromium	2.0	1.0	0.9	0.9	0.8	1.2	33	2.0
Cobalt	-	0.4	0.4	0.8	0.4	0.4	38.4	41.5
Copper	2.0	69	49	49	47	2.1	64.3	38.0
Iron	300	284	284	232	189	565	1600	915
Lead	1.0	0.5	0.7	0.4	0.4	0.7	1.1	0.5
Lithium	-	1.7	1.7	1.8	1.5	0.8	22.3	25.6
Magnesium	-	3000	3040	2740	2560	1790	5350	6840
Manganese	-	12.0	11.8	15.9	8.8	36.0	363	506
Mercury	0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Molybdenum	-	2.1	2.0	2.0	1.9	0.1	12.0	13.0
Nickel	25.0	2	2	3	2	2	17	17
Potassium	-	848	874	1020	852	520	9410	11000
Rubidium	-	2.5	2.5	2.7	2.4	2.1	18.8	17.4
Selenium	1.0	< 1	< 1	< 1	< 1	< 1	6	7
Silver	0.1	< 0.05	< 0.05	< 0.05	< 0.05	0.13	0.35	0.89
Sodium	-	3980	4170	7590	4550	2390	256000	336300
Strontium	-	54.4	53.5	60.2	51.8	34.0	836	1030
Tellurium	-	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	0.4	0.4
Thallium	-	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Tin	-	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Titanium	-	9	8	6	5	5	9	7
Thorium	-	0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.08	< 0.05
Uranium	-	0.11	0.11	0.10	0.09	< 0.05	0.16	0.24
Vanadium	-	< 1	< 1	< 1	< 1	< 1	< 1	1
Zinc	30.0	5	6	6	6	13	92	69

 Exceeds CCREM (1987) guidelines for the protection of freshwater aquatic life

In terms of the other laboratory analyses presented herein, typical per sample analytical costs, based on undiscounted, single sample pricing, are as follows:

• acidity	- \$12
• alkalinity	- \$14
• ammonia	- \$16
• sulphate	- \$41
• chloride	- (included in sulphate analysis by IC scan)
• nitrate/nitrite	- \$19 (or nitrate may be included with sulphate in IC scan)
• phosphorus	- \$17
• total dissolved solids	- \$12
• total suspended solids	- \$9
• dissolved organic carbon	- \$15
• cyanide	- \$34
• hardness	- <u>\$12</u>
TOTAL	\$201

Collectively, these additional analyses can be costly for a large number of samples. Accordingly, care should be taken to be judicious in parameter selection to maximize cost-effectiveness. Strategies for cost reduction in this example might include, for example:

- reduction in dissolved solids analyses, if major ions (e.g., sulphate, calcium) are individually analyzed;
- elimination of chloride analysis (it is not a conventional mine-related contaminant); and
- selective removal of some parameters at some locations in subsequent sampling runs (e.g., reduced analysis of ammonia, sulphate, hardness at stations upstream of mine sources).

5.1.4 Evaluation of Methods

Costs for water quality analysis depend on the numbers of samples analyzed, the parameters analyzed and the detection limits desired. In the case of this field evaluation, several water

quality samples were necessary to address two study areas (rivière Noire and rivière Bousquet) and several potential sources of impact (Complexe Bousquet, Mine Doyon, Mine Dumagami, two inactive mines and the town of Cadillac). In terms of water quality parameters, full metal scans and extensive characterization of general water quality parameters was appropriate to facilitate identification of treated effluent concentrations (e.g., sulphate, hardness, TDS) and ARD effects (pH, acidity, metals).

In terms of parameters and detection limits, analyses presented herein for trace elements were carried out by ICP - Mass Spectroscopy (ICP-MS), a relatively new technology which provides lower detection limits than more conventional ICP (or ICAP) methods. To evaluate the costs associated with these methods, a request was made to Barringer Laboratories, since they offer both methodologies. The information provided is presented in Appendix B. The ICP-MS provides for analysis of more elements than ICP, and typically provides for detection limits which are 1,000 fold lower than provided by ICP. Thus, ICP-MS solves detection limit problems with ICP, which typically requires supplementary analyses (by other methods) to achieve detection limits equal to or lower than water quality criteria (e.g., As, Cd, Cu, Pb). This is true only for mercury with ICP-MS.

Based on cost-effectiveness considerations, metal determination by ICP-MS is generally recommended over other methods. It is also recommended that environmental effects monitoring programs provide for site-specific parameter selection, and for adjustment of parameter lists in subsequent sampling based on the outcome of more intensive analyses during initial sampling programs.

Based on this field evaluation, the importance of water quality sampling in aquatic effects monitoring is evident. Here, water quality data were used to identify movement and dispersion of effluent and to indicate potential sources of biological impact.

5.2 Sediment Quality

Like water quality, sediment quality may be affected by mining through the release of effluent and by metal contamination from ARD. Also, any direct deposition of solids (e.g., tailings, sludges, etc.) unintentionally released to the environment or released historically (prior to implementation of modern environmental control measures) may be identified by

sediment quality sampling. Sediment quality conditions are evaluated for the rivière Bousquet/lac Chassignolle system and in the rivière Noire system to identify potential contaminants and sediment quality factors which may influence biological conditions (fish and benthic invertebrates). Evaluation of sediment core sampling methods is presented in Section 4.0 of this report, while Section 7.0 examines possible relationships between sediment quality and biological effects.

5.2.1 Methods

Stations where sediment samples were collected for analysis in September and October 1995 are presented in Figures 5.1 to 5.4.

During the preliminary survey, surficial sediment samples were collected in the deep basin of lac Bousquet (reference Station LB1), in deeper water in the eastern portion of lac Chassignolle (downstream Station LC1) and upstream in rivière Bousquet in the treated effluent streams of Complexe Bousquet (Station MB) and Mine Doyon (Station MD). Samples from LB1 and LC1 were collected by petite Ponar, while MB and MD samples were collected directly in hand-held bottles. All samples were secured in clean, 500 mL PET bottles, stored refrigerated in the field and forwarded to RPC laboratories for analysis within six days of sample collection. At the laboratory, samples were air-dried, lightly pulverized, sieved through a 1 mm mesh to remove pebbles and vegetation and digested in HNO₃/HF. Resulting solutions were diluted to volume and analyzed for trace elements by ICP-MS. Mercury was analyzed independently by cold vapour atomic absorption spectrophotometry.

During the main (October) survey, surficial sediments were collected at the 16 stations monitored for water quality and biological effects in the rivière Noire system (Figure 5.4). Samples were collected either directly in clean, hand-held PET bottles (shallow sites) or by petite Ponar grab. For grab samples only the surface 3 cm of sediment was collected from two to three grabs. Two 500 mL bottles of sediment were collected per site. One sample bottle was stored refrigerated and the other sediment sample jar was topped up with water from the site and frozen (sample for partial digestion). All samples were forwarded to RPC Laboratories within ten days of collection. At RPC, samples were analyzed for total trace metals, as described above for the September survey, as well as for moisture, total organic

carbon, loss-on-ignition (550°C and 850°C) and grain size (sieve and pipette). In addition, all sediments were also analyzed for metals (ICP-MS) after partial extraction, as described in Appendix B.

Munsell colour of sediments were recorded on moist samples, as listed in Appendix B.

In addition, a principal components analysis was carried out based on total and partial metals to identify differences between reference, near-field and far-field areas.

5.2.2 Results

5.2.2.1 Preliminary Reconnaissance

Results of the analysis of preliminary reconnaissance surficial sediment samples are shown in Table 5.8. Overall, results show no obvious increases in metal concentrations in lac Chassignolle relative to lac Bousquet (reference). Sediments collected from the effluent streams showed low metal concentrations overall, with no indication of significant contamination relative to lake sediments. As observed in sediment cores (Section 4), some metals (including Cd and Pb) appear to be higher in concentration in the reference lake than in lac Chassignolle, which is influenced by discharges from Mine Doyon and Complexe Bousquet.

5.2.2.2 Main Survey

Sediment chemistry results from the main survey samples (October, 1995) collected in rivière Noire are compared to Environment Canada 1995 Interim Sediment Quality Guidelines in Tables 5.9 and 5.10 for total metals and other parameters.

Sediments in rivière Noire and lac Preissac were either a sandy-silt or silty-sand, depending on location, with ruisseau Dormenan sediments being somewhat finer at Stations 19 and 15. Total organic carbon contents were relatively low (<2%) in rivière Noire and lac Preissac, and somewhat higher in Dormenan Creek (up to 6.68%).

TABLE 5.8: SEDIMENT QUALITY RESULTS - PRELIMINARY RECONNAISSANCE
(September, 1995 (mg/kg))

Station	lac Bousquet	lac Chassignolle	Complexe Bousquet	Mine Doyon
	(reference)		Effluent Discharge	Effluent Discharge
	LB1	LC1	MB	MD
Aluminum	64200	71700	-	-
Antimony	0.55	0.33	0.16	0.1
Arsenic	5	11	2	1
Barium	501	583	438	343
Beryllium	1.2	1.6	1.2	0.8
Bismuth	2.36	9.94	0.26	0.12
Cadmium	2.21	1.73	0.92	0.16
Calcium	15700	17000	20800	57800
Chromium	122	137	170	77
Cobalt	28.3	32.8	24.1	41.8
Copper	45.6	74.6	70.2	114
Iron	43100	48500	30250	32780
Lead	85.2	48.9	12.2	9.1
Lithium	25	42	24	19
Magnesium	11000	14700	13700	7800
Manganese	675	1470	1270	1430
Mercury	0.1	0.1	0.02	0.03
Molybdenum	1	16.3	0.7	5.3
Nickel	53.9	68	76	36
Phosphorus	894	921	483	799
Potassium	15600	22700	15000	11500
Rubidium	71	112	67	43
Selenium	<2	<2	<2	<2
Silver	0.55	0.95	0.3	0.72
Sodium	19100	19900	21600	17000
Strontium	272	264	343	291
Tellurium	2.5	2	0.4	0.6
Thallium	0.4	0.6	0.3	0.3
Tin	4.9	2.8	1.1	0.8
Uranium	0.8	2	0.9	1.4
Vanadium	93	97	75	55
Zinc	157	222	390	47

TABLE 5.9: SEDIMENT CHEMISTRY AND GRAIN SIZE RESULTS FROM RIVIÈRE NOIRE, OCTOBER 1995

Concentration mg/Kg (dry wt.)												
Sample I.D.	TEL	PEL	1	2	3	4	5	6*	7	8	9	10
Aluminium	-	-	7300	9840	11000	12100	14700	16400	11900	13000	15300	14500
Antimony	-	-	0.02	0.04	0.88	0.40	0.46	0.22	0.35	0.36	0.36	0.41
Arsenic	5.9	17.0	2	3	1730	512	498	48	392	568	512	381
Barium	-	-	35.6	55.6	37.9	71.2	71.4	52.9	52.6	60.4	72.4	68.4
Beryllium	-	-	0.13	0.19	0.18	0.22	0.22	0.33	0.21	0.24	0.28	0.26
Bismuth	-	-	0.11	0.22	0.23	0.22	0.26	0.18	0.19	0.25	0.29	0.32
Boron	-	-	2	2	1	2	2	3	2	2	2	2
Cadmium	0.596	3.53	0.18	0.28	0.18	0.26	0.37	0.48	0.35	0.36	0.50	0.64
Calcium	-	-	1960	2620	6610	6150	4340	6220	4760	5430	6440	4830
Chromium	37.3	90.0	41	54	61	60	69	67	67	73	68	75
Cobalt	-	-	6.3	8.0	16.2	15.4	17.0	18.5	16.1	17.2	18.3	19.2
Copper	35.7	196.6	7.1	16.7	40.4	28.4	29.8	113.4	44.3	42.5	59.9	57.8
Iron	-	-	9530	12900	36000	26800	30000	23200	24600	26300	29400	28600
Lead	35.0	91.3	7.1	16.1	7.4	10.5	11.7	8.6	8.5	10.1	10.7	10.9
Lithium	-	-	8.9	12.7	12.8	18.5	17.9	23.6	19.0	21.2	22.8	22.8
Magnesium	-	-	3650	4600	7780	8260	8790	6720	8160	9000	9520	9300
Manganese	-	-	113	163	357	370	338	313	232	305	309	263
Mercury	0.174	0.486	0.013	0.022	0.054	0.105	0.135	0.084	0.103	0.126	0.084	0.111
Molybdenum	-	-	0.29	0.34	0.47	0.36	0.40	0.88	0.48	0.46	0.53	0.47
Nickel	18.0	35.9	21.1	28.8	52.8	40.2	41.9	36.0	40.5	45.0	46.7	48.3
Phosphorus	-	-	320	570	480	490	520	520	570	580	730	570
Potassium	-	-	490	750	1000	2190	1990	1200	2060	2260	2690	2380
Rubidium	-	-	5.6	8.9	7.5	15.0	14.3	14.7	13.1	14.5	18.5	16.8
Selenium	-	-	<1	<1	<1	1	<1	1	1	1	1	1
Silver	-	-	0.03	0.05	0.09	0.14	0.15	0.53	0.19	0.20	0.32	0.27
Sodium	-	-	130	230	120	200	260	790	350	380	400	380
Strontium	-	-	10.3	15.5	21.4	19.6	18.3	27.7	17.2	19.4	24.3	21.7
Tellurium	-	-	<0.5	<0.5	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Thallium	-	-	<0.05	0.07	0.07	0.13	0.15	0.13	0.10	0.11	0.16	0.14
Thorium	-	-	1.33	2.06	1.97	2.77	2.88	3.97	2.29	2.68	3.06	3.03
Tin	-	-	0.2	0.3	0.3	0.2	0.3	0.2	0.4	0.2	0.2	0.3
Titanium	-	-	425	568	395	670	721	919	681	777	865	911
Uranium	-	-	0.24	0.37	0.24	0.35	0.38	0.61	0.37	0.38	0.52	0.43
Vanadium	-	-	17	21	40	45	58	48	54	56	53	67
Zinc	123.1	314.8	28	46	45	89	67	172	76	79	99	129
Organic Carbon (%)			1.24	1.35	0.82	0.72	1.01	2.29	1.10	1.20	1.89	1.86
Moisture (%)			39.4	36.1	31.8	33.6	39.6	41.6	39.5	40.6	45.2	45.0
LOI (550 °C) (%)			2.30	3.56	2.21	2.35	3.00	5.78	3.01	3.30	4.67	4.21
LOI (850 °C) (%)			0.42	0.53	1.44	1.01	0.85	0.76	0.91	0.93	1.06	0.96
% Gravel			0.600	0.240	0.110	0.070	0.150	0.190	0.090	0.050	0.040	0.150
% Sand			58.89	24.27	44.10	44.25	33.28	49.26	32.66	30.68	27.12	17.10
% Silt			33.3	66.7	51.8	48.1	58.1	32.3	57.6	58.5	60.6	74.2
% Clay			7	9	4	8	8	18	10	11	12	9

* sample taken from mouth of Doremnan Creek

Exceeds TELs (Threshold Effect Levels) Environment Canada (1995)
 Exceeds PELs (Probable Effect Levels) Environment Canada (1995)

TABLE 5.10: SEDIMENT CHEMISTRY AND GRAIN SIZE RESULTS FROM LAC PREISSAC AND DORMENAN CREEK, OCTOBER 1995

Concentration mg/Kg (dry wt.)			Lac Preissac				Dormenan Creek		
Sample I.D.	TEL	PEL	Ref-13	Ref-14	11	12	Ref-19	15	6
Aluminium	-	-	10000	7760	15300	24100	20200	17700	16400
Antimony	-	-	0.08	0.05	0.62	0.33	0.03	0.37	0.22
Arsenic	5.9	17	66	71	798	701	9	21	48
Barium	-	-	56.7	42.1	81.5	115	121	86.9	52.9
Beryllium	-	-	0.27	0.22	0.22	0.45	0.35	0.33	0.33
Bismuth	-	-	5.59	4.07	1.10	5.06	0.35	3.24	0.18
Boron	-	-	2	2	2	3	3	3	3
Cadmium	0.596	3.530	0.82	0.68	0.56	1.97	0.39	1.49	0.48
Calcium	-	-	3230	2920	8180	4440	4340	5600	6220
Chromium	37.3	90.0	42	33	69	104	79	81	67
Cobalt	-	-	9.9	8.3	22.2	28.0	18.0	15.0	18.5
Copper	35.7	196.6	31.0	23.3	53.3	107.1	36.3	653.3	113.4
Iron	-	-	15100	12500	36600	44900	19700	24200	23200
Lead	35.0	91.3	18.6	17.6	11.1	31.1	12.4	61.4	8.6
Lithium	-	-	14.6	11.2	23.9	38.5	25.7	21.0	23.6
Magnesium	-	-	4790	3730	11700	15700	7030	6050	6720
Manganese	-	-	565	526	439	1760	406	248	313
Mercury	0.17	0.49	0.047	0.030	0.101	0.327	0.049	0.076	0.084
Molybdenum	-	-	6.23	5.13	1.11	7.77	0.49	2.58	0.88
Nickel	18.0	35.9	29.3	23.9	48.7	62.6	41.9	43.0	36.0
Phosphorus	-	-	590	580	470	790	600	610	520
Potassium	-	-	1000	770	3350	2450	1240	1120	1200
Rubidium	-	-	11.9	8.5	19.2	21.7	16.0	12.6	14.7
Selenium	-	-	1	1	1	1	1	2	1
Silver	-	-	0.14	0.11	0.31	0.42	0.16	2.56	0.53
Sodium	-	-	250	190	250	330	340	1040	790
Strontium	-	-	15.5	13.1	24.1	21.8	29.3	25.9	27.7
Tellurium	-	-	< 0.5	0.6	< 0.5	0.9	< 0.5	5.4	< 0.5
Thallium	-	-	0.10	0.06	0.18	0.22	0.17	0.13	0.13
Thorium	-	-	2.83	2.26	2.66	4.61	2.36	4.20	3.97
Tin	-	-	0.2	0.2	0.3	0.6	0.1	0.3	0.2
Titanium	-	-	539	457	911	950	944	739	919
Uranium	-	-	0.91	0.59	0.36	0.99	0.62	1.10	0.61
Vanadium	-	-	25	20	76	99	35	42	48
Zinc	123.1	314.8	70	56	89	167	84	361	172
Organic Carbon (%)			1.15	0.63	1.02	1.24	4.99	6.68	2.29
Moisture (%)			40.0	41.8	42.2	50.8	60.8	69.0	41.6
LOI (550 °C) (%)			2.87	2.00	2.66	3.91	12.4	13.0	5.78
LOI (850 °C) (%)			0.59	0.48	1.61	1.40	0.85	1.35	0.76
% Gravel			0.34	0.23	0.33	0	2.23	0.48	0.05
% Sand			26.18	32.79	51.11	10.99	24.09	31.31	28.28
% Silt			60.88	56.89	38.7	65.19	38.09	44.75	61.77
% Clay			12.6	10.09	10	23.83	35.59	23.45	9.9

Exceeds TELs (Threshold Effect Levels) Environment Canada (1995)
 Exceeds PELs (Probable Effect Levels) Environment Canada (1995)

FIGURE 5.5A: COMPARISON BETWEEN FULL EXTRACTION AND PARTIAL EXTRACTION IN SEDIMENT ANALYSIS FROM THE RIVIÈRE NOIRE

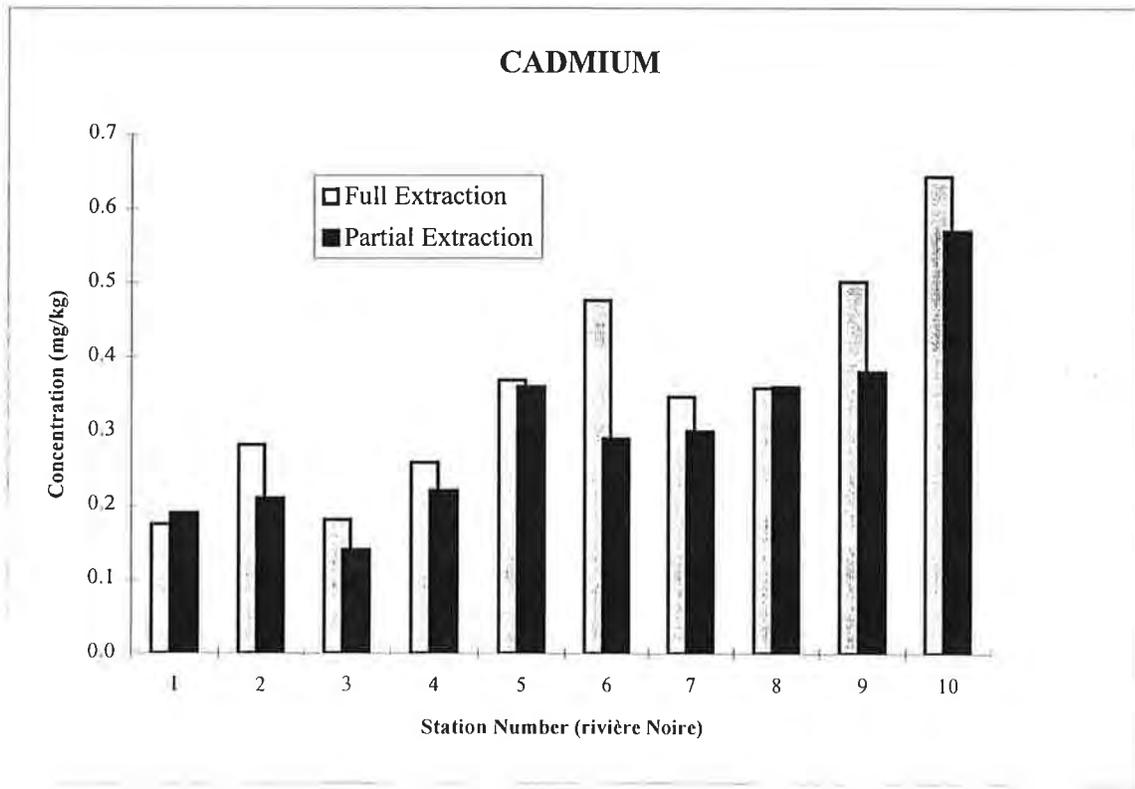
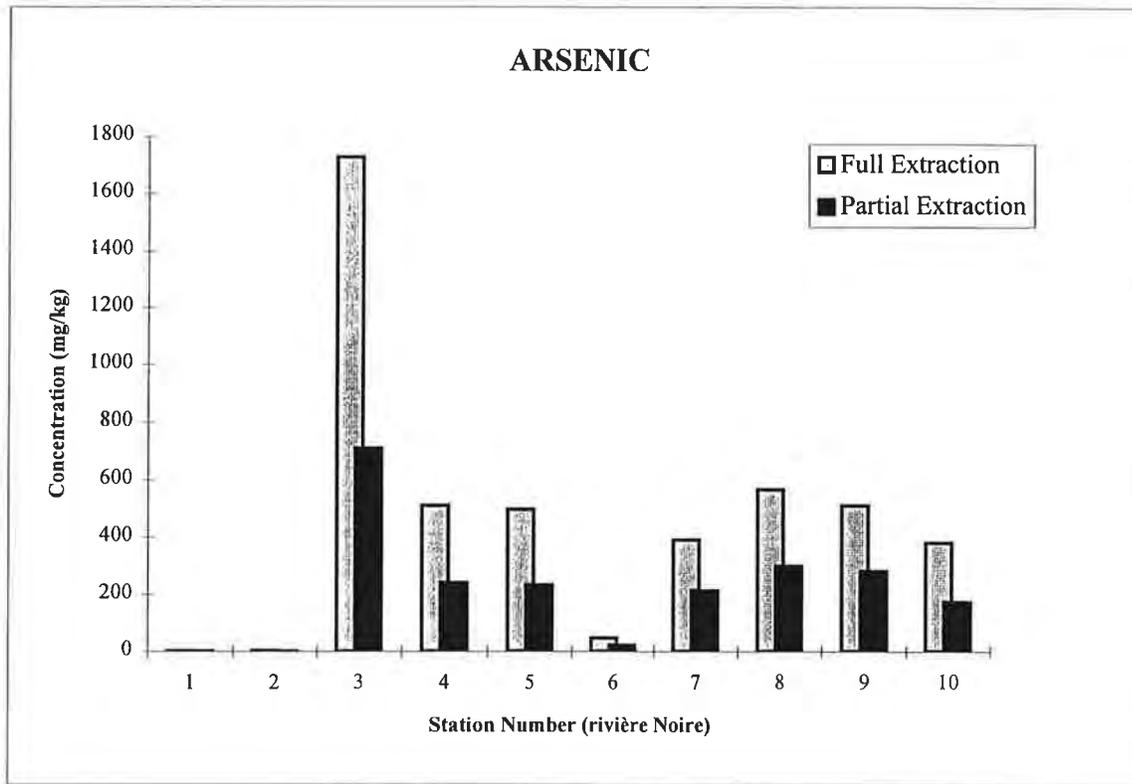


FIGURE 5.5B: COMPARISON BETWEEN FULL EXTRACTION AND PARTIAL EXTRACTION IN SEDIMENT ANALYSIS FROM THE RIVIÈRE NOIRE

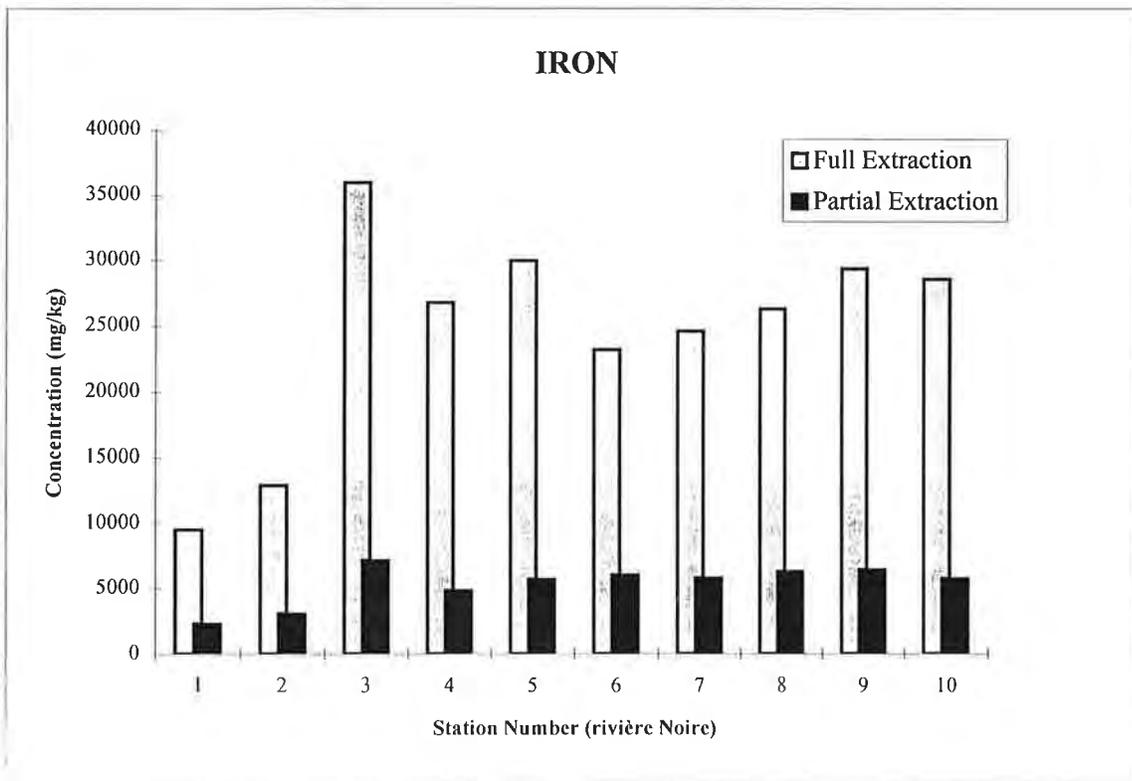
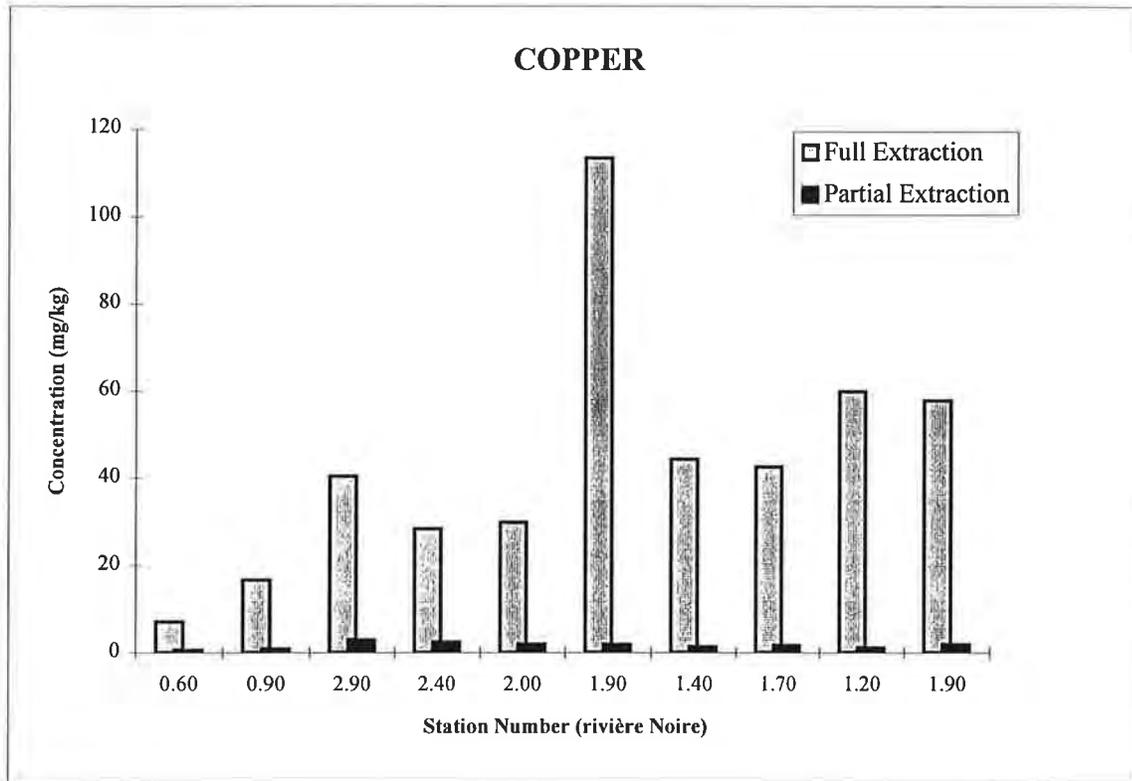


FIGURE 5.5C: COMPARISON BETWEEN FULL EXTRACTION AND PARTIAL EXTRACTION IN SEDIMENT ANALYSIS FROM THE RIVIÈRE NOIRE

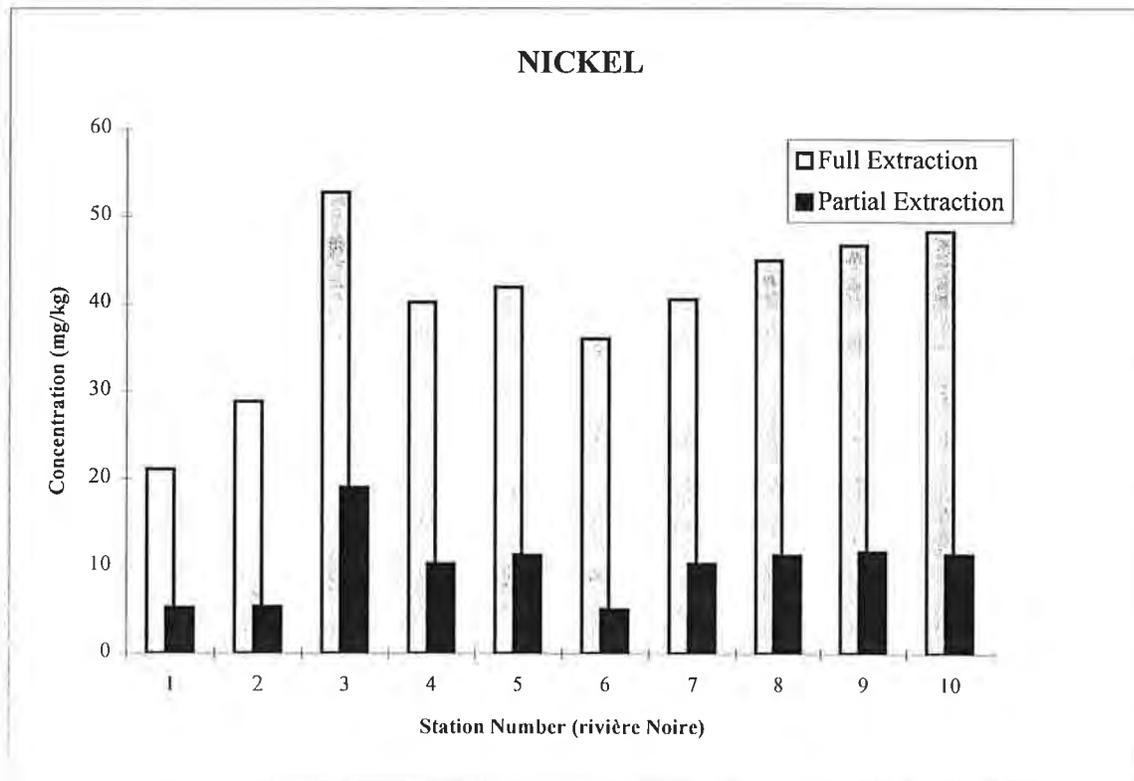
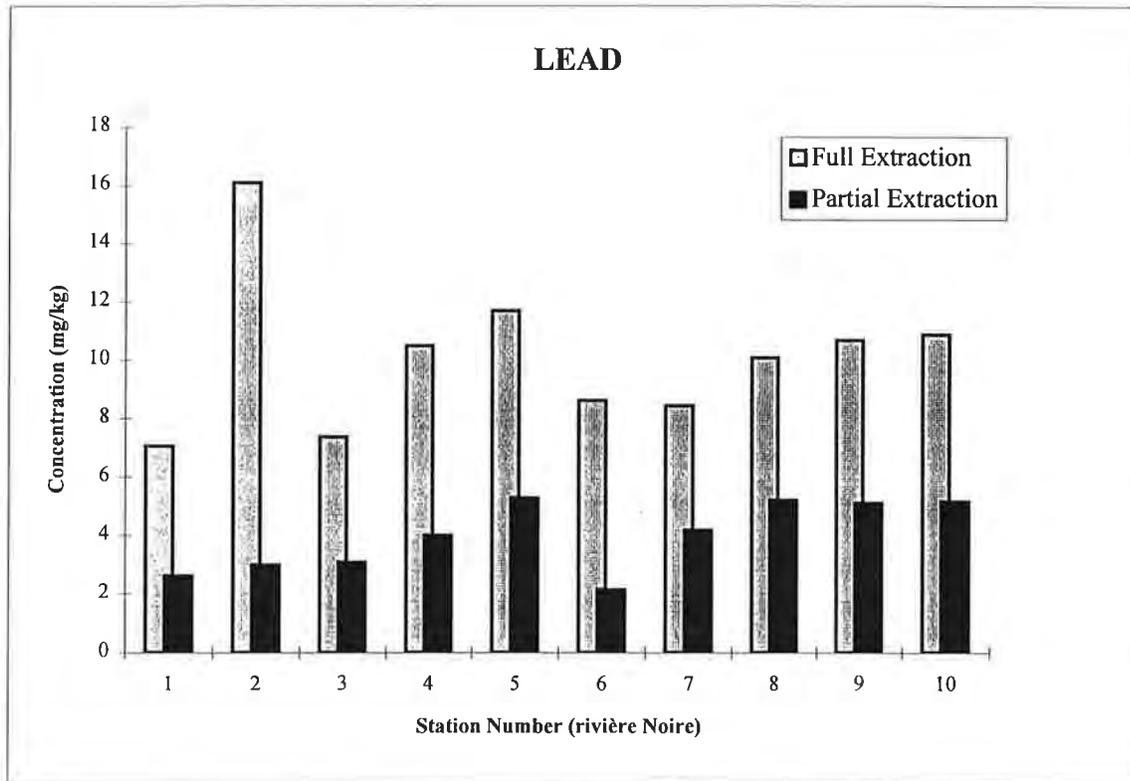
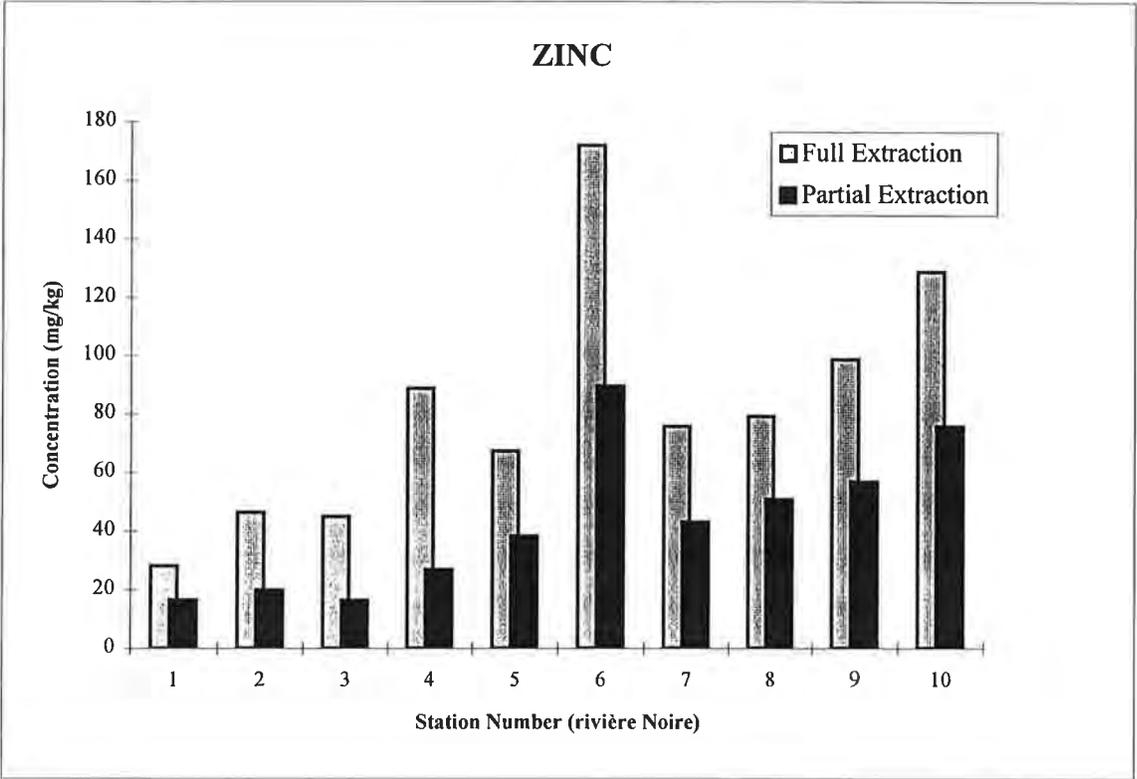


FIGURE 5.5D: COMPARISON BETWEEN FULL EXTRACTION AND PARTIAL EXTRACTION IN SEDIMENT ANALYSIS FROM THE RIVIÈRE NOIRE



In terms of metal concentrations, arsenic concentrations were strongly elevated in rivière Noire and the river mouth area (up to 1,730 mg/kg at Station 3), with lower levels in Dormenan Creek (less than 50 mg/kg), indicating that the sources were from one or both of the inactive mines (Table 5.9). Other metals appearing to be slightly enriched downstream of the mine sites (including Mine Dumagami) include nickel, copper and zinc. Metals extensively exceeding their probable effects level (PEL) guidelines include arsenic and zinc. Overall, copper, nickel, lead and cadmium levels were higher in lac Bousquet and lac Chassignolle sediments than in rivière Noire sediments (refer to Tables 5.8 and 5.9).

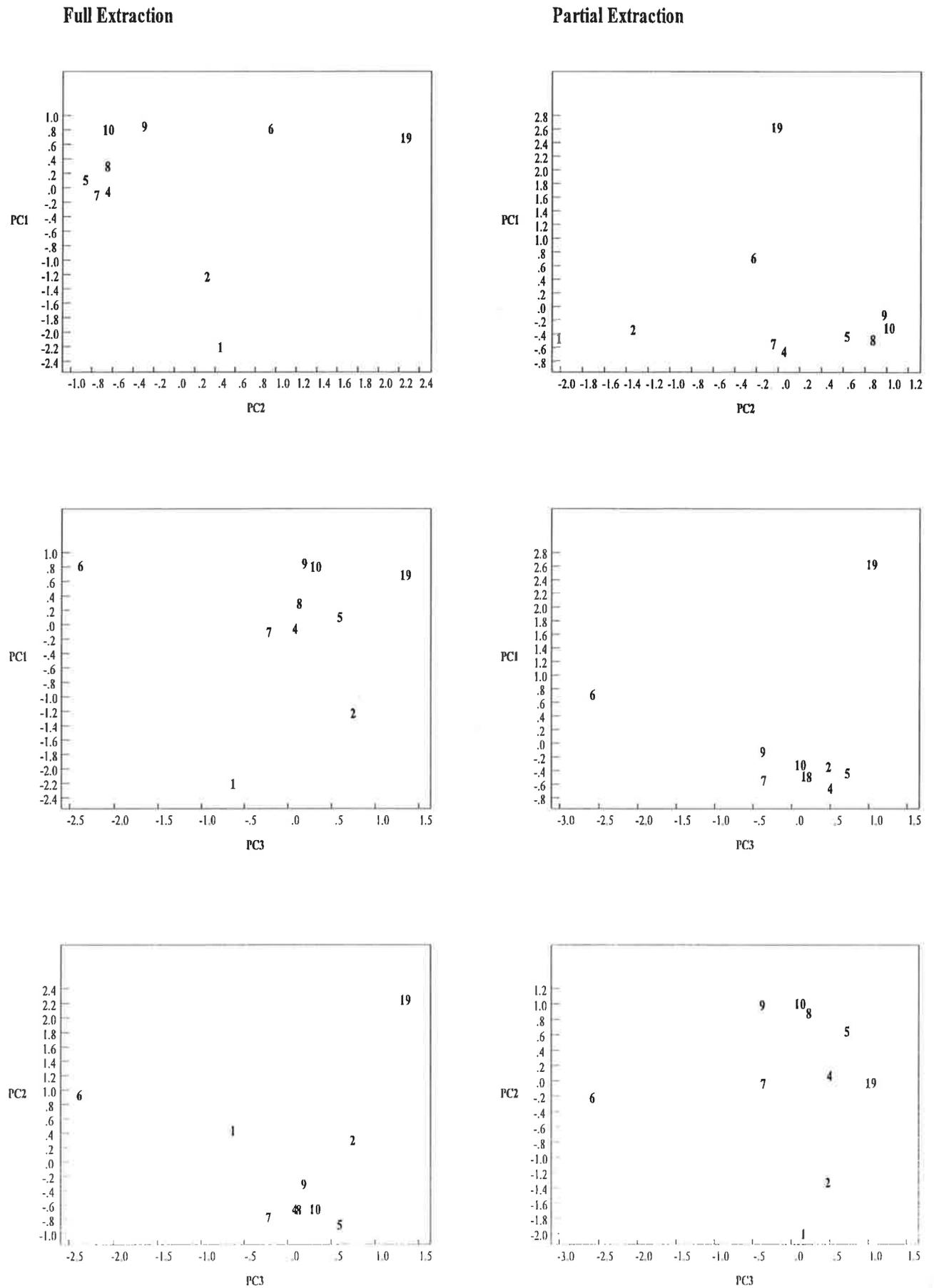
Partial extraction results are compared with total extraction results for selective stations and metals in Figure 5.5 (a to d), with a complete listing of all results in Appendix B, Tables B4 and B5. Partial extraction generally extracted most of the cadmium (generally $\geq 80\%$), little of the copper (generally $\leq 5\%$), about half of the arsenic and zinc, and about 30% of the iron and nickel.

The slight differences in fractional abundance of metals between Station 6 (ruisseau Dormenan at mouth) and other locations in rivière Noire may reflect the different metal sources from Mine Dumagami compared to the two inactive mines. This is most apparent for cadmium, copper and nickel (Figure 5.5).

PCA of Sediment Data

There were a large number of metals analyzed in both the full extraction and the partial extraction methods, consequently, principal components analysis (PCA) was used to summarize variation in each of these data sets. The variables used in the PCA analyses and output data are presented in Appendix B. For the purpose of PCA analysis the stations were grouped into reference, near-field and far-field areas. The station groupings that defined the areas were based on similarities in benthic invertebrate community structure (refer to Section 7). However, with the exception of Station 19, the station groupings based on full and partial extraction were similar to those based on benthic communities (Figure 5.6 and Figure 7.3, Section 7.0). Stations 1, 2 and 19 represented the reference area, Stations 5, 7 and 8 represented the near-field area, and Stations 9 and 10 represented the far-field area.

FIGURE 5.6: BIPLLOTS OF THE ASSOCIATIONS BETWEEN SAMPLING LOCATIONS RELATIVE TO THE PCA AXES BASED ON THE FULL EXTRACTION AND PARTIAL EXTRACTION OF SEDIMENT QUALITY SAMPLES



Full Extraction (Total Metals)

Chromium, nickel, cadmium, zinc and copper were all important positive contributors to the structure of PC1, which accounted for 53.2% of the variation in the raw data matrix. Arsenic and silt were strong negative contributors to PC2, while TOC, gravel and clay were positive contributors, which accounted for 20.5% of the variation. Sand and lead were negative contributors to PC3, but this latter PC axis explained only 13.3% of the variation (Table 5.11).

Reference and near-field areas had significantly different scores on PC2 (ANOVA; $p < 0.05$), and were also significantly different ($p < 0.05$) in the MANOVA test of all three components. The *a posteriori* power associated with these differences was greater than 0.8, indicating a high probability of a real difference between the two areas.

Reference and far-field areas were not significantly separated by any of the three principal components but were significantly different ($p < 0.05$) in the overall MANOVA test (Table 5.11). The *a posteriori* power associated with this difference was greater than 0.8, indicating a high probability of a real difference between the two areas.

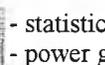
Partial Extraction (Partial Metals)

Clay, TOC and gravel were important positive contributors to PC1, which accounted for only 36.8% of the variation. Important metals in the PCA of the partial extraction data set were lead, nickel, cadmium, arsenic, and copper. All of these had strong positive contributions to PC2, which explained 32.8% of the variation in the raw data matrix. Zinc had a strong negative contribution to PC3, while chromium had a positive contribution. This last principal component accounted for 13.2% of the variation in the data.

Reference and near-field areas were significantly different in the overall MANOVA of the three PCA axes analyzed (Table 5.11). However, the *a posteriori* power associated with this difference was low suggesting that it may not be a real difference. There were no significant differences between the reference and far-field areas.

TABLE 5.11: VARIATION IN SEDIMENT METAL AND TOC CONCENTRATIONS AND GRAIN SIZE COMPOSITION BY PRINCIPAL COMPONENT ANALYSIS (PCA), AND TESTS OF AREA DIFFERENCES IN PCA SCORES BY M(ANOVA)

Sediment Chemistry Metric	Full Extraction Method		Partial Extraction Method			
	Probability	Power	Probability	Power		
Reference vs Near-field						
PC1	0.302	0.152	0.289	0.158		
PC2	0.006	0.970	0.065	0.485		
PC3	0.641	0.071	0.430	0.110		
PC1,2,3	0.012	0.990	0.045	0.680		
Reference vs Far-field						
PC1	0.211	0.203	0.575	0.077		
PC2	0.169	0.247	0.066	0.492		
PC3	0.778	0.056	0.148	0.276		
PC1,2,3	0.031	0.880	0.125	0.390		
Component Structure						
Eigenvalue (%)	PC1 20.2 (53.2)	PC2 7.8 (20.5)	PC3 5.1 (13.3)	PC1 9.9 (36.8)	PC2 8.9 (32.8)	PC3 3.6 (13.2)
metal (loading), rank	chromium (0.912), 6 nickel (0.892), 7 cadmium (0.807), 15 zinc (0.786), 19 copper (0.713), 24	TOC (0.863), 1 gravel (0.854), 2 clay (0.851), 3 arsenic (-0.814), 4 silt (-0.639), 7	sand (-0.682), 1 lead (-0.651), 4	clay (0.987), 1 TOC (0.980), 2 gravel (0.895), 5	lead (0.811), 4 nickel (0.802), 5 cadmium (0.779), 6 arsenic (0.771), 7 copper (0.598), 11 sand (-0.572), 13	zinc (-0.737), 1 chromium (0.596), 3

 - statistically significant difference at p=0.05
 - power greater than 0.800

5.2.3 Costs

In this study, sediment metals were determined by ICP-MS analysis of sediment extracts. Metal analyses by either ICP-MS or by conventional ICP are approximately the same as described for water, plus an allowance for the extraction (typically \$20 to \$25 per sample additional). Per sample costs for analyses, based on single samples, are typically as follows:

• total organic carbon	-	\$24
• moisture	-	\$10
• loss-on-ignition	-	\$34
• grain size by sieve and pipette	-	\$75
• total metals by ICP-MS (includes digestion)	-	\$85
• partial metals by ICP-MS	-	\$100
• mercury	-	<u>\$18</u>
TOTAL		\$346

5.2.4 Evaluation of Methods

Sediment chemistry analyses are important to elucidate possible cause-effect relationships in biological measures. Unlike water chemistry analyses, conventional ICP is capable of achieving satisfactory detection limits (e.g., \leq Environment Canada interim sediment quality guidelines) for the most important metals, with the exception of arsenic, selenium and mercury which are typically not done by ICP (see Appendix B). If the parameter list can be shortened to around five parameters, based on previous sediment quality information, costs for metal analysis may be reduced. In terms of other sediment quality parameters, it is probably redundant to measure both total organic carbon and loss-on-ignition, as the latter represents a surrogate measure of the former. Also, the measurements of sediment moisture is subject to substantial measurement variability and is of limited value in assessing aquatic biological effects. The measurement of Munsell colour also appears to be of somewhat limited value, except where it may be important in identification of visual differences that may have a basis in mineral composition. Our understanding is that Munsell colour is typically used for soils and not aquatic sediments.

Sediment chemistry parameters that may be worth considering in future, by the AETE Technical Committee, include porewaters in sediments tested for toxicity, and limited but more complete fractional analysis of metal fractions (perhaps also in limited numbers of sediments tested for toxicity). Data from these analyses provide additional insight into the relative bioavailability and mobility of metals. Petrographic analysis may also be of value to assist in identifying the major minerals present and to provide data on direct versus indirect sources of sediment contamination.

At a minimum, future AETE field studies should include total metals, grain size distribution and total organic carbon, with further consideration given to other analyses of potential value in determining the metal fractions present.

Overall, it appears that full extraction of metals coupled with TOC and grain size analyses was better able to detect significant differences with greater power among reference, near-field and far-field areas.

6.0 SEDIMENT TOXICITY TESTING

6.1 Introduction

Sediment samples for toxicity testing were collected at the same stations sampled for benthic invertebrates (refer to Figure 5.4). The purpose of the sediment toxicity component was to assess the ability of selected single species tests to predict mining-related impacts on the benthic invertebrate community in the natural receiving environment and determine whether these effects could be related to sediment chemistry.

6.1.1 Selection of Reference Sediment and Sites for Toxicity Testing

The selection of adequate reference sites is one of the key components of a biological testing program. The primary purpose for collecting reference sediments in any study is to provide a geochemically similar sediment to measure the effects, which are not contaminant-related (i.e., physical characteristics) of test sediments on the organism (Environment Canada, 1995).

Sediment toxicity can be influenced by factors, such as total organic carbon (TOC) and grain size, which are considered the most important parameters by Environment Canada, and therefore, reference sites that appeared to have similar grain size and TOC levels to those observed in sediments from the exposed stations, were selected for this study.

6.1.2 Collection and Preservation of Samples

Samples were collected using a petite-Ponar grab. Only surface sediment (the upper 3 cm) was used for toxicity testing. Therefore, a preferred penetration depth of at least 6 to 8 cm was required to ensure minimum disturbance of the upper layer during sampling.

At each station, 10 L of sediment were collected which necessitated several grabs (at least ten) that were homogenised on site in a clean high-density polyethylene container lined with a high-density polyethylene bag using a stainless steel spoon. Conductivity, dissolved oxygen, pH and temperature were measured in the field at each station. Sediment texture,

organic content, and general appearance were noted on standard data sheets.

Between stations, all sampling equipment was cleaned thoroughly and rinsed several times with clean water. All samples were kept cool in the field and during transport, and were forwarded to the BEAK Ecotoxicity laboratory within five days of collection. In the lab, samples were stored at 4°C until testing.

6.2 Toxicity Test Methods

Sediment toxicity was evaluated by exposing organisms to sediment and measuring survival and growth in the amphipod *Hyaella azteca*, survival and reproduction in the tubificid oligochaete *Tubifex tubifex* and inhibition of photoluminescence in the Microtox® bacterium *Vibrio fischeri*.

Hyaella azteca - Growth and Survival Test

Hyaella azteca, an epibenthic detritivore, was used as one of the test species. These freshwater amphipods are easily cultured and have a short generation time. Tests were conducted according to Environment Canada's draft biological test method: "Test for Growth and Survival in Sediment Using the Freshwater Amphipod *Hyaella azteca*" February 1995 Preview to Final Manuscript. Ten juvenile amphipods were placed in each of five replicate sediment samples with overlying water for 14 days. The test conditions are provided in Appendix C. At the end of the test the number of surviving organisms was recorded, removed, dried and weighed.

Tubifex tubifex - Survival and Reproduction Test

The aquatic oligochaete worm *Tubifex tubifex* used in the sediment toxicity test, belongs to the family Tubificidae, an important component of benthic invertebrate communities in freshwater and estuarine sediments. It has proven to be an ideal test species in terms of handling and sensitivity.

Tests were conducted according to the ASTM protocol, "E 1384 - 94a Standard Guide for

Conducting Sediment Toxicity Tests with Freshwater Invertebrates". Tests were conducted at the National Water Research Institute in Burlington under the direction of Dr. Kristin Day since the BEAK cultures did not meet our QA/QC performance quality objectives at the time of sediment testing.

Four mature adult worms, eight weeks of age were added to each of five replicate sediment samples with overlying water and kept in darkness for 28 days at 23°C. The vessels were aerated gently, and temperature and dissolved oxygen were monitored. The sediment was sieved at the end of the test to remove and count adults, young and cocoons.

Microtox® Chronic Test - Photoluminescence Bacteria Test

The Microtox® Chronic Test uses the Microtox® strain of luminescent bacteria, *Vibrio fischeri*, (formerly called *Photobacterium phosphoreum*). Hydrated cells of the test reagent, when grown in a special medium, initiate induction of all enzyme pathways (including those required for luminescence) needed for cell division, growth and metabolism. These numerous metabolic pathways, including DNA replication, become available for inhibition by toxic chemicals. The close metabolic association of the luciferinase system with cell division and other physiological processes within the cell, provides an endpoint for measuring inhibition of cellular activity (i.e., chronic toxicity).

The Microtox® Solid-Phase test measures the toxicity of contaminants that are bound to particles in soil or sediment. This procedure allows the test bacterium to come in direct contact with toxicants in an aqueous suspension of the test sample, detecting both the soluble and insoluble organic and inorganic material. As in the liquid-phase test, the measure of light reduction at 5 minutes after a 20-minute exposure period to the test sample is taken as a toxicity induced end-point.

The exposure method followed the protocol outlined in "Microtox® Manual, A toxicity Testing Handbook Volume II Detailed Protocols - Microbics Corporation (1992)".

6.3 Sediment Toxicity Results

Four sites were identified as reference sites which were upstream or some distance from all known contaminant sources, they were Station 1 on rivière Noire and Station 19 on ruisseau Dormenan, upstream of the Mine Dumagami, and Stations 13 and 14 on the far shore of lac Preissac. The remaining test sites have been described earlier and locations were presented in Figure 5.4. The toxicity data have been summarized in Table 6.1 and are compared to sediment metal concentrations and benthic invertebrate indices from the same sites.

Hyaella Survival

Survival of amphipods at the reference sites ranged from 66% to 92% (Figure 6.1 and Table 6.1). The test sites on the rivière Noire to the junction of ruisseau Dormenan showed no effect on amphipod survival when compared to 66% survival at reference Station 1, located furthest upstream on rivière Noire. However, if the data are compared to Stations 2 and 19 and the test reference sediment collected from Long Point, Ontario, the effects on amphipods begin at Station 4, located just downstream of the idle mine sites. Survival at Station 1 was also significantly lower than the Long Point reference sediments. Station 6 on ruisseau Dormenan produced complete mortality and downstream of the confluence with rivière Noire all stations to the mouth (Station 10) also produced significantly higher mortality in exposed amphipods. Survival was not significantly lower at the lake Stations 11 and 12, indicating no effect of the rivière Noire discharge in this area of lac Preissac.

Hyaella Growth

There is little point considering growth in amphipods which exhibited little or no survival (Stations 6 to 12) therefore, the only sites of interest are Stations 1, 2, 4 and 5 on rivière Noire, upstream of ruisseau Dormenan and the sites in lac Preissac. Amphipods exposed to sediment from reference Station 1 exhibited comparable growth to the reference Station 19 but lower than at Station 2 (Figure 6.2 and Table 6.1), immediately downstream, suggesting no effect on growth or survival from the joining stream which receives municipal wastewater from the town of Cadillac. Station 4 on the rivière Noire about 1.5 km upstream of the confluence with Dormenan Creek produced reduced growth but Station 5,

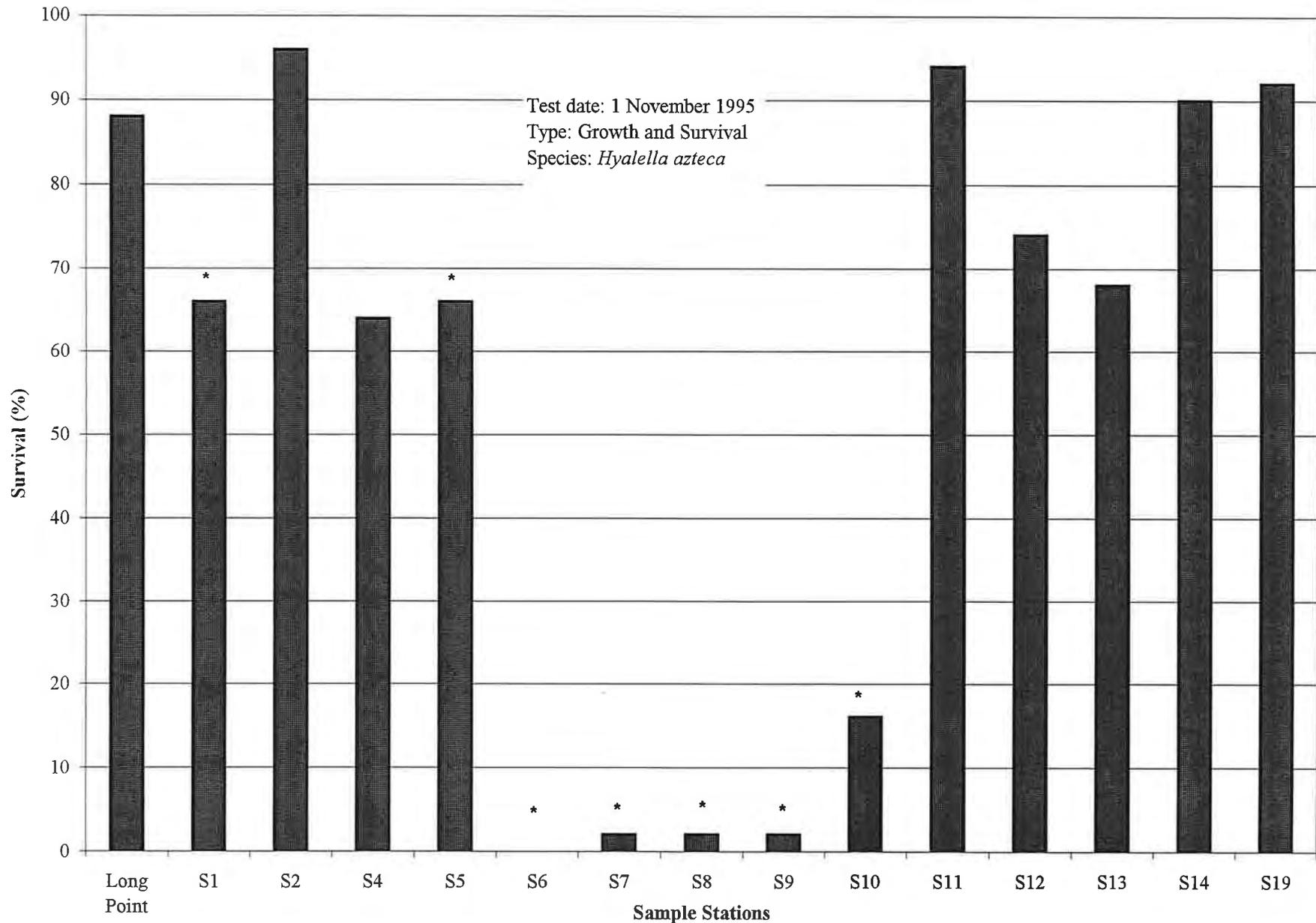
TABLE 6.1: SUMMARY OF BENTHIC, TOXICITY AND METALS DATA FOR SEDIMENTS COLLECTED IN RIVIÈRE NOIRE
(benthic data from 200 µm sieve, species level identification)

Stations	Stream Stations										Lake Stations			
	1-Ref	19-Ref	2	4	5	6	7	8	9	10	13-Ref	14-Ref	11	12
Toxicity Results														
<i>Hyalella azteca</i> % Survival	66	92	96	64	66	0	2	2	2	16	68	90	94	74
Mean weight (mg)	0.233 0.138	0.124	0.121	0.084	0.171	-	0.04	0.02	0.03	1.076	0.074	0.087	0.073	0.078
<i>T. tubifex</i> Mean Offspring/Adult	34.6	34.1	39.9	0.2	31.2	6.0	24.1	20.6	29.0	34.8	36.4	36.8	17.5	10.4
% Inhibition	0	0	-12	99	12	83	32	42	18	2	0	0	51	71
Microtox IC50 (mg/kg)	13000	3700	2400	8700	1600	200	880	1400	3700	2700	14900	31800	7800	7700
Benthic Indices														
Density/m ²	19170	11140	13033	12576	8300	152	352	96	27244	26361	16078	27148	34961	8282
Number of Taxa	55	68	37	44	17	11	8	4	6	7	71	80	55	58
<i>T. tubifex</i> Density/m ²	3023	657	139	1000	7404	4	261	83	27192	26326	44	378	18291	17
Metals Results (µg/g)														
Aluminium	7300	20200	9840	12100	14700	16400	11900	13000	15300	14500	10000	7760	15300	24100
Arsenic	2	9	3	512	498	48	392	568	512	381	66	71	798	701
Cadmium	0.18	0.39	0.28	0.26	0.37	0.48	0.35	0.36	0.5	0.64	0.82	0.68	0.56	1.07
Chromium	41	79	54	60	69	67	67	73	68	75	42	33	69	104
Copper	7.1	36.3	16.7	28.4	29.8	113.4	44.3	42.5	59.9	57.8	31	23.3	53.3	107.1
Iron	9530	19700	12900	26800	30000	23200	24600	26300	29400	28600	15100	12500	36600	44900
Lead	7.1	12.4	16.1	10.5	11.7	8.6	8.5	10.1	10.7	10.9	18.6	17.6	11.1	31.1
Lithium	8.9	25.7	12.7	18.5	17.9	23.6	19	21.2	22.8	22.8	14.6	11.2	23.9	38.5
Magnesium	3650	7030	4600	8260	8790	6720	8160	9000	9520	9300	4790	3730	11700	15700
Mercury	0.013	0.049	0.022	0.105	0.135	0.084	0.103	0.126	0.084	0.111	0.047	0.03	0.101	0.327
Nickel	21.1	41.9	28.8	40.2	41.9	36	40.5	45	46.7	48.3	29.3	23.9	48.7	62.6
Selenium	<1	1	<1	1	<1	1	1	1	1	1	1	1	1	1
Silver	0.03	0.16	0.05	0.14	0.15	0.53	0.19	0.2	0.32	0.27	0.14	0.11	0.31	0.42
Zinc	28	84	46	89	67	172	76	79	99	129	70	56	89	167

Exceeds TELs (Threshold Effect Levels) Environment Canada (1995)

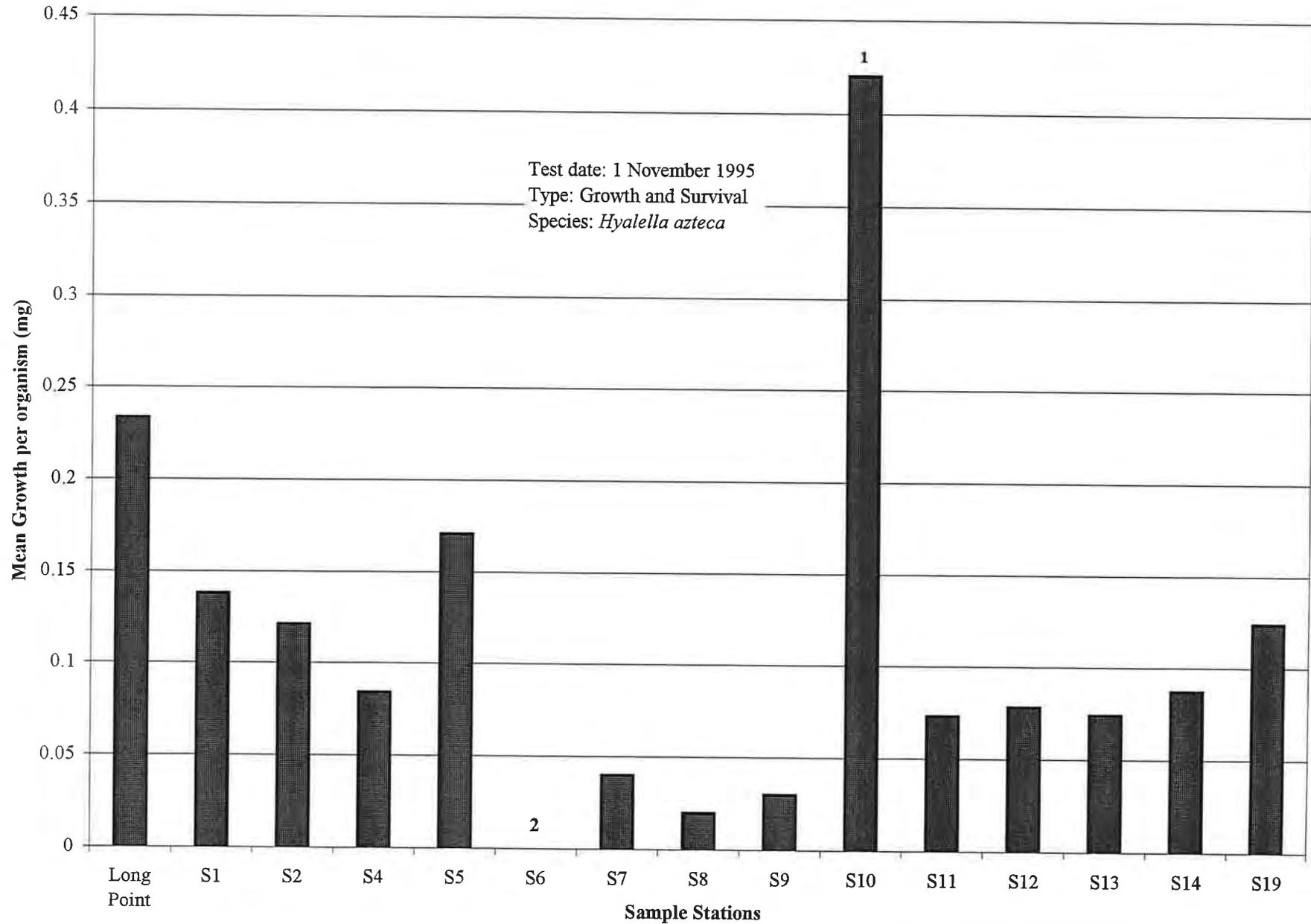
Exceeds PELs (Probable Effect Levels) Environment Canada (1995)

FIGURE 6.1: MEAN SURVIVAL (%) OF *Hyalella azteca* FOR 15 SEDIMENT SAMPLES



* - indicates that sample mean is significantly different ($p < 0.05$) from the control (Long Point sediment)

FIGURE 6.2: MEAN GROWTH OF *Hyalella azteca* FOR 15 SEDIMENT SAMPLES



1: Significantly above the control (Long Point)

2: No observed growth due to 100% mortality of organisms

about 1 km upstream, showed similar growth to the reference sites (Figure 6.2). *Hyaella* growth response suggests there is a contaminant source upstream of Station 4 which is diluted or neutralized (complexed) at Station 5. There was a notable increase in most sediment metal levels, especially arsenic, from Station 2 to Station 4, believed to be originating from the idle mine sites in that area (discussed in Section 5).

Tubifex Survival

Survival of *Tubifex tubifex* at all stations was comparable to the reference sites with the exception of Station 12 in lac Preissac, which produced only 25% survival in oligochaetes (Appendix C). This station is almost 1.5 km from the mouth of rivière Noire but does show elevated sediment levels of arsenic, chromium, cadmium and copper compared to sediment levels at the other sites.

Tubifex Reproduction

The number of empty (hatched young) cocoons produced by oligochaetes exposed to sediment at all stations was comparable to that at reference sites except at Station 4 where there were about 60% fewer cocoons produced (Appendix C).

The number of young counted per adult was also lower than reference site production by about 99% at Station 4 in rivière Noire but production was back up to reference levels 1 km downstream at Station 5. This trend was also shown with *Hyaella azteca* growth.

Oligochaetes exposed to sediment from Station 6 in ruisseau Dormenan exhibited an 83% reduction in young produced per adult which appeared to carry through to downstream sites in rivière Noire at Stations 7 and 8 which showed about 32% to 42% reduction in young production, respectively, while production levels were back up to reference levels further downstream at Station 9 and at the mouth of the river at Station 10. *Tubifex tubifex* densities in the sediments at Stations 9 and 10 observed during the benthic invertebrate assessment were also found to be the highest in the study area (Table 6.1).

Sediment from the lake showed incremental inhibition at Station 11 with about 50% reduced

production and Station 12 with about 70% reduced production. In contrast, Station 11 was found to have significantly higher *Tubifex tubifex* densities than found at reference Stations 13 and 14 (Table 6.1).

***Microtox*[®]**

Sediment from all stations on rivière Noire showed some level of inhibition of luminescence when compared to IC50 values for reference Stations 1, 13 and 14. The response at Stations 4, 11 and 12 were most similar to the response at the reference sites. Sediment from Station 6 at the mouth of ruisseau Dormenan produced the most toxic response of all *Microtox*[®] readings, while downstream of the confluence with rivière Noire at Stations 7 and 8 the inhibition was less. All other stations exhibited responses comparable to reference sediments, which showed an IC50 range of 3700 mg/kg at reference Station 19 to 31800 mg/kg at reference Station 14.

Statistical Analysis

Based on the benthic invertebrate community structure the stations on rivière Noire were grouped into reference, near-field and far-field areas (refer to Section 7.0). Station 1,2 and 19 represented the reference area, while Stations 5, 7 and 8, and Stations 9 and 10 represented the near-field and far-field areas, respectively.

Statistical tests to detect significant differences between areas indicated that only *Tubifex tubifex* showed significant differences between the reference area and the near-field (Table 6.2). However, the power associated with these differences was only 0.6.

Percent *Hyaella* survival was the only test which showed a significant difference between reference and far-field areas (Table 6.2). The power associated with the difference was quite high suggesting that there is a true difference.

TABLE 6.2: TESTS OF AREA DIFFERENCES (by ANOVA) FOR ACUTE AND CHRONIC TOXICITY FROM RIVIÈRE NOIRE SEDIMENTS

	Probability	Power
Reference vs Near-Field		
<i>Hyalella azteca</i>		
% Survival	0.079 ¹	-
Mean Weight (mg)	0.289 ¹	-
<i>Tubifex tubifex</i>		
Mean Offspring/Adult	0.040	0.620
%Inhibition	0.028	0.715
Microtox	0.269 ¹	-
Reference vs Far-Field		
<i>Hyalella azteca</i>		
% Survival	0.011	0.954
Mean Weight (mg)	0.372	0.118
<i>Tubifex tubifex</i>		
Mean Offspring/Adult	0.275	0.158
%Inhibition	0.174	0.240
Microtox	0.447 ¹	-

¹ Calculated using t-test assuming unequal variance

statistically significant difference at p=0.05

power greater than 0.800

A 10

Indicated Areas of Stress

The toxicity data indicate that the sediments from ruisseau Dormenan are highly toxic and that a somewhat reduced toxic effect extends into rivière Noire from the confluence with the creek. There also appears to be some low level of toxicity in lake sediment at Stations 11 and 12.

Upstream of the Dormenan confluence there is a slight indication of toxicity at Station 4 on rivière Noire which is dissipated through dilution or additional complexation about a kilometre downstream at Station 5. The benthic invertebrate data indicate an opposite effect with reduced numbers of taxa at Station 5 compared to Station 4 (refer to Section 7.0).

6.4 Comparison of Methods

Hyalella survival appears to provide the most sensitive response and sensitivity may have improved if Reference Station 1 did not have such a low % survival which would have resulted in less variability among reference stations. The *Tubifex* production of young per adult provided slightly less sensitivity than *Hyalella* survival but did provide the best graded response to environmental stress and showed less variability among reference stations. *Hyalella* growth was a reasonable indicator of stress where the organisms showed comparable survival to reference sediment exposure but did not exhibit the same sensitivity as *Tubifex* reproduction. The Microtox® test identified the most toxic sediment samples and sediments of intermediate toxicity from Stations 7 and 8 but revealed no significant difference between reference, near-field and far-field areas.

The Microtox® tests represents a good screening level toxicity indicator but rapid testing characteristics are offset by reduced sensitivity compared to whole organism sublethal tests.

6.5 Costs

The unit costs for the various tests were as follows:

- *Hyaella* \$600
- *Tubifex* \$400
- Microtox® \$270

The amount of effort required for the *Tubifex* test in measuring and recording all the endpoints reported in this study would probably require a future higher charge by about two times that indicated above. The reason the costs were lower is because the test is still in the development phase and not routinely offered by commercial labs. The professional time required for the *Tubifex* test is greater than that required for the *Hyaella* test.

The Microtox® test reflected the results of the benthic invertebrate assessment at the most impacted sites for the least cost, but failed to identify 60% of the exposure sites that produced a stress response with the other tests combined. It would identify very toxic locations characterized by low numbers of benthic taxa, but might not be cost-effective for long-term environmental monitoring.

The *Tubifex* test indicated stress at 78% of the stations that produced effects in all the test systems combined while the *Hyaella* test indicated stress at 56% of the stations that produced effects in all of the test systems combined. It failed to identify effects in the lake samples.

Clearly, there are complimentary features to the *Hyaella* , Microtox®, and *Tubifex* tests. All responded slightly differently to contaminated environments, especially when comparing impacted areas suggested by the benthic invertebrate community structure. Together, the *Hyaella* and *Tubifex* tests identified nine sites that produced toxic responses. Consequently, omitting the use of one or more of the three tests would result in some underestimate of areas that were impacted.

6.6 Evaluation of Methods

All of the toxicity data combined provides a very similar, although not exact, representation of environmental quality to that indicated by the benthic invertebrate survey (refer to Section 7.0). Obviously, the duration of exposure for an indigenous organism compared to a representative test species is considerably different (months vs days) so expectation of an absolutely accurate reflection of community conditions by toxicity tests is unreasonable. Also, the benthic invertebrate community reflects the combined effects of water quality and sediment quality conditions, while the sediment toxicity tests do not reflect the water quality problems in rivière Noire and ruisseau Dormenan.

The value of using an organism that is reasonably tolerant to lethal conditions (e.g., *Tubifex*) ensures that graded sublethal responses can be measured (reproduction). In comparison, the *Hyaella* is more sensitive but cannot tolerate the lethal conditions and growth becomes irrelevant and the test cannot, under these lethal conditions, measure relative differences in toxicity.

While the Tubificid test does provide useful sublethal data the test procedure is more complex than other tests using similarly tolerant organisms (e.g., *Chironomus tentans*). The chironomid growth test is simpler in that the organism is exposed for ten days, collected and weighed. The usual numbers and replicates are utilized but the effort required to generate the final data is about half that for the Tubificid test. Past experience indicates similar tolerance but a nicely graded growth response under conditions that would be toxic to more sensitive organisms.

It is important to maintain very sensitive test organisms in the test battery because the community response is more sensitive than any of the laboratory tests and it is important to identify and quantify lethal environmental conditions. *Hyaella* appear to be one of the best laboratory organisms for sensitive lethality testing of sediments. Another sensitive mayfly species (*Hexagenia limbata*) is also commonly used.

While the laboratory tests cannot predict the health and quality of the benthic invertebrate community, they can differentiate between toxicity residing in the sediment vs the liquid

phase (water) while the benthic invertebrate community will respond to both. The toxicity test then becomes valuable in identifying or confirming sources of toxicity or accounting for community responses by differentiating between habitat differences, chemical differences or differences in media quality. Generally, sediment toxicity tests are used to further investigate impacted areas that have been identified through benthic invertebrate analysis, in order to establish cause-effect relationships.

The degree of concurrence between sediment toxicity, sediment chemistry, and benthic invertebrate community conditions shown here indicates the possibility of using data on natural community impacts to selectively reduce the number of stations or areas where sediment toxicity measurements are undertaken in environmental effects studies. The cost to characterize the natural benthic invertebrate community at a site are less than the cost of a single species toxicity test.

7.0 BENTHIC INVERTEBRATE COMMUNITY ASSESSMENT

7.1 Methods

7.1.1 Field and Laboratory Methods

Rivière Bousquet

During the preliminary reconnaissance survey, benthic samples were collected by petite-Ponar grab (0.023 m²) at both reference and exposure stations on the rivière Bousquet. Six replicate samples, each consisting of two pooled grabs of sediment were collected at each site. All samples were sieved in the field with a 200 µm mesh, preserved to a level of 10% buffered formalin and forwarded to BEAK's Benthic Ecology Laboratory in Brampton, Ontario. Processing methods are provided in Appendix D.

Artificial substrates consisting of cobble-filled barbecue baskets of the type identified as preferable in the CANMET literature review were installed on 09 September in depositional habitats at an upstream reference area and a downstream exposure area in rivière Bousquet. Six baskets were installed at each location, and were suspended above the bottom from sturdy overhanging trees. The installations did not appear to have been tampered with during the duration of exposure. Exposure was confirmed at the time of installation by conductivity measurements. Dissolved oxygen levels were also notably lower at the exposure site compared to the reference site.

Artificial substrates, after approximately six weeks of colonization, were removed from the exposure area on 20 October, 1995 and from the reference area on 22 October, 1995. Each artificial substrate was removed by placing a 200 µm mesh net around it and removing it from the river while in the net. The basket was placed in a large receiving tub and the cobble was taken from the basket and placed loosely in the tub. Invertebrates which were not clinging, or attached to the rocks were removed first by pouring water into the receiving tub and then pouring off the water (and free invertebrates) into a 200 µm box sieve. After this process was repeated several times, each individual piece of cobble was rinsed and inspected. If necessary, invertebrates still clinging to the cobble were scraped or picked off

and placed in the 200 μm box sieve. The net was also rinsed into the 200 μm box sieve and then inspected to ensure invertebrates were not being transferred to other samples. Once the entire sample was transferred into the box sieve, the sample was gently sieved in water to remove material and invertebrates smaller than 200 μm . The remaining sample was then transferred into a benthic jar along with a tag labelled with the project number, station number and replicate number. The outside of the jar was immediately labelled with the same information. Samples were then taken to a secure storage area where they were preserved in 10% buffered formalin.

Rivière Noire

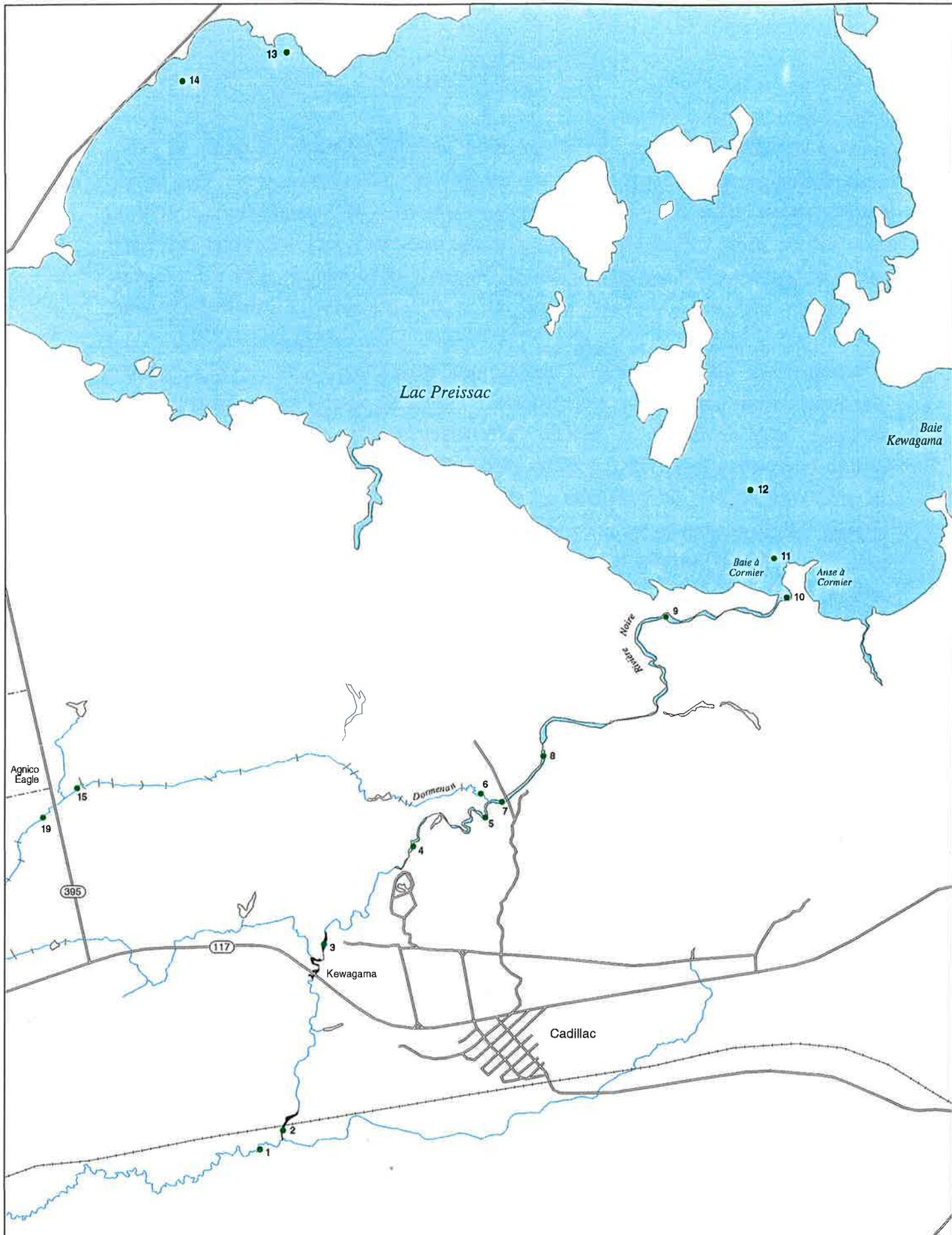
During the October field program, benthic invertebrates were sampled at sixteen sites on ruisseau Dormenan, rivière Noire and lac Preissac waterbodies (Figure 7.1). Sediment samples for toxicity testing and sediment chemistry characterization were also collected at each site. In an effort to assess the effectiveness of different invertebrate processing techniques to delineate mining-related impacts, sampling occurred throughout an expected impact gradient in the study area. Five samples were collected at each site, each consisting of two pooled petite-Ponar grabs (total area 0.046 m^2). All samples were sieved in the field with a 200 μm mesh, preserved to a level of 10% buffered formalin and returned to BEAK's Benthic Ecology Laboratory in Brampton, Ontario. Processing methods are provided in Appendix D.

7.1.2 Statistical Methods

Ponar Grab Samples from rivière Noire and ruisseau Dormenan

Over the past several years, considerable research and protracted discussions have focused upon the design of such methods for monitoring the environmental effects of industries. The conclusions of many studies and workshops led to the recommendation to Environment Canada of several sets of indices for the monitoring of benthic communities.

Two simple statistics included in these recommendations are the density of organisms/ m^2 at a sampling station, and the number of different taxa collected at a station. These simple



Benthic Invertebrate, Toxicity, Water and Sediment Chemistry
Collection Sites on Rivière Noire and Dormesun Creek



beak
consultants
limited

Figure
7.1

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measures are easily understandable and sometimes appear to be as meaningful as more complicated ratios or data summaries used for characterizing the abundance and diversity of a benthic community. In cases of slight to moderate impacts these two metrics are sometimes misleading especially when sensitive taxa are replaced by more tolerant taxa, such that the total number of taxa and density do not change but the structure of the community dramatically changes.

There is a large suite of multivariate methods that have been used for investigations of natural and man-made variation in biological communities. One common method currently used in environmental effects monitoring studies is Correspondence Analysis. Correspondence analysis (CA) is a multivariate ordination technique that appears to have a number of features that make it most suitable for describing the composition of benthic communities. Among these features are several that minimize or avoid problems inherent in other common multivariate methods, such as the use of relative, rather than absolute abundances in the calculations, and less sensitivity to non-linear relationships between environmental gradients and taxonomic abundances. Additionally, the CA method may be less sensitive to the presence of rare taxa - a common dilemma in the analysis of biological communities. Finally, a number of widely available commercial or academic software packages implement the CA method, so it is widely available to most consultants and industry and government researchers.

The results of CA reduce the large number of benthic taxa found commonly in most freshwater or marine samples to a few summary measurements of the variation in the community. In the present study, for example, 187 benthic taxa were identified at the species level. It is very difficult to summarize differences between stations or areas from visual inspection of such a large matrix of data. The CA method calculates values or "scores" for each of the sampling stations and for each taxon found in the original data matrix. These scores reflect the variation in the original data matrix and, typically, the three most important new or "derived" variables from the CA contain one-half to three-quarters of the total variation in abundance of all the original variables (i.e., taxa). The three scores at each station are then used as benthic summary indices to compare and contrast different sampling stations or areas.

In order to evaluate the effectiveness and sensitivity of the various benthic invertebrate processing methods it was necessary to group stations with similar community structures which reflected similar impacted areas. This was done with both cluster analysis and correspondence analysis using the 200 μm species level data, which was the most detailed data available. Stations which displayed substantial similarity were considered as groups for further analyses. These groups included a set of three stations which were upstream of known mine discharges and drainage, and which had benthic communities characteristic of good water quality. This group of stations represented the reference area. Stations immediately below known discharges, with impoverished benthic communities, were designated as a "Near-Field" group of stations, and stations near the mouth of rivière Noire which were determined to have a different benthic community, reflecting signs of recovery, were assigned to the "Far-Field" group of stations.

The above indices (number of taxa, density, and the three CA axis scores) were used as dependent variables in MANOVA and ANOVA comparisons of Reference and Near-Field and Reference and Far-Field groups of stations. Power analysis statistics were calculated to evaluate the reliability of these indices in distinguishing differences between areas. Different benthic sampling and processing techniques (level of taxonomy, mesh size, and number of replicates) were evaluated for their efficiency in distinguishing areas by comparison of relative power values from the MANOVA and ANOVA analyses. Additionally, the indices were used in correlation and regression analyses with sediment metal variables and toxicity variables.

These groups of stations representing reference, near-field and far-field areas were also used to test for significant differences in sediment quality and sediment toxicity between areas.

Comparison of Ponar Grab and Artificial Substrates Samples

Numbers of taxa were compared between the reference and exposure areas and between methods (Ponar grabs and artificial substrates) using a factorial ANOVA design. Comparisons between the reference and exposure areas for each method individually were conducted using t-tests.

7.2 Detection of Differences Between Areas

7.2.1 Characterization of Reference, Near-Field, and Far-Field Areas

Confounding Factors

Two possible confounding factors in rivière Noire drainage system were apparent. These were municipal sewage effluent and impacts from historic mining operations.

The sewage discharge from the town of Cadillac into rivière Noire was judged most likely to affect the benthic community of Station 2, which is just below the confluence of ruisseau Beauchemin and rivière Noire (Figure 7.1). However, no substantive impacts were found in the analyses performed below and therefore, Station 2 was included in the reference area.

The drainage from historic mining operations on the east and west sides of rivière Noire upstream of Station 4 apparently have substantial effects on the benthic community of rivière Noire. The effects of these historic mines and the effects of the Dumagami Mine discharging to ruisseau Dormenan were evident in rivière Noire below the mouth of ruisseau Dormenan. These combined discharges receive little dilution from the mouth of ruisseau Dormenan to lac Preissac, and effects from the river extend into the lake.

Initial analyses confirmed that the lac Preissac stations (11, 12, 13, 14) supported dramatically different communities than were found in rivière Noire. This is not surprising since benthic communities of lakes and rivers are often quite different. Further tests (Correspondence Analyses) on this subset of stations using reference Stations 13 and 14 showed altered benthic communities at Stations 11 and 12. Benthic communities at these latter stations are likely affected by the discharge of rivière Noire, but the two stations also showed considerable differences between each other, indicating a gradient in the effect. The benthic community at Station 12 was more similar to the reference stations. This large variation in benthic indices, coupled with the fact that only one station was located in each area, made it difficult to form any conclusions about the effectiveness of different processing techniques to identify mining-related impacts in a lacustrine environment. Accordingly, most analytical effort was focused upon the stations in the flowing waters of the rivière Noire system.

Benthic Indices Calculated

Rationale for Selection of Benthic Indices

Since organism density, number of taxa, and the three axis scores derived from CA are accepted and are in widespread use for the analysis of benthic communities in EEM studies, BEAK focused on these benthic indices for the 1995 Pilot Field Program. Future field programs should also focus on other commonly used benthic indices.

Many methods exist for comparing differences in benthic communities. These range from simple measurements of community richness, such as number of taxa per station or per area, to complex multivariate tools to relate the abundance of all benthic taxa to all environmental measurements. Effective methods must be easily available, robust, and interpretable.

Benchmark Benthic Data Set

The benchmark for the evaluation of different techniques for the collection, processing and identification of benthic invertebrate samples was the most detailed of the combinations available. This combination (species-level identification, 200 μm mesh sieve, 5 replicate samples per station) results in benthic summary indices that contain the greatest possible amount of information on the communities that were sampled.

Three levels of identification were analyzed:

- Species-level identifications are difficult and time-consuming, and require a high level of expertise available in only a few benthic laboratories, such as BEAK's Brampton facility.
- Genera-level identification also require extensive expertise, but is less demanding and time consuming than species identification.
- Family-level identification is relatively straightforward for most invertebrate groups and requires little effort and expertise.

Mesh sizes used for sample reduction (separation of benthic organisms from fine silts or sands) that are commonly used are 200 to 250, 500 to 600, and 1000 μm meshes. Most studies on freshwater systems use a 500 μm sieve. This is also the maximum allowable sieve size for pulp and paper EEM programs in both freshwater and marine systems. As mesh size increases, the risk of losing small invertebrates through the mesh increases; at smaller mesh sizes, smaller individual organisms are retained, providing more information about the environment at the sampling station, especially in terms of total invertebrate density. These smaller organisms are composed of both small sized taxa (i.e., number of taxa increases with smaller sieve size), and of small, immature individuals of larger sized taxa (i.e., density increases with smaller sieve size). Smaller organisms require more laboratory processing and identification resources and consequently, processing costs increase with decreasing sieve size.

Replicate samples are generally regarded as a way to adequately sample the inherent variation at a single sampling station. To estimate the inherent variation within an area replicate stations are sampled. A single Ponar grab sample from a station will inevitably miss collecting individuals from a number of taxa that actually occur at the station. The number of samples required to collect all, or almost all, of the taxa present is a matter of ongoing debate among field biologists, and may vary from one type of community or location to another. Five samples were collected from each station in the 1995 AETE Pilot Study.

The various combinations of sampling variables in the present study: three levels of taxonomy, three different mesh sizes, and five replicate samples can be combined in 45 possible combinations. This would result in 45 different data sets from which to calculate benthic indices to evaluate sampling techniques. Since this scope was too broad, BEAK targeted combinations of these variables at the extremes of the cost-effectiveness continuum for initial comparison. Further comparisons were selected, based upon the results of the initial combinations tested and on our extensive experience with benthic sample processing.

Benchmark Data Set: Results

Organism Density and Number of Taxa

In total, 47,102 organisms were collected in the benthic samples from the 1995 AETE Pilot Study. These organisms represented 187 taxa at the species level of identification. The river and creek stations are identified by station number in the plot of number of taxa against density ($\log [x+1]$ transformed) in Figure 7.2. Station 19, in the upper reaches of ruisseau Dormenan (above the Dumagami Mine discharge) has the greatest number of taxa. Station 8, in rivière Noire, below the confluence with ruisseau Dormenan, has the fewest number of taxa and the lowest density of invertebrates. Stations 1, 2, 4, and 19 have relatively high numbers of taxa and moderately high density. This usually is indicative of relatively unimpacted environments. Stations 9 and 10 have low numbers of taxa and have the greatest density of all river stations, which under some circumstances is characteristic of organic enrichment in the environment. Stations 6, 7, 8, and perhaps Station 5 are suggestive of severely stressed communities. These latter stations have low numbers of taxa and low or relatively low densities of organisms.

Correspondence Analysis

A screening process was used to remove rare taxa from the data set before analysis. Of the 187 original taxa, 61 taxa occurred at more than one lotic (river) station or comprised more than 5% of the total organisms collected. CA of this, the most detailed data set (species/200 $\mu\text{m}^2/5$ replicates), resulted in three important new variables, or "scores" per station. These three new variables accounted for a total of 66% of the variation in the original data set. The three variables accounted for 27, 23, and 16% of the variance, and correspond to Axis 1, 2 and 3, respectively.

Station scores were plotted to show stations with similar benthic communities and to aid in the delineation of a reference area in the unaffected zone, and a near-field area and far-field area in the effluent-exposure zone. Figure 7.3 shows three biplots of station scores. The top panel, plotting CA2 against CA1 scores, is the most important plot because most of the community variation (27% + 23% = 50%) is portrayed in this plot. The middle panel

FIGURE 7.2
CANMET AETE 1995: PLOT OF STATION VALUES OF NUMBER OF TAXA AND DENSITY.

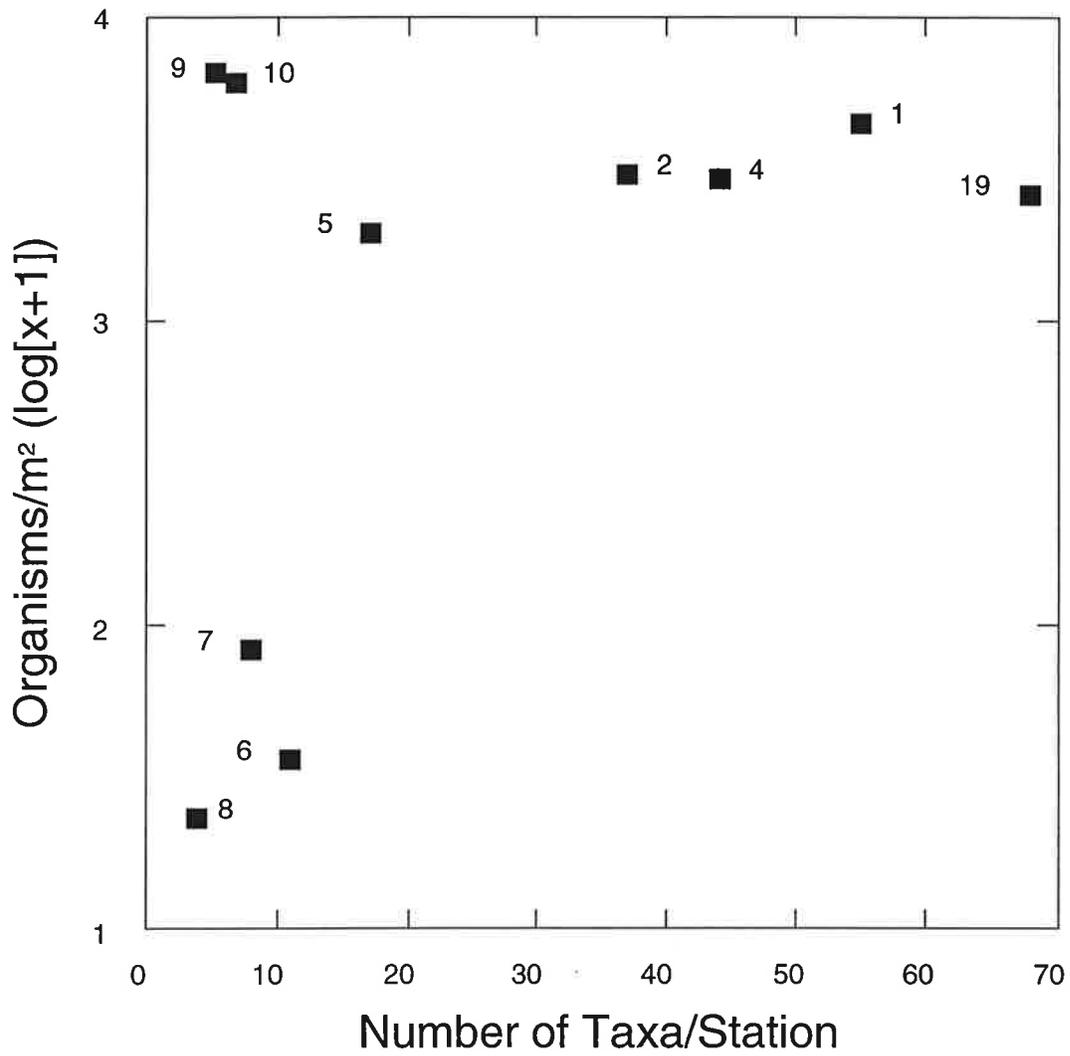
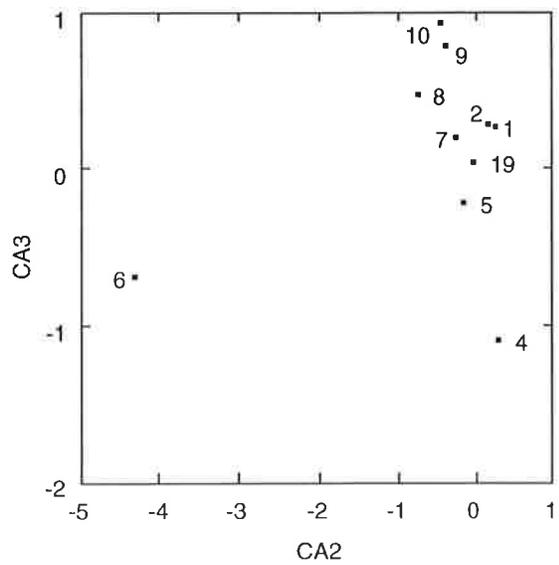
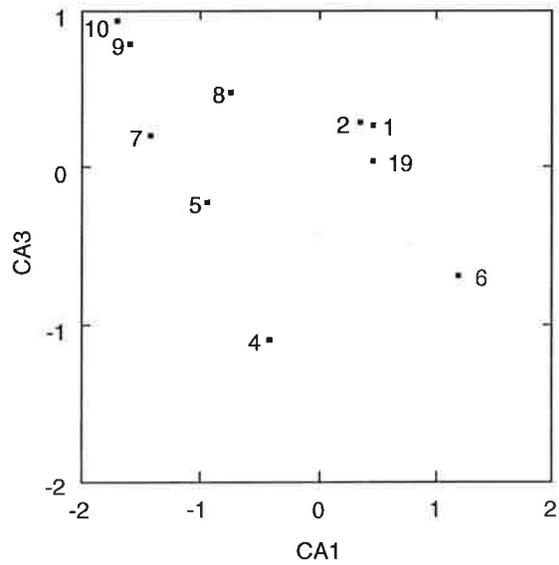
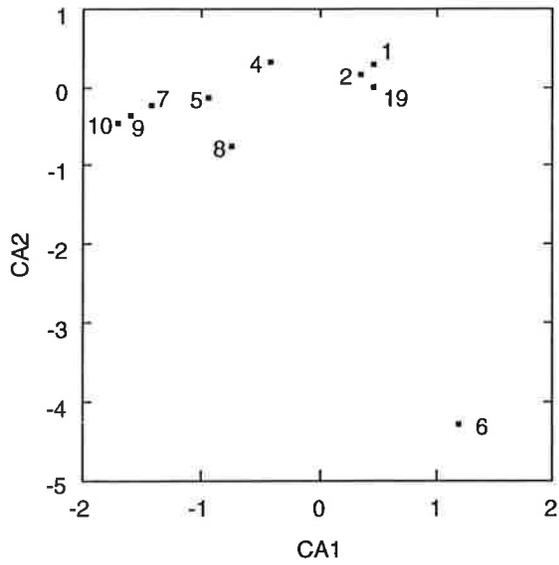


FIGURE 7.3
CANMET AETE 1995: PLOTS OF STATION LOCATIONS ON CA AXES.
STATIONS LYING CLOSE TOGETHER HAVE SIMILAR BENTHIC COMMUNITIES.



biplot, CA3 vs. CA1, portrays 43% of the variation, and the last biplot accounts for 39% of the variation (23% from CA2 and 16% from CA3).

It is immediately apparent that Station 6 has a radically different community than any of the other stations, illustrating severe impacts. This community is near the mouth of ruisseau Dormenan. Effluent from the Dumagami Mine is discharged to this small stream and receives little dilution until the stream meets rivière Noire. As in Figure 7.2 (the plot of density vs. number of taxa), Stations 1, 2, and 19 show strong similarities in all the biplots of Figure 7.3. The similar communities at these stations upstream of the mine discharge suggest that they can represent a reference community reflecting an unimpacted zone.

Stations 9 and 10 are farthest downstream from the mine discharge while remaining in a riverine environment. These two stations show similar CA scores in all biplots and have similar density and numbers of taxa (Figure 7.2). These similarities suggest that Stations 9 and 10 are in a recovery zone and can represent a far-field area, where effluent impacts likely remain significant, but may not be as severe as at stations closer to the discharge.

In between the reference and far-field groups of stations in the biplots of Figure 7.3 are the Stations 5, 7, and 8. They show moderately strong community similarities and are found in rivière Noire between the reference and far-field stations. These three stations represent the most severely impacted area of rivière Noire and were designated as the near-field area for the present study.

Station 4, although it shows similarities to the near-field and reference area stations in the upper panel biplot, is distinct in the remaining biplots. This suggests that this station is notably different from the designated groups, perhaps because of the historic mining activities on the east side of rivière Noire. It was not included in any of the three groups designated above for comparison of area differences in the ruisseau Dormenan/riivière Noire system. Table 7.1 lists the taxa which were important contributors to the variables (CA Axes) extracted from the CA. The taxa are listed under the CA Axes where they were strong contributors, and the direction of their association (positive or negative) is indicated in the column labelled "Association". For example, reference area stations (1, 2, 19) have high scores on CA Axis 1 and thus have low relative abundance of both immature tubificids

TABLE 7.1: ECOLOGICAL CHARACTERISTICS¹ OF TAXA REPRESENTING COMMUNITY DIFFERENCES BETWEEN NEAR-FIELD, FAR-FIELD, AND REFERENCE AREAS, BASED ON CA-ORDINATION OF ABUNDANCE DATA. TAXA LISTED ARE STRONG CONTRIBUTORS TO THE CA AXIS INDICATED. DATASET: SPECIES/200µm/5 REPLICATES

Taxon	Association	Substrate	Habitat	Habit	Trophic Relationships
CA AXIS 1					
<i>Tubifex tubifex</i>	-	organic depositional	lakes and slow rivers	burrower	detrivore
immature tubificids with hair chaetae	-	organic depositional	lakes and slow rivers	burrower	detrivore
CA AXIS 2					
<i>Culicoides</i>	-	org. depos. & marsh	margins of slow rivers	burrowers, occ. planktonic	predator (engulfer)
<i>Thienemannia pilinucha</i>	-	variable erosional	lotic erosional	sprawler	collector - gatherer
Enchytraeid worms	-	organic depositional	lakes and slow rivers	burrowers, occ. planktonic	detrivore
<i>Bezzia</i>	-	organic depositional	lakes and slow rivers	burrowers, occ. planktonic	predator (engulfer)
CA AXIS 3					
<i>Polypedilum scalaenum</i>	-	vascular hydrophytes	lentic littoral	climbers and clingers	herbivore (shredder)
<i>Brillia</i>	+	organic depositional	lakes and slow rivers	burrower (lotic) & sprawler (lentic)	predator (engulfer)
<i>Tubifex tubifex</i>	+	organic depositional	lakes and slow rivers	burrower (lotic) & sprawler (lentic)	detrivore
<i>Ablabesmyia</i>	-	organic depositional	lakes - littoral	sprawler	predator (engulfer/piercer)

*compiled from Merritt and Cummins (1984); Rooke and Mackie (1982a,b)

with hair chaetae and adult *Tubifex tubifex*. Stations 9 and 10, which have the lowest scores on CA Axis 1, have the highest relative abundance of these taxa, and the near-field stations 5, 7, and 8 tend to have intermediate density of these taxa.

A complete listing of the raw data set may be found in Appendix D. Also included in this appendix are tables with the results of the correspondence analysis of this data set and those of the least detailed data set (family level taxonomy/1000 μm mesh/5 replicates). Since the appendix tables for only these two data sets occupy 74 pages, tables for the other six data sets (220 pages) used in comparative analyses have been retained in data archives at BEAK. These can be made available upon request.

Summary of Results

The selected benthic indices (density, number of taxa, and the three axes from CA) support the selection of Stations 1, 2, and 19 as a set of unimpacted, Reference-area stations. Stations 5, 7, and 8 have similar communities and can be considered as representative of the severely impacted near-field area. Stations 9 and 10 are the most geographically distant river stations from the mine effluent and have similar communities that suggest some recovery in the benthic invertebrate community and may characterize the far-field area of impact. Station 6 on ruisseau Dormenan has few living organisms, and Station 4 may be influenced by confounding historic mining operations. These latter two sites were not included in any of the above groups for analysis.

7.2.2 Effect of Number of Replicate Samples

Pooled and Unpooled Replicates

Replicate samples are those samples collected from a single station in a sampling area. They are taken to account for or to include the natural variability in the distribution of discretely distributed, individual organisms. Replicates may be handled in two ways in a sampling program. In the first instance, replicates may be collected in the field at a single station, then physically combined to give a single, composite sample at a station. Alternately, replicates may be taken at a station and labelled, preserved, identified, and

analyzed separately. This latter method results in multiple observations of taxon abundance at a single station.

Pooled Replicates

Pooled replicates, where the individual replicate samples are composited to form a single larger sample, may estimate with increased precision the number of taxa and true density at a station. As replicates are collected, new taxa are added to the number known from previous samples. New taxa are added in smaller and smaller increments until (theoretically) all are collected. This relationship, between cumulative number of taxa collected and increasing total number of replicates (area), is sometimes called a "species-area curve". Figure 7.4 presents the species-area curve for a mean of five individual stations (these are presented in Appendix D). Although the number of taxa collected nearly doubles, from 21 to 41, as the number of replicates increases from 1 to 5, there is no real asymptote to the curve. This suggests that even with a composite of five replicate Ponar grabs, not all taxa at the station are accounted for.

As the number of pooled replicates is increased, the estimate of the benthic organism density at the station should become a more precise estimate of the true value. In Figure 7.4, mean coefficient of variation for five stations is shown as error bars on the estimate of the mean five-station density. The CV values are generally consistent over the range of 2 to 5 replicate samples. The Central Limit Theorem predicts that the 5-station mean of 5 replicate samples is the most accurate estimate of the true mean density at the five stations.

Comparison of a data set with fewer replicates (species/200 μm /3 replicates) with the more replicated data set at the same level of taxonomy and sieve size (species/200 μm /5 replicates) can provide some information on the value of the information added to the data set with two additional replicates. Table 7.2 compares the statistical power of these two combinations in an analysis of variance (ANOVA) of the differences in benthic indices between reference and near-field and between reference and far-field areas. Comparisons of reference to either of the effluent-exposed areas are more often statistically significant ($p < 0.05$) and are more often associated with high probability of finding a true difference (power > 0.80) when five replicates are used. This strongly suggests that the added

Figure 7.4

CanMet: Evaluation of Replicate Data, for 5 Stations.

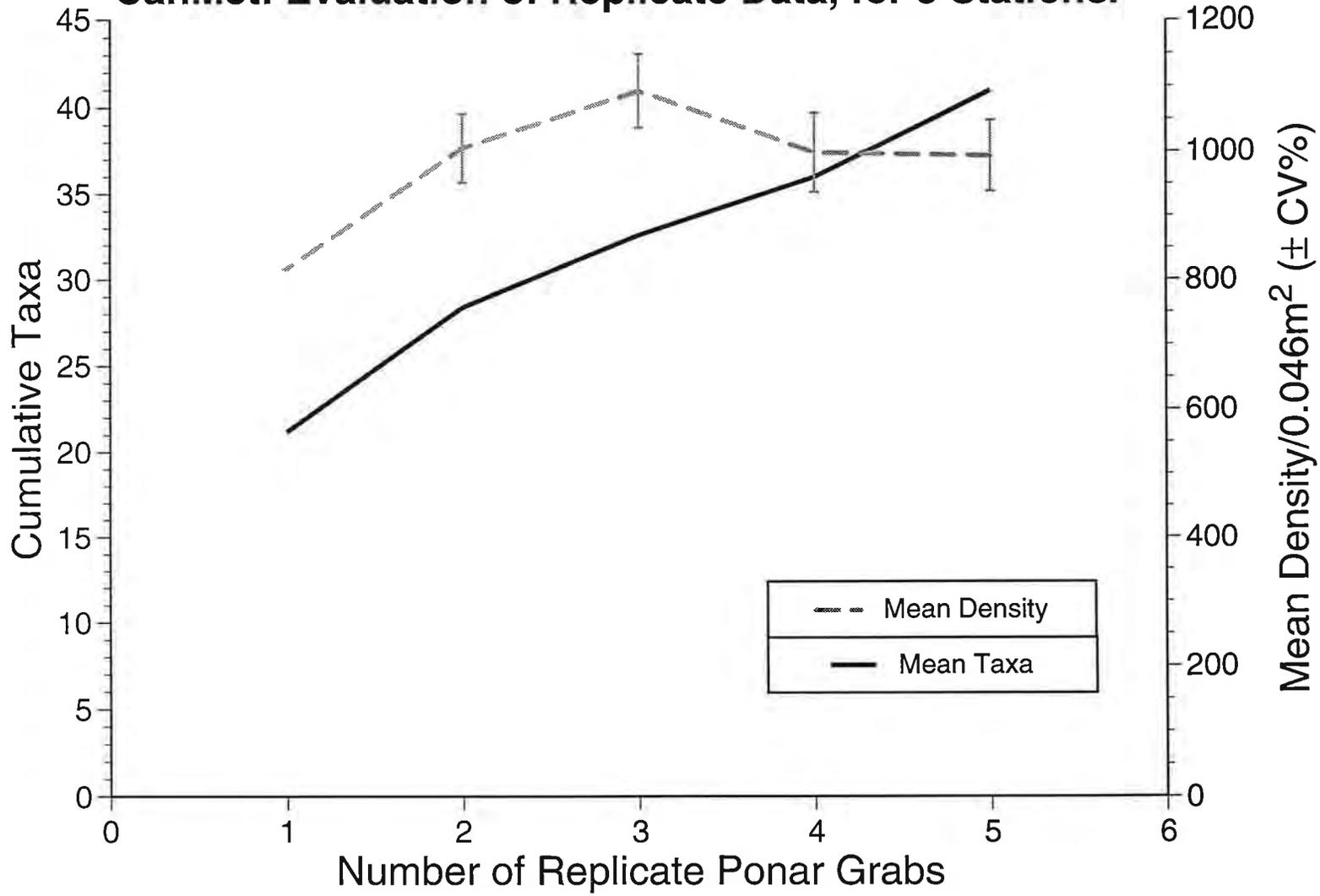


TABLE 7.2: DETECTION OF AREA DIFFERENCES WITH DIFFERENT NUMBERS OF POOLED SAMPLES/STATION

Benthic Community Metric*	Species / 200-μm mesh / 5 Replicates		Species / 200-μm mesh / 3 Replicates	
	Probability	Power	Probability	Power
Reference vs. Near-Field				
Number of Taxa	0.011	0.908	0.016	0.849
Organism Density	0.033	0.668	0.033	0.674
MANOVA	0.050	0.610	0.033	0.760
CA1	0.002	0.999	0.003	0.996
CA2	0.066	0.481	0.623	0.073
CA3	0.863	0.053	0.948	0.051
MANOVA	0.038	0.730	0.113	0.370
Reference vs. Far-Field				
Number of Taxa	0.027	0.761	0.014	0.920
Organism Density	0.029	0.741	0.099	0.375
MANOVA	0.097	0.410	0.085	0.450
CA1	<0.0005	1.000	0.001	1.000
CA2	0.014	0.913	0.701	0.062
CA3	0.010	0.961	0.162	0.255
MANOVA	0.024	0.980	0.097	0.500

*may have been analysed as transformed values such as log (x+1), square root, or ranks to satisfy assumptions of M(ANOVA).

Power \geq 0.800

p < 0.05

information in the five replicate samples aids in the detection of area differences, even though the replicates are pooled so that the samples at a station are comparable to a single, composited sample.

Separate Replicates

When replicate samples are not composited and are processed and identified as individual entities, the variation of stations within areas may be estimated with a nested ANOVA design. The aim of this technique is to separate variation at stations from that variation considered to be part of the natural (error) variation. If variation of stations within areas is large and is not considered separately, the detection of differences between areas may not be detectable. Table 7.3 compares the probabilities and power of detecting differences in benthic indices between areas using pooled replicates and unpooled replicates. Reference and near-field areas are separated about as well in both cases, but the differences are more detectable with higher levels of power with pooled replicate samples for the reference vs. far-field comparison. This result implies that variation of stations within areas may not be masking area differences in the present study.

Summary

The relationship between number of replicates and cumulative number of taxa suggests that five samples will not collect all taxa at a site. However, the information added by increasing the number of pooled samples from three to five appears to increase the ability to detect real differences between areas. In the present study, the use of a nested ANOVA design with unpooled replicate data did not appear to enhance the detection of area differences.

7.2.3 Effect of Sieve Size and Level of Taxonomy

The Interdependence of Sieve Size and Taxonomy

Most benthic studies use 500 μm mesh sieves for sample reduction. A 500 μm sieve is presently the maximum size allowable for EEM studies required under the *Fisheries Act*.

**TABLE 7.3: DETECTION OF AREA DIFFERENCES WITH FIVE POOLED SAMPLES/STATION (1-way ANOVA),
OR FIVE UNPOOLED SAMPLES/STATION (Nested ANOVA). DATA SET IS: SPECIES / 200 µm-MESH / 5 REPLICATES**

<u>Benthic Community Metric*</u>	<u>Pooled Replicates</u>		<u>Unpooled Replicates</u>	
	<u>Probability</u>	<u>Power</u>	<u>Probability</u>	<u>Power</u>
<u>Reference vs. Near-Field</u>				
Number of Taxa	0.011	0.908	0.010	0.929
Organism Density	0.033	0.668	0.026	0.734
<u>Reference vs. Far-Field</u>				
Number of Taxa	0.027	0.761	0.029	0.747
Organism Density	0.029	0.741	0.075	0.452

* may have been analyzed as transformed values such as log (x+1), square root, or ranks to satisfy assumptions of ANOVA

 p<0.05

 power greater than 0.800

Smaller mesh sieves will, of course, catch smaller individual organisms. Some of these are taxa that would not be collected adequately by the larger meshes, and others are smaller, younger individuals of taxa that would be collected by coarser mesh sieves. These smaller, immature or earlier instar individuals are often unidentifiable because the characteristics used in taxonomic keys are not always present on immature and younger instar individuals. Consequently, these immatures and earlier instars cannot be assigned to a known taxon and are placed in an additional and somewhat artificial taxon grouping of indeterminates or only identified to genus or family levels. An example of this is the mayfly taxon Caenidae listed in the detailed data set (species/200 μm /5 replicates) in Appendix D. The individuals of *Caenis* are probably immatures of *Caenis latipennis*, but cannot be positively assigned to this taxon because the nymphs are not fully developed. The additional taxon can result in misleading conclusions about the composition of communities if one station has many more immatures of a taxon than does another, even if the total number (identifiable and unidentifiable nymphs) is similar at both stations.

Sieve Size

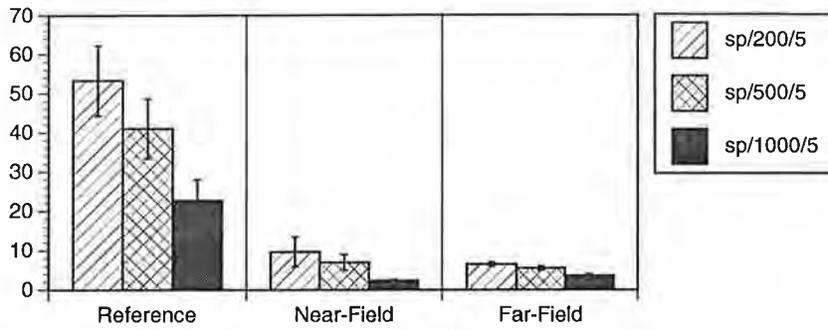
In all three river areas (reference, near-field, and far-field) the mean number of taxa per area is greatest with samples taken using 200 μm mesh sieves (Figure 7.5a). As sieve size increases to 500 and to 1000 μm mesh, the number of taxa collected decreases and the differences between areas decline as well.

Number of organisms (i.e., density) also is affected. Fewer individuals are collected with coarser mesh sieves (Figure 7.5). Most of the decrease in density is associated with the change from 200 μm mesh to 500 μm mesh. This change reflects the loss of small, oligochaete worms, nematodes and tiny arthropods (mainly chironomids).

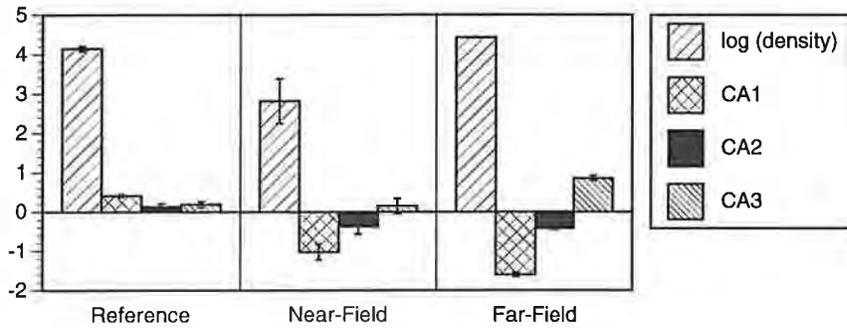
The magnitude and direction of the multivariate benthic indices (CA scores) appears to be relatively stable across the mesh sizes examined, but subtle changes in the values of CA scores, density, and number of taxa are more rigorously tested using ANOVA and power analysis in comparisons of the reference area to either of the effluent-exposure areas.

FIGURE 7.5
CANMET AETE 1995: MEAN VALUES FOR BENTHIC INDICES IN REFERENCE AND EFFLUENT
EXPOSURE AREAS, AND THE EFFECT OF DIFFERENT MESH SIZES ON INDEX VALUES.

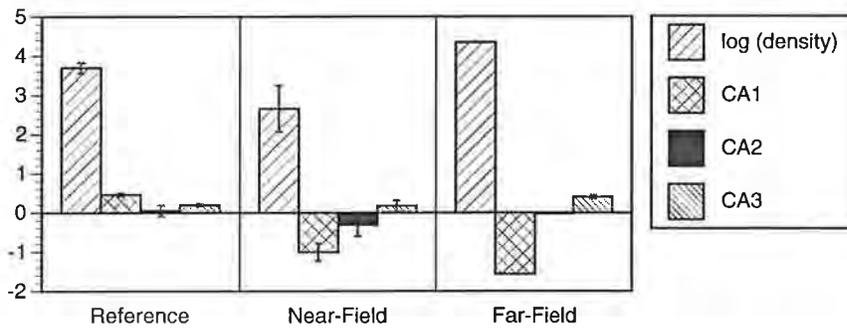
a) Number of Taxa/Area at three mesh sizes



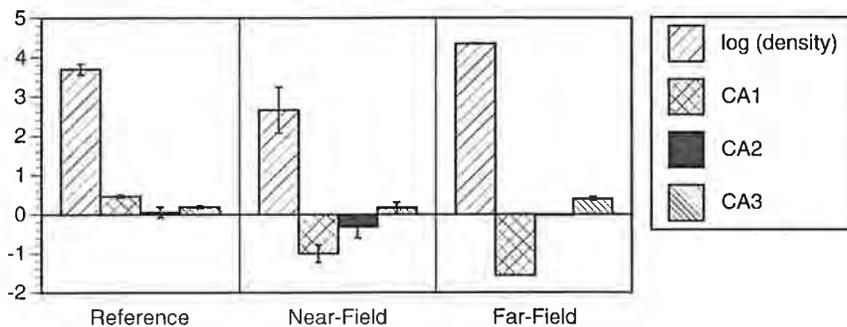
b) Species taxonomy; 200- μ m mesh; 5 replicates



c) Species taxonomy; 500- μ m mesh; 5 replicates



d) Species taxonomy; 1000- μ m mesh; 5 replicates



Detection of Area Differences

The influence of mesh size on the ability to detect area differences was examined by comparing the number of indices in a comparison with a given level of power (the probability of reliably detecting a true difference).

The actual value of the power statistics associated with ANOVA of reference versus near-field comparisons are provided in Figure 7.6. Comparisons using species-level taxonomy, with five replicate samples, were carried out at each mesh size. For all three mesh sizes, significant ($p < 0.05$) community differences (measured by CA1) were detected with high power. Other CA axes had low power and were not significantly different between reference and near-field areas. Both density and number of taxa distinguished areas with high power in the 200 μm mesh comparison, but only number of taxa showed acceptable power to distinguish areas when larger mesh sizes were used. Average power was highest in the 200 μm mesh comparison and lowest in the 500 μm mesh comparison.

Power values that exceed 0.50, 0.80 and 0.95 are enumerated from Figure 7.6; this summary (Figure 7.7) shows the frequency of occurrence or "hits" at the three power levels. The power levels for the assessment of hits are somewhat arbitrarily chosen, as there is no real standard method. Thus, the 1000 μm mesh comparison appears the best method at high power levels although 200 μm mesh is better at power > 0.50 . Mean power (from Figure 7.6) suggests that the 200 μm mesh is best; this and the other information from the previous figure should be kept in mind when interpreting the summary in Figure 7.7.

Comparison of reference and far-field areas is illustrated in the same manner as for the previous comparison: individual power values are shown in Figure 7.8, and the summary of "hits" at the three power levels are shown in Figure 7.9. In this comparison the superiority of the 200 μm mesh sieve is readily apparent. The reference area and far-field area are reliably distinguished by all three CA indices and by number of taxa and density. The 500 μm and 1000 μm mesh comparisons of reference and far-field areas show fewer distinguishing indices and lower overall mean power than the 200 μm mesh. The number of "hits" at the three power levels (Figure 7.9) also shows that the 200 μm mesh is the best

Figure 7.6

CanMet: Testing the statistical power of sampling variables on the ability to distinguish Reference and Near-field Areas.

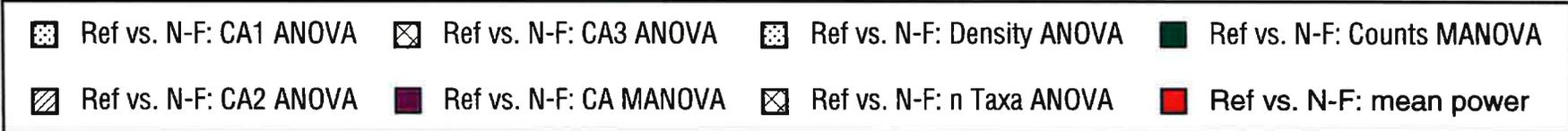
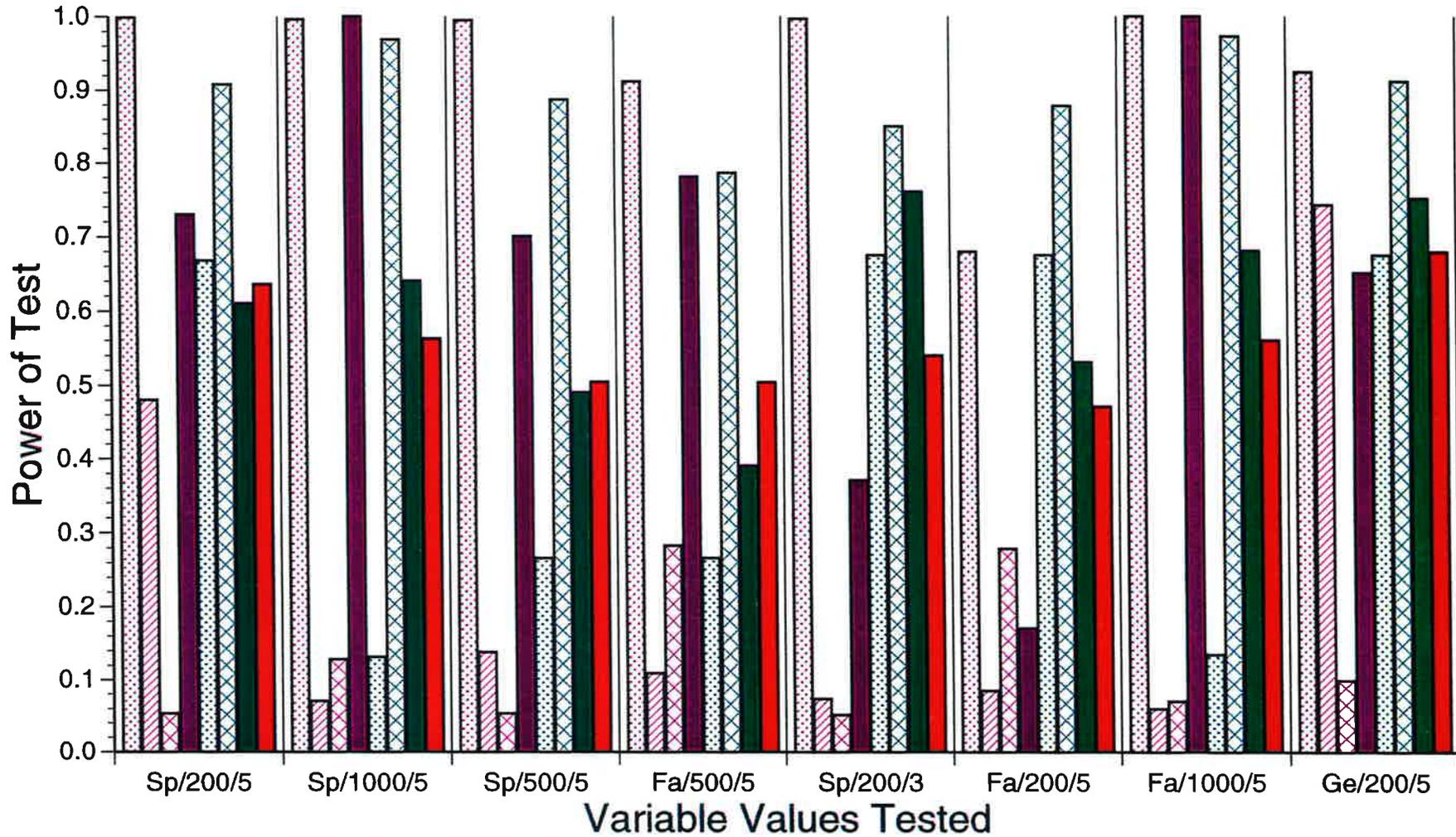


Figure 7.7

CanMet: FREQUENCY OF POWER VALUES EXCEEDING SELECTED LIMITS IN COMPARISON OF REFERENCE AND NEAR-FIELD AREAS.

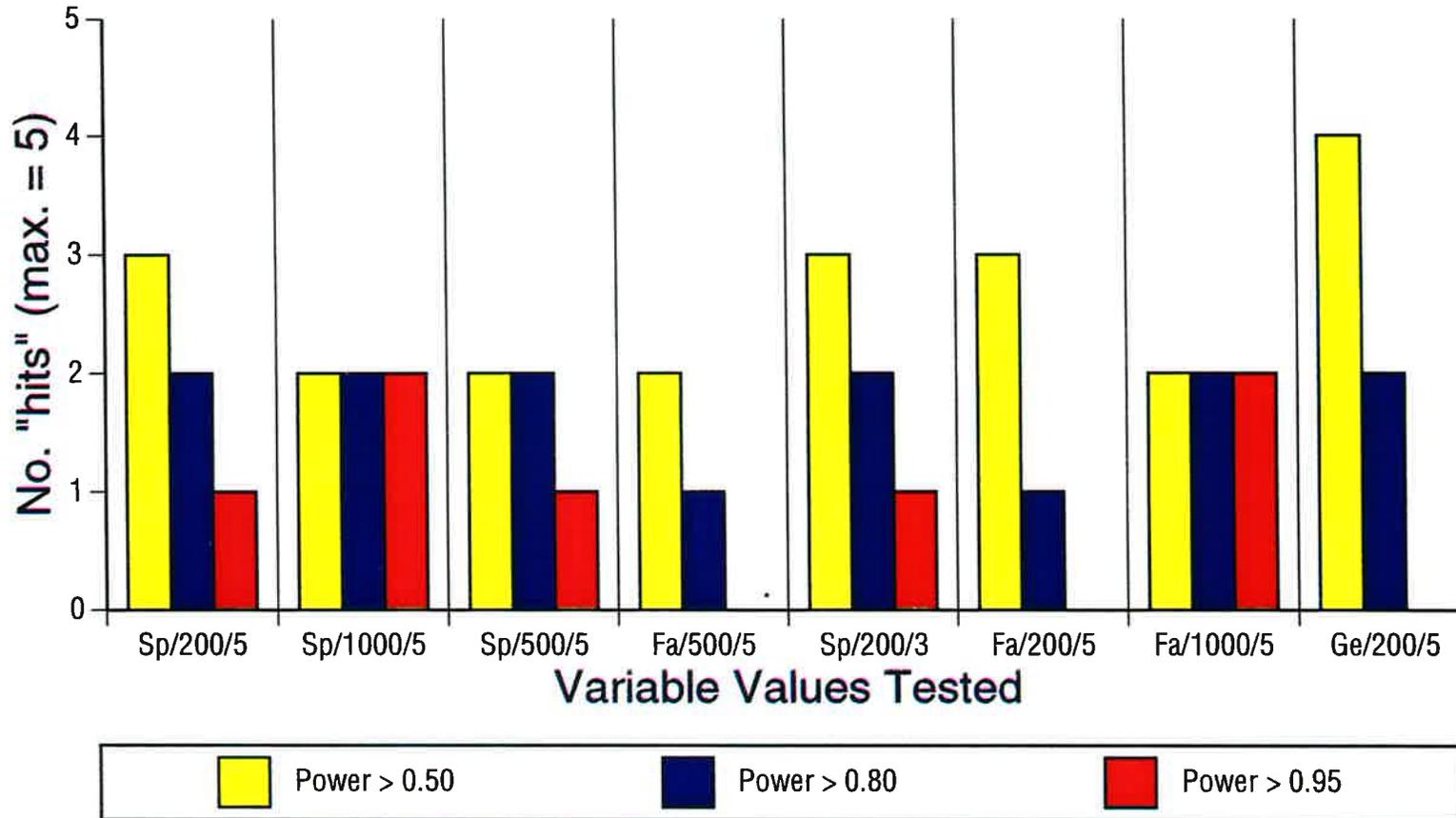


Figure 7.8

CanMet: Testing the statistical power of sampling variables on the ability to distinguish Reference and Far-field Areas.

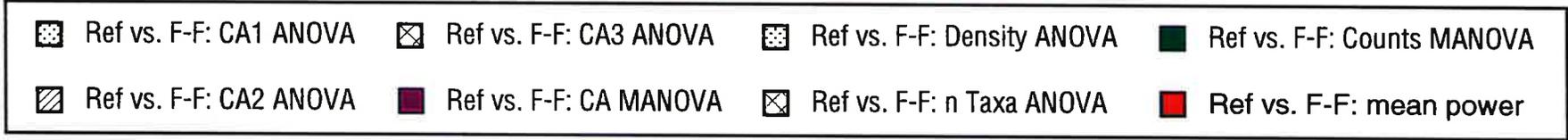
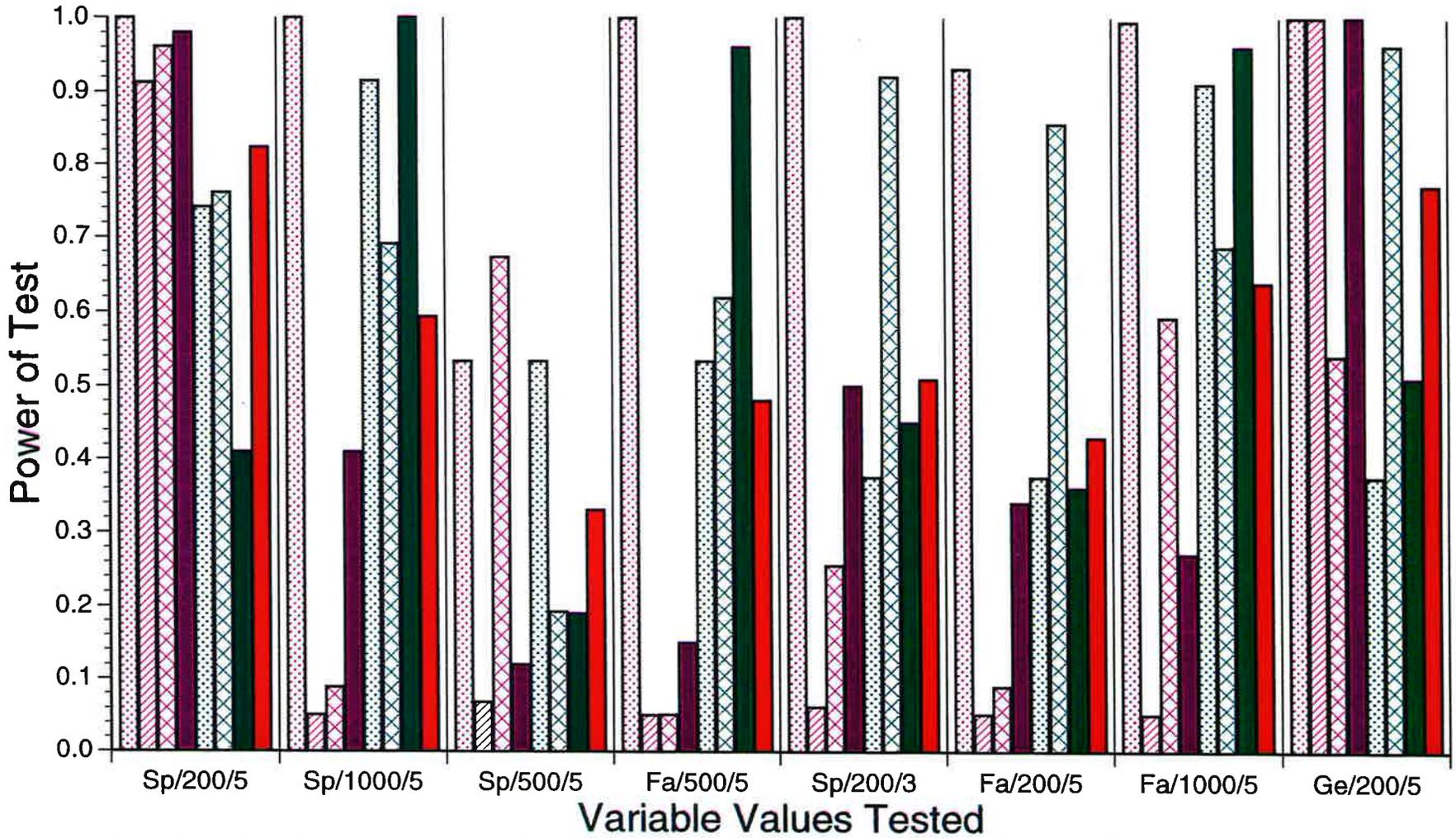
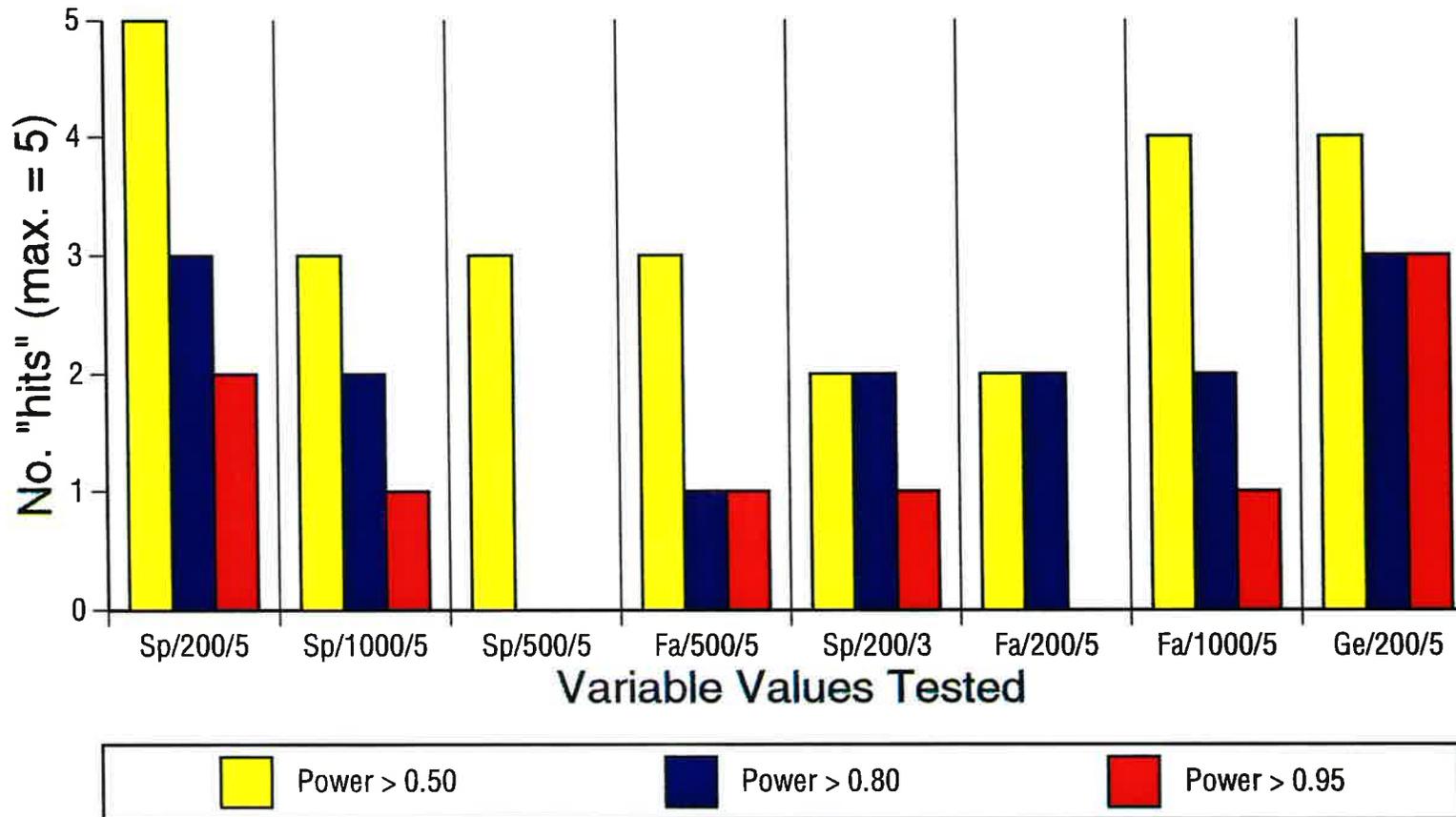


Figure 7.9

CanMet: FREQUENCY OF POWER VALUES EXCEEDING SELECTED LIMITS IN COMPARISON OF REFERENCE AND FAR-FIELD AREAS.



for distinguishing area differences at any power level; 500 μm and 1000 μm mesh have lower and similar ability at power = 0.5, but the 500 μm mesh sieve is not as reliable owing to fewer hits at higher power levels.

Summary

The 200 μm mesh sieve collects more individual organisms and more taxa than either the 500 μm or 1000 μm mesh sieves. Although more unidentifiable immature and earlier instar organisms are collected with the finest mesh, the ability to distinguish between reference and near-field, and between reference and far-field areas is generally still the best with the 200 μm mesh sieve.

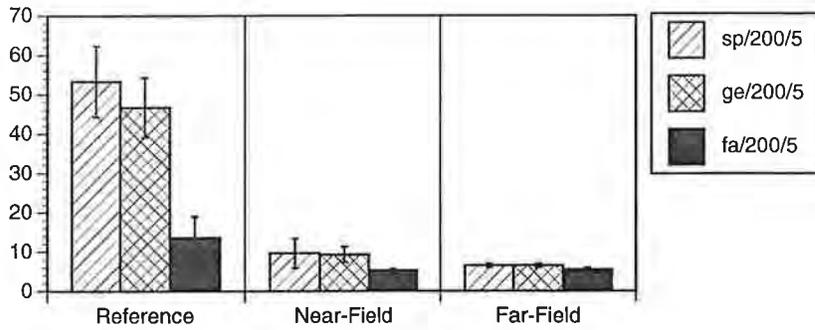
Level of Taxonomic Identification

High levels of taxonomic precision, such as identification to the species level, requires considerable expertise and more time resources than are necessary for genus or family-level identification. Even with highly experienced personnel, substantial numbers of organisms (usually immatures, early instars and groups which cannot be identified to species while in the aquatic phase of their life cycle) may not be identifiable at the species level, and immatures of several taxa may have to be combined as a new taxon identified only to genus or to family. Obviously, with the greater effort at the species level, more taxa are recorded in each sample, station, or area than are found with lower level taxonomy (Figure 7.10a). Decreasing the level of taxonomic detail to the family level of identification minimizes the difference between areas.

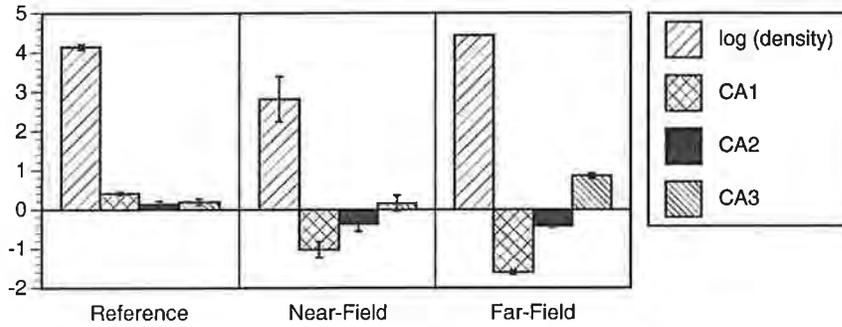
The density of organisms sampled is unaffected by changing the level of taxonomic detail. The pattern of CA indices between reference and effluent-exposure areas (measured by CA1, CA2, and CA3) remains similar over the three levels of identification, although variation between areas appears to be less dramatic with lower taxonomic precision (Figure 7.10).

FIGURE 7.10
CANMET AETE 1995: MEAN VALUES FOR BENTHIC INDICES IN REFERENCE AND EFFLUENT
EXPOSURE AREAS, AND THE EFFECT OF DIFFERENT TAXONOMY LEVELS ON INDEX VALUES.

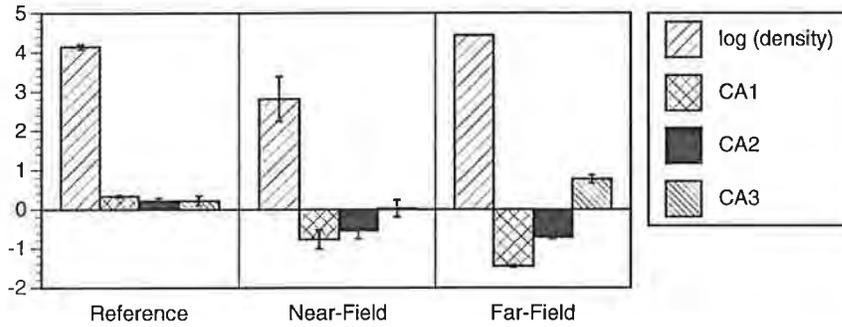
a) Number of Taxa/Area at three levels of taxonomy



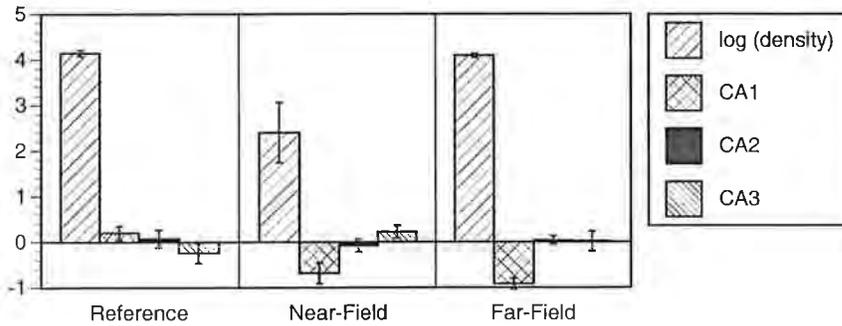
b) Species taxonomy; 200- μ m mesh; 5 replicates



c) Genus taxonomy; 200- μ m mesh; 5 replicates



d) Family taxonomy; 200- μ m mesh; 5 replicates



Detection of Impacts

The influence of different levels of taxonomic detail on the ability to detect area differences was examined in the same manner as was used for comparisons of mesh sizes. Individual power values for comparisons of benthic indices between reference and near-field areas are illustrated in Figure 7.6, and a summary of the values exceeding selected power values ("hits") is given in Figure 7.7 for species, genus, and family level taxonomy using five replicate samples taken with a 200 μm mesh sieve.

The comparison of reference and near-field areas showed similar results for species and genus-level taxonomy. Both levels of taxonomy had similar mean power for the indices tested (Figure 7.6). Genus and family taxonomy resulted in fewer occurrences of high power tests, but were similar to species taxonomy at lower powers. Only species and family were compared at other mesh sizes, and here these radically different levels of taxonomy showed substantial similarity at the 500 μm and 1000 μm mesh sizes.

Reference and far-field comparisons of benthic indices suggested that species-level taxonomy gave substantially greater power than genus or family taxonomy in the detection of area differences (Figures 7.8 and 7.9), although again there was less difference apparent between species and family taxonomy in comparisons of areas using coarser mesh sizes.

Summary

Although individually, species-level taxonomy and 200 μm mesh sieves are generally more powerful at detecting differences between areas, the two variables are not independent. At fine mesh sizes, species taxonomy is more powerful than family, and may be slightly more powerful than genus-level identification. Using 500 μm or 1000 μm mesh, these differences in taxonomic precision are not so apparent.

Best Combinations of Sieve Size and Taxonomic Level

Reference vs. Near-Field

In this comparison, genus-level taxonomy and 200 μm mesh sieves most reliably distinguished between areas. Of the five benthic indices tested, number of taxa, density, and CA1 and CA2 had high power in detecting significant differences between areas. The more detailed taxonomy, at species-level, was almost as effective at this mesh size. A much less intensive method: family/1000 μm /5 replicates, also distinguished adequately between areas in this study (Figure 7.6), based upon number of taxa and CA1 station scores. This is not surprising given the severity of the impacts in the near-field. In situations where impacts on benthic invertebrate communities are more subtle family level taxonomy and 1000 μm mesh size may not be able to distinguish the differences in community structure.

In summary, power is uniformly high for CA1 in all combinations tested, indicating that the communities in these areas are significantly different. It is not surprising that low-precision methods can detect such large differences in benthic communities when impacts are severe. Number of taxa also has high power in all combinations, but density does not have high power to distinguish between areas except at fine mesh sizes.

Reference vs. Far-Field

The combination of species taxonomy, 200 μm mesh, and 5 replicates had the highest overall power in detecting differences between the reference and far-field areas. All three CA scores as well as density and number of taxa had high power. Genus level taxonomy also was effective at the 200 μm mesh. As in the preceding comparison, CA1 always had high power, and number of taxa was reliable as well. Other indices (CA2, CA3, density) varied with individual combinations of taxonomy, mesh size, and number of replicates.

Overall, any combination of the techniques tested was able to reliably distinguish between areas based upon CA1 scores, but only more intensive methods (lower taxonomic level and smaller mesh size) detected differences in the other four indices.

7.3 Ponar Grabs Versus Artificial Substrates

The results of the full factorial ANOVA indicated that there were significant differences ($p=0.008$) between the methods when estimating numbers of taxa. Numbers of taxa tended to be greater using the artificial substrate samplers than when using the Ponar sampler in each area.

Results of the Ponar samples indicated no significant differences in total abundance, numbers of taxa, diversity measures and EPT (Ephemeroptera, Plecoptera, Trichoptera) Index ($p > 0.05$) between the reference and exposure areas, suggesting no effect of the Mine Doyon discharge on the benthic invertebrate community in rivière Bousquet. However a comparison of the community structure suggested that there are substantial differences between the two areas which tends to suggest a slight impact.

In the Ponar samples, the changes from the reference to the exposure area were primarily observed as an increase in the relative abundance of pollution-tolerant organisms, such as tubificid oligochaetes (*Aulodrilus limnobioides* and immatures with hair chaetae), ostracods, and clams. Conversely, decreases in the pollution-sensitive organisms, such as the mayfly *Hexagenia* and the chironomids (in particular those belonging to the metal pollution-sensitive sub-family Tanytarsini) occurred in the exposure area relative to the reference area.

Results of the artificial substrate samples indicated no significant differences in total abundance, numbers of taxa, diversity measures and EPT index ($p > 0.05$) between the reference and exposure areas. A comparison of the percent composition of the major taxonomic groups indicated no substantial differences between the two areas. However, the generic composition of two groups, the mayflies and chironomids, was altered substantially between the two areas. Mayfly composition indicated a shift to increased abundances of the tolerant species - *Eurylophella temporalis*, together with lower abundances of the relatively less tolerant taxa *Stenacron interpunctatum* and *Leptophlebia* in the exposure zone. Chironomids also indicated a similar change, with increases in *Microtendipes pedellus*, *Nanocladius* and *Helopelopia*, three relatively pollution-tolerant taxa, coincident with decreases in the abundance of *Micropsectra*, a relatively sensitive taxa, in the exposure zone. There were no Tanytarsini present in either area using the artificial substrates.

In general, changes in the benthic macroinvertebrate community which a benthic ecologist would generally attribute to mining impacts were slightly more obvious in the Ponar samples than in the artificial substrates, likely reflecting the additional influence of sediment quality on the Ponar collected community. The artificial substrates are only affected by water quality which was found not to change substantially between the reference and exposed areas. The data support the general consensus in the scientific community that it is better to sample the community colonizing the natural substrate, if possible and to only use artificial substrates in situations where samples cannot be collected from the natural substrate or when sediment quality effects are not an issue.

All of these results were based on the results derived from the 200 μm mesh which was found with the rivière Noire samples to be the most sensitive in detecting differences between reference and exposure areas. In addition, since the 200 μm mesh size revealed few differences between the two communities, it is unlikely that these small-scale changes would be detected by coarser sampling and processing methods.

7.4 Relationships Among Sediment Chemistry, Toxicity and Benthic Invertebrate Community Structure

7.4.1 *Comparability of Toxicity Tests with Sediment and Water Chemistry and Benthic Communities*

Sediment from Station 6 in the mouth of ruisseau Dormenan appeared to be the most toxic producing complete mortality in *Hyalella*, 83% inhibition of *Tubifex* reproduction and 95% inhibition of luminescence in Microtox[®]. These data correspond to a severely impacted benthic invertebrate community found at this site. Chemical analyses of sediments indicate this station contained the highest concentrations of copper and zinc compared to the other stations, however, the levels were below the Environment Canada Probable Effects Level (PEL) but exceeded the Threshold Effects Level (TEL). Water quality at this site exceeded the CCREM guidelines for the protection of aquatic life for ammonia, copper, iron, selenium and zinc. The highest concentrations of these parameters, with the exception of iron, were found at this site.

There was a considerable impairment of *Tubifex* cocoon production (60%), greater inhibition of young production (99%), and slight impairment of *Hyalella* growth at Station 4 in rivière Noire. The next station, downstream Station 5, showed improved quality through recovery of *Hyalella* growth, and *Tubifex* young production to reference levels. Benthic invertebrate data showed an opposite trend where the number of taxa and density were notably higher at Station 4 than Station 5. Also at Station 4 there were approximately 1,000 *Tubifex tubifex*/m² which is in contrast to the 99% inhibition of young production determined with toxicity tests. Microtox[®] results also showed the opposite trend where sediments at Station 5 were more toxic than at Station 4. There were no obvious changes in sediment or water chemistry between the two sites.

The environmental stress exhibited by, Microtox[®], oligochaete production and *Hyalella* mortality at Stations 7 and 8 is supported by the low number of taxa and lower density of all organisms and tubificids. Sediment and water concentrations of arsenic were also elevated at these sites and consistent with those at Stations 4 and 5. Copper levels in the sediments were higher than at Stations 4 and 5. Ammonia concentrations in water were also notably higher and well above the CCREM guideline for the protection of aquatic life at Stations 7 and 8 compared with Stations 4 and 5. This helps to explain the more impacted benthic community at Stations 7 and 8.

Stations 9 and 10 which exhibited high toxicity with *Hyalella* showed lower numbers of taxa but densities were at reference levels. Tubificids showed no toxic response, and *Tubifex* densities at these stations were the highest in the study area. Microtox[®] IC50s were similar to reference Station 19. Again, arsenic was at the same concentration as at upstream stations. The *Hyallela* response more accurately reflected the response of the benthic invertebrate community.

Only *Tubifex* young production appeared to exhibit inhibition when exposed to lac Preissac samples from Stations 11 and 12 which are likely depositional areas contaminated from sediment losses from the rivière Noire system. Microtox[®] IC50s when compared to reference Stations 13 and 14 also suggested that there may be environmental stress in this area of lac Preissac. The number of taxa at Stations 11 and 12 were notably lower than at reference Stations 13 and 14. Stations 11 and 12 contained the highest concentrations of

arsenic, chromium and copper. Station 12 was particularly contaminated and had the highest concentrations of all metals. However, the benthic invertebrate community at this site appeared to be more healthy than at Station 11 (Section 7). There was no difference in water quality between Stations 11 and 12 with the exception of ammonia which exceeded the CCREM guideline for the protection of aquatic life at Station 11.

The Microtox® test was capable of identifying the most toxic sediment sample at Station 6 and identified the next stations downstream, Stations 7 and 8, as being somewhat less toxic. The Microtox® test showed little or no inhibition at the other stations.

Toxicity endpoints also showed correlation with sediment chemistry variables. In analyses of full metal extraction methods, the Microtox® test results were negatively correlated with chromium concentration (-0.713; $p = 0.021$). *Hyalella* mortality was positively correlated with copper concentration (0.688; $p = 0.028$). Partial metal extractions showed a significant negative correlation between copper concentration and Tubifex reproduction (-0.702; $p = 0.024$), and positive correlations between *Hyalella* mortality and both zinc concentration (0.762; $p = 0.010$). These results are not unexpected, given the known effects of heavy metals on invertebrates; however, the results indicating relationships between toxicity endpoints and sediment metal concentrations should be viewed with caution since sample sizes are small and because of the number of correlations performed.

In the most detailed benthic data set (200 μm , species level) number of taxa showed a significant, negative correlation with *Hyalella* percent mortality and a significant positive correlation with the Microtox® test, suggesting that low numbers of taxa may be explained by toxicity of sediments to invertebrates or general toxicity to metabolic pathways (Table 7.4). The results of the Tubifex reproduction test also were positively correlated with CA3 station scores, which may indicate that stations with high CA3 scores (Stations 9 and 10: the far-field Area) have conditions suitable for Tubifex reproduction. Examination of the raw data shows that tubificids and immatures with hair chaetae are more common at these stations than at any other river station. *Tubifex tubifex* also is a strong positive contributor to CA axis 3 (Table 7.1).

The least detailed benthic data set (1000 μm , family level) also shows significant negative

**TABLE 7.4: COMPARISON OF MOST DETAILED AND LEAST DETAILED BIOLOGICAL SAMPLING METHODS:
PEARSON CORRELATION^a OF SEDIMENT TOXICITY ENDPOINTS TO BIOLOGICAL COMMUNITY VARIABLES**

Toxicity Test	Most Detailed: species / 200 µm / 5 replicates					Least Detailed: family / 1000 µm / 5 replicates				
	No. Taxa	Density	CA1	CA2	CA3	No. Taxa	Density	CA1	CA2	CA3
Tubifex reproduction test	-	-	-	-	0.755	-	-	-	-	-
Microtox test	0.639	-	-	-	-	0.766	-	-	-	-
Hyallolela % mortality test	-0.846	-	-	-	-	-0.682	-	-0.766	-	-

^a Only significant relationships are shown.

correlations of *Hyalella* percent mortality with number of taxa and CA1 scores and significant positive correlation with the Microtox® test.

7.4.2 Benthic Indices and Sediment Chemistry

Relationship to Level of Benthic Detail

Full Extraction

Gravel/coarse material was significantly positively correlated with number of taxa, while copper, zinc, arsenic, silt and nickel were all significantly negatively correlated with benthic indices (either CA1 or CA2) from the detailed benthic data set (species/200 μm /5 replicates) (Table 7.5). The least detailed benthic data set (family/1000 μm /5 replicates) had significant negative correlations between number of taxa and arsenic and nickel concentration, and between CA1 and arsenic, copper, and nickel concentration. These individual correlation could be spurious if not supported by other evidence, because of the large number of possible correlations between all metals and all benthic indices.

Accordingly, correlation of PCA station scores and benthic indices also was examined (Table 7.5). PC2, dominated by the strong positive contributions of TOC, gravel/coarse material and clay, and the negative contributions of arsenic and silt, was significantly correlated with the CA1 scores from the detailed benthic data set. This indicates that high levels of arsenic in sediments are associated with high abundance of the benthic taxa important in the CA, namely *Tubifex tubifex*, immature tubificids with hair chaetae, and several other taxa (refer to Table 7.1, Section 7.0). This correlation also was apparent with number of taxa from the least detailed benthic matrix (family/1000 μm /5 replicates).

Metal concentrations TOC and grain size, summarized as PC scores, also had significant explanatory power in regression analysis with benthic indices. In the detailed benthic data set, PC2 (arsenic, TOC, gravel, clay) was related to a decline in the number of taxa and changes in the composition of the benthic community (CA1). PC3 also had a relationship with CA2 (Table 7.5). The least detailed benthic data set showed no relationships with the metal data set in regression analyses.

TABLE 7.5: COMPARISON OF MOST DETAILED AND LEAST DETAILED BIOLOGICAL SAMPLING METHODS: RELATIONSHIPS^a OF SEDIMENT METAL VARIABLES (FULL EXTRACTION METHOD) TO BIOLOGICAL COMMUNITY VARIABLES BY PEARSON CORRELATION AND BY MULTIPLE REGRESSION.

	Most Detailed: species / 200 µm / 5 replicates					Least Detailed: family / 1000 µm / 5 replicates				
	No. Taxa	Density	CA1	CA2	CA3	No. Tax	Density	CA1	CA2	CA3
Correlation of Individual Metals^b										
arsenic	-	-	-0.801	-	-	-0.758	-	-0.704	-	-
cadmium	-	-	-	-	-	-	-	-	-	-
chromium	-	-	-	-	-	-	-	-	-	-
copper	-	-	-	-0.909	-	-	-	-0.692	-	-
lead	-	-	-	-	-	-	-	-	-	-
nickel	-	-	-0.677	-	-	-0.675	-	-0.670	-	-
zinc	-	-	-	-0.806	-	-	-	-	-	-
TOC	-	-	-	-	-	-	-	-	-	-
Gravel	0.749	-	-	-	-	-	-	-	-	-
Sand	-	-	-	-	-	-	-	-	-	-
Silt	-	-	-0.762	-	-	-	-	-	-	-
Clay	-	-	-	-	-	-	-	-	-	-
Correlation of PCA station scores										
PC1 (cobalt, lithium, rubinium, titanium, thallium, chromium, nickel, strontium, aluminum, beryllium, calcium, thorium)	-	-	-	-	-	-	-	-	-	-
PC2 (TOC, gravel, clay, arsenic, antimony, mercury, potassium, silt)	0.664	-	0.743	-	-	-	-	-	-	-
PC3 (sand, molybdenum, copper, lead)	-	-	-	0.815	-	-	-	-	-	-
Regr. of PCA station scores: p-value and (sign of regr. coeff.)										
PC1 (cobalt, lithium, rubinium, titanium, thallium, chromium, nickel, strontium, aluminum, beryllium, calcium, thorium)	-	-	-	-	-	-	-	-	-	-
PC2 (TOC, gravel, clay, arsenic, antimony, mercury, potassium, silt)	0.036 (+)	-	0.014 (+)	-	-	-	-	-	-	-
PC3 (sand, molybdenum, copper, lead)	-	-	-	0.004 (+)	-	-	-	-	-	-
PC1, PC2, PC3	0.023	-	0.015	0.002	-	-	-	-	-	-

^a Only significant relationships are shown.

^b Individual correlations may be suspect unless supported by PC score correlations and by F statistics from regressions

In summary, the station groups identified from the benthic indices (reference, near-field, and far-field), also tended to be distinguishable on the basis of their sediment TOC, grain size and metal concentrations. Indices from the detailed benthic data set (species/200 $\mu\text{m}/5$ replicates) were more consistently related to the sediment metal chemistry by correlation and regression than were the indices from the least detailed benthic data set (family/1,000 $\mu\text{m}/5$ replicates).

Partial Extraction

Arsenic, cadmium, chromium, lead, nickel, zinc, gravel and silt concentrations from the partial extractions of metals were significantly related to indices from the detailed benthic data set (number of taxa, CA1, and CA2). In the least detailed benthic data set, more metals were correlated with number of taxa, but chromium was not correlated with CA1 and no metals were correlated with CA2 (Table 7.6).

Summaries of the TOC, grain size and metal variation with PCA were more easily interpreted. PC2 (nickel, arsenic, lead) was significantly correlated with CA1 scores, for both the most and least detailed benthic data sets and with number of taxa for the least detailed benthic data set. Additionally, in the most detailed benthic data set, PC3 (zinc) was correlated with CA2 of the benthic data.

Regression analysis revealed that, in the detailed benthic data set, PC2 and PC3 had significant relationships with either CA1 or CA2. The least detailed benthic data set had significant relationships of number of taxa and CA1 to PC2.

Overall, partial metal extractions less reliably characterized the differences between reference and effluent-exposure areas while having stronger relationships with values of the benthic indices.

Relationships of TOC, grain size and metal concentrations and related indices were stronger with benthic indices calculated on detailed benthic data (species/200 $\mu\text{m}/5$ replicates) than on the least detailed benthic data set (family/1000 $\mu\text{m}/5$ replicates).

TABLE 7.6: COMPARISON OF MOST DETAILED AND LEAST DETAILED BIOLOGICAL SAMPLING METHODS: RELATIONSHIPS^a OF SEDIMENT METAL VARIABLES (PARTIAL EXTRACTION METHOD) TO BIOLOGICAL COMMUNITY VARIABLES BY PEARSON CORRELATION AND BY MULTIPLE REGRESSION.

	Most Detailed: species / 200 µm / 5 replicates					Least Detailed: family / 1000 µm / 5 replicates				
	No. Taxa	Density	CA1	CA2	CA3	No. Taxa	Density	CA1	CA2	CA3
Correlation of Individual Metals^b										
arsenic	-0.637	-	-0.807	-	-	-0.761	-	-0.708	-	-
cadmium	-	-	-0.653	-	-	-	-	-0.769	-	-
chromium	-	-	-0.665	-	-	-	-	-	-	-
copper	-	-	-	-	-	-	-	-	-	-
lead	-	-	-0.900	-	-	-0.890	-	-0.876	-	-
nickel	-	-	-0.913	-	-	-0.843	-	-0.817	-	-
zinc	-	-	-	-0.791	-	-0.713	-	-0.792	-	-
TOC	-	-	-	-	-	-	-	-	-	-
Gravel	0.749	-	-	-	-	-	-	-	-	-
Sand	-	-	-	-	-	-	-	-	-	-
Silt	-	-	-0.762	-	-	-	-	-	-	-
Clay	-	-	-	-	-	-	-	-	-	-
Correlation of PCA station scores										
PC1 (clay, TOC, aluminum, lithium, gravel)	-	-	-	-	-	-	-	-	-	-
PC2 (iron, cobalt, thorium, lead, nickel, cadmium, arsenic, uranium)	-0.632	-	-0.679	-	-	-0.634	-	-0.742	-	-
PC3 (zinc)	-	-	-	0.916	-	-	-	-	-	-
Regr. of PCA station scores: p-value and (sign of regr. coeff.)										
PC1 (clay, TOC, gravel)	-	-	-	-	-	-	-	-	-	-
PC2 (iron, cobalt, thorium, lead, nickel, cadmium, arsenic, uranium)	-	-	0.031 (-)	-	-	0.049 (-)	-	0.022 (-)	-	-
PC3 (zinc)	-	-	-	0.0002 (+)	-	-	-	-	-	-
PC1, PC2, PC3	0.005	-	0.027	0.002	-	-	-	-	-	-

^a Only significant relationships are shown.

^b Individual correlations may be suspect unless supported by PC score correlations and by F statistics from regressions

It is uncertain whether the partial extractions more accurately reflect bioavailability than the total fractions, although there is some evidence, based on correlation with benthic community indices with total and partial metal fractions, that partial metal fractions explain more of the variation among benthic communities than do the total metals.

7.5 Relative Costs of Benthic Sampling Techniques

Table 7.7 summarizes the laboratory costs of sample processing for benthic invertebrate samples. The sample processing costs are based on the minimum QA/QC requirements for environmental effects monitoring programs conducted under the *Fisheries Act*. (i.e., sorting with a microscope at a magnification of at least 10 times, 20 times for 200 μm , minimum of 95% recovery of benthic invertebrates from the sample, 25% minimum subsample size, taxonomy completed by highly qualified individuals and other associated QA/QC). Costs for field sampling are independent of the costs in this table. Artificial substrates have comparable processing costs, but substantial added costs may be incurred because of the requirement of an additional field trip to retrieve the substrates.

As expected, samples processed through 200 μm mesh and identified to species level are the most expensive, with an estimated median cost of \$295. The methods used in the least detailed data set (family taxonomy, 1000 μm mesh) are the least costly at an estimated median of \$75. The "industry standard" method used for the current Environment Canada EEM programs (genus taxonomy which is basically the same as "lowest practical level" and 500 μm mesh) has a median cost of \$175.

7.6 Evaluation of Benthic Sampling and Analytical Techniques

Conclusions from the AETE 1995 Pilot Study

- Unpooled replicate samples allow for characterization of variation of stations within groups, but had little additional value in the present study.
- Replicate samples (pooled or unpooled) add information and precision to estimates of benthic indices.
- Taxonomic identifications are influenced by sieve size.

TABLE 7.7: COST FOR BENTHIC SAMPLE ANALYSIS

Taxonomic Detail	Sieve Size (μm)		
	200	500	1000
Species	\$260 to \$330	\$180 to \$250	\$160 to \$230
Genus	\$230 to \$280	\$150 to \$200	\$110 to \$160
Family	\$170 to \$220	\$90 to \$140	\$50 to 100

Note: Costs will vary from laboratory to laboratory.

Assumption: Samples sorted by microscope with a 95% sorting recovery efficiency.

- 200 μm mesh sieves distinguished area differences most reliably.
- Species or genus-level taxonomy distinguished area differences most reliably.
- Where severe impacts on the benthic community exist, family-level taxonomy and 1,000 μm mesh sieves may adequately detect differences between reference and effluent-exposure areas. However, they will be less able to distinguish slight to moderate impacts.
- Ponar-grab samples appeared more likely to detect differences between benthic communities than were suspended artificial substrates, probably because of exposure to sediment contaminants. Artificial substrates were exposed to water quality differences only.
- Costs of artificial substrate sampling programs may be considerably higher than for other methods because of the additional trip required for retrieval of substrates.
- Laboratory costs for the least detailed benthic techniques (family/1000 μm) are approximately 25% of the cost of the most detailed technique (species/200 μm).
- Partial extraction of sediment metals showed more reliable separation of reference and impact areas and was better related to benthic indices than was the case for full (total) sediment metal extraction.
- Detailed benthic invertebrate data (e.g., species level taxonomy and 200 μm mesh sieves) showed stronger relationships with sediment chemistry than coarse benthic data (e.g., family level taxonomy and 1000 μm mesh sieves).
- Agreement between benthic indices and toxicity endpoints was most notable for *Hyaella* mortality and *Tubifex* reproduction tests, with either detailed or coarse benthic data sets.

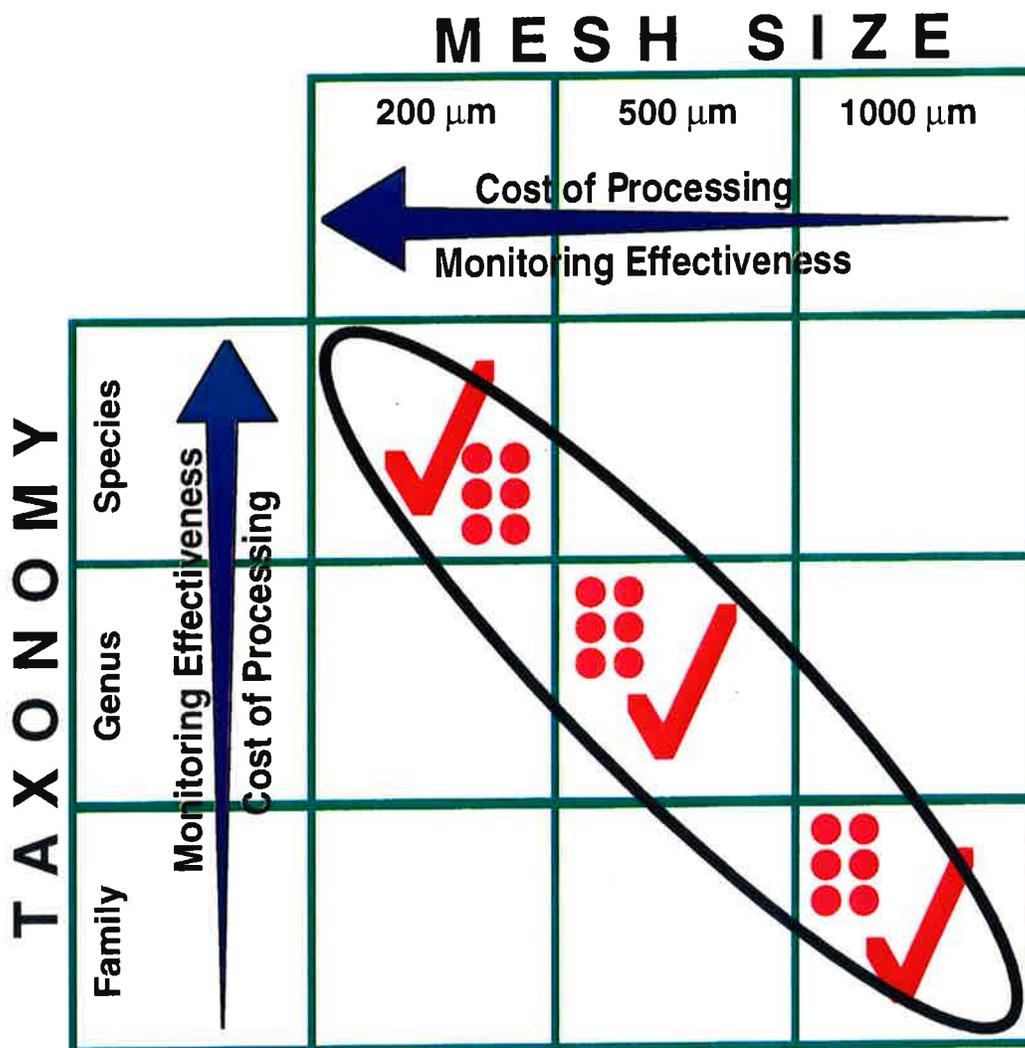
Considerations for Future Studies

- Detection of severe impacts is possible with the least costly, least detailed benthic techniques; however, slightly to moderately impacted areas, as well as moderate improvements in benthic communities as a result of improvement in effluent treatment may not be detected without more detailed processing

and identification.

- Comparison of an initial monitoring survey using one combination of benthic techniques or level of detail, with later surveys using other methods, will be problematic. Therefore, since the main purpose of an EEM program is to monitor temporal and spatial changes in the health of biological communities then it is advisable to start the monitoring program with more a detailed level of processing and identification.
- If samples were sieved in the field using 200 μm mesh, the fine fraction could be separated by further sieving with coarser mesh and saved for more detailed processing if coarser and cheaper processing methods were unable to resolve monitoring issues.
- Further study of the value of replicate sampling at a station may be in order, as both pooled and unpooled replicate Ponar grabs may add information and/or statistical power to the analyses used in monitoring programs.
- Testing of all possible combinations of taxonomic level identification and sieve size at a single location is unlikely to yield a method applicable to all field sites. It may be more useful to test several promising combinations from the cost/effectiveness continuum at other field sites (see Figure 7.11).
- If new field sites are chosen for testing benthic methods, it is advisable to select sites where there is a well-defined gradient of impact from a severely impacted area to areas that are moderately and slightly impacted so that the sensitivity of the methods can be adequately assessed.

**FIGURE 7.11
POSSIBLE SELECTION OF BENTHIC SAMPLING
TECHNIQUES FOR EVALUATION IN FUTURE STUDIES.**



REPRESENTS REPLICATE SAMPLES AT EACH STATION

8.0 FISH SURVEY

The fish survey was carried out to evaluate various methods for measuring mining-related fish community impacts. The survey included the collection of information on the general health, condition and reproductive status of fish from various environments, and the collection of information on metal bioaccumulation in various tissues and metallothionein (MT) responses in those tissues. Also, samples of various tissues were collected for histopathological analysis. Preliminary sampling and analysis of fish tissues were carried out during the September reconnaissance program in order to evaluate the availability of various fish species and to identify general responses in terms of tissue metals and MT.

8.1 Methods

8.1.1 Preliminary Reconnaissance

The fish community was sampled in upstream (reference) and downstream (exposure) areas of rivière Bousquet, with sampling sites located immediately downstream of lac Bousquet, downstream of the rapids and the Mine Doyon effluent discharge, and downstream of the Complexe Bousquet discharge (see Figure 8.1 for fish collection areas). Experimental gillnets (46 m long x 1.5 m deep, mesh sizes 4 cm to 13 cm) were used at each location to identify the species present, relative catch-per-unit-effort between areas and to obtain tissues to determine suitability for metallothionein/histopathology assessments. Seining was also carried out at the first two locations noted above to assess the availability of forage fish and young-of-the-year sport fish and small forage species.

Preliminary samples of three large fish species (silver redhorse, white sucker, walleye) and two small fish (young-of-the-year northern pike, young-of-the-year brown bullhead) were collected from reference and exposure areas, and samples were dissected and processed for metal analysis and metallothionein analysis based on the procedures outlined in Appendix E. Samples for metallothionein analysis were frozen with dry ice immediately after dissection. The numbers and types of samples submitted for metal and metallothionein analysis are as follows:

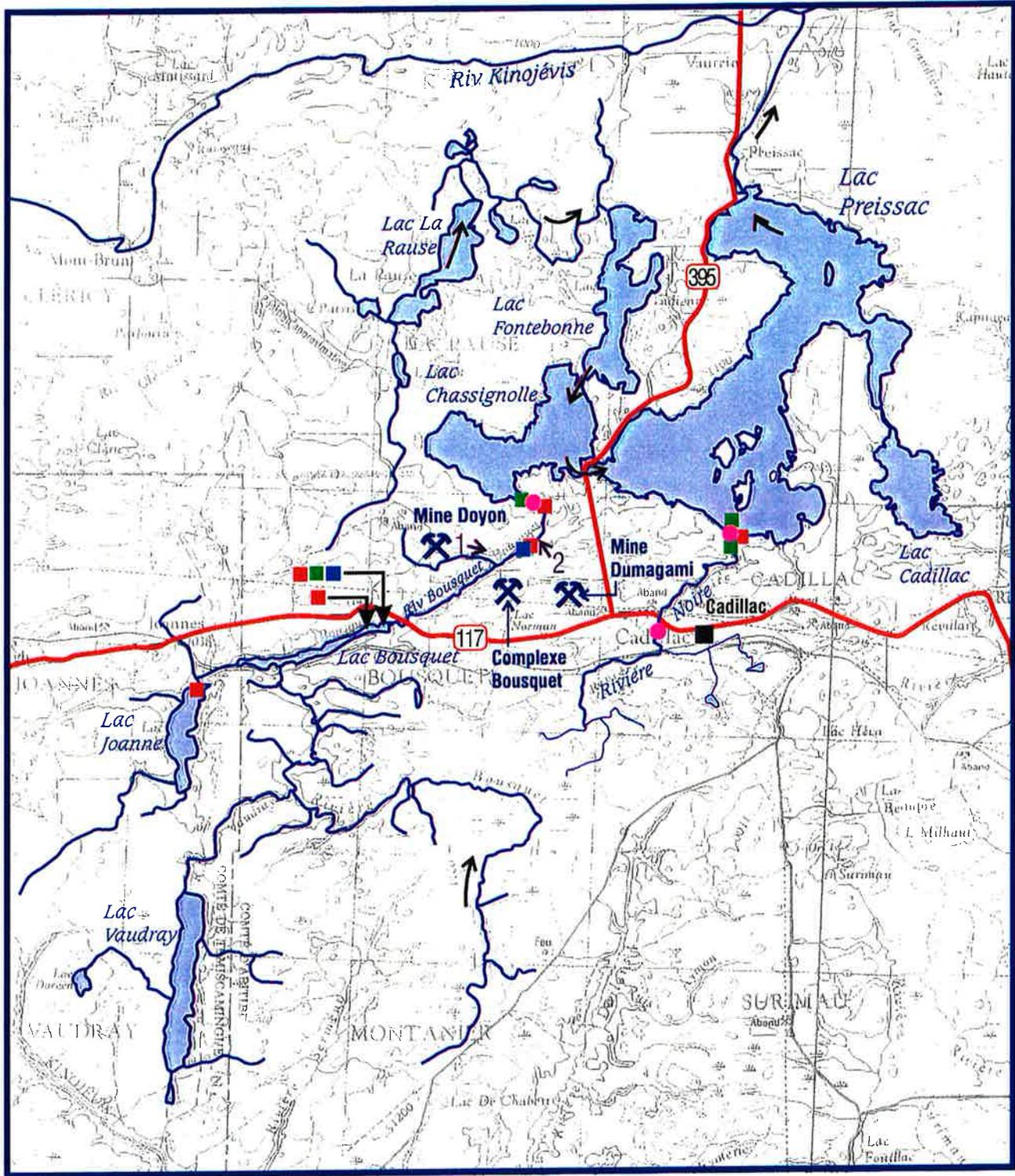


FIGURE 8.1
Fish Collection Areas-
September and October 1995



↗ Treated Effluent Discharges

- Seine Nets- October
- Electrofishing- October
- Gill Nets- October
- Gill Nets/Seine Nets- September
- Gill Nets- September

TABLE 8.1: NUMBER OF FISH CAUGHT AND TISSUE TYPES COLLECTED DURING THE PRELIMINARY FIELD SURVEY.

Area	Species	No. of Fish	Tissues Types			
			Gill	Liver	Kidney	Gut (young-of-the-year)
Reference	Pike	1				X
	Pike	1				X
	Brown bullhead	3				X
	Brown bullhead	3				X
	Brown bullhead	3				X
	White sucker	1	X	X	X	
	White sucker	1	X	X	X	
	Walleye	1	X	X	-	
	Walleye	1	X	X	X	
	Silver redhorse	1	X	X	X	
Exposure (both sites combined)	Northern Pike	1				X
	Northern Pike	1				X
	Brown bullhead	3				X
	Brown bullhead	3				X
	Brown bullhead	3				X
	White sucker	1	X	X	X	
	White sucker	1	X	X	X	
	Walleye	1	X	X	X	
	Walleye	1	X	X	X	
	Silver redhorse	1	X	X	X	

Fish were readily captured by experimental gill net (larger fish) and seine (young-of-the-year fish, juvenile yellow perch) both upstream and downstream of the mine effluent discharge points in rivière Bousquet. Common species captured by gill net included walleye, sauger, silver redhorse, white sucker, yellow perch and northern pike. Species most readily captured in good numbers by seine net were young-of-the-year northern pike and brown bullhead. Juvenile perch were present in lower numbers in seine hauls.

No fishing was carried out in lac Chassignolle or in lac Bousquet, due to restrictions imposed on BEAK's experimental fishing permit. However, the dominant species present were expected to be the same as those found in the river.

Based on this survey, it was concluded that capture of sufficient numbers of large and small fish for histology, metallothionein and metal analysis would likely be possible. Small fish captured by seine could be readily kept viable for several hours prior to processing (all species). Among the large fish, species remaining viable for the greatest period post-netting

included silver redhorse, followed by white sucker and walleye, with northern pike showing reduced survival.

Based on the results of the preliminary survey, no differences in tissue metal concentrations were apparent between reference and exposure areas in the rivière Bousquet system (Section 8.2.1). Accordingly, it was concluded by the AETE Technical Committee based on recommendations made by BEAK, that a portion of the fish survey component of this study be re-allocated to the nearby rivière Noire system, where greater biological impacts were expected and where the benthic invertebrate, sediment chemistry and sediment toxicity surveys were being conducted.

8.1.2 Main Survey

The main survey focused on the capture and analysis of fish from both the rivière Noire and rivière Bousquet systems, with possible collection and analysis of small fish also from lac Joannès, located upstream of lac Bousquet. The fishing effort focused on the use of experimental gill nets, as described in Section 8.1.1, for the capture of large fish, and electrofisher and/or seine for the capture of small fish at various locations (Figure 8.1).

It is sometimes difficult to collect a pre-determined number of fish of both sexes within a reasonable time period. Of the time allocated for the field component of the main survey, about 75% was to be allocated for the collection and processing of fish (the equivalent of 12 ten-hour days for two crews of two). If, after the first few days of fishing effort, the field crew found that catches were low and were unable to achieve targeted numbers (for example due to reduced catch-per-unit-effort relative to the preliminary survey or to poor catches in the rivière Noire), consideration would be given to re-allocation of the remaining effort (and analytical allotment) to the collection of greater numbers of species and/or locations for small fish collections, particularly in the rivière Noire watershed where multiple impact sources and contaminant gradients were expected to yield valuable results. Fishing methods used were as described in Section 8.1.1 for the preliminary reconnaissance, with the additional use of electrofishing to sample shallow areas.

Given previous success in capturing adult pike, walleye, sucker and redhorse in the rivière Bousquet, BEAK targeted one bottom-feeding fish and one predator for the Phase II (main)

Study, with the final selection of species dependent upon their relative catchabilities. Final species selection would be made in the field after the first two to three days of fishing effort. In terms of small fish, BEAK proposed collection of the range of species found in relative abundance at all locations sampled.

Sample Processing - Field

Adult fish were measured on a standard measuring board. Total length (TL), fork length (FL), and standard length (SL) were recorded to the nearest millimetre. Small fish (young-of-the-year or forage fish) were measured in the same manner. Only fresh specimens remaining alive after capture were used for metal and metallothionein analyses due to the perishability of metallothionein and histopathology samples. Fish that were dead were used for the standard measurements, such as body weight and length, liver and gonad weight, aging and fecundity.

External and internal abnormalities, such as black spot, tumours, liver discolouration or scarring were noted on the field sheets.

Fish were weighed on Pesola™ scales which were calibrated each day using standardized, brass weights. Absolute precision of recorded weights varied with the weight of the fish. However, the scales were selected to permit a relative precision of 2% of wet weight or better. Scale capacities ranged from 0 to 10 g, 0 to 100 g, 0 to 1 kg, and 0 to 2.5 kg. Weights were accurate to within 0.1 g for the 10 g scale (1%), 0.5 g for the 100 g scales (0.5%), 5 g for the 1 kg scales (0.5%) and to within 50 g for 2.5 kg scales (2%).

For all adult suckers, the first left pectoral spine was removed and cleaned for ageing. Cleithra were collected from northern pike and dorsal spines were collected for walleye. Aging structures from each fish were packaged together, with some scales as back-up aging structures, in a labelled scale envelope.

White sucker livers, which are diffuse in the mesenteries of the digestive tract, were removed by separating the liver tissue from intestine with dissection instruments. Livers in northern pike and walleye are distinct organs and were readily removed by dissection. Obvious external fat deposits and the gall-bladder were removed from liver tissue. Liver

tissue was weighed to the nearest 0.1 g using an Ohaus electronic tare balance, which usually permitted measurement precision within $\pm 1\%$.

Adult fish gonads were removed and weighed on an Ohaus electronic tare balance to the nearest one-tenth of a gram (0.1 g). For females, gonad weight was also measured on a tare balance and gonad volume was measured by placing the entire gonad in a 250 mL graduated cylinder containing a known volume of water and measuring and recording the displacement. A subsample of the ovary was then removed, and its volume measured in the same manner using a 100 mL graduated cylinder. This subsample was placed in a polyethylene (PET) bottle and preserved in 10% formalin for subsequent counting of eggs for fecundity estimates. Each sample was labelled by fish number.

Liver, kidney, gill filaments and skeletal muscle samples were dissected from males and females of each of three large fish species from the three sampling areas (rivière Noire, rivière Bousquet downstream, rivière Bousquet upstream reference). Fish were dissected in the field using stainless steel instruments, frozen and submitted to the analytical laboratory for metal analysis. Tissues were only collected from fish that were alive at the time of collection.

The whole gut of the most abundant small fish species collected from each of four areas was also removed for analysis (rivière Bousquet reference and exposure, rivière Noire, lac Joannès). Small fish were dissected in the field, frozen and submitted to the analytical laboratory for metals determination.

Samples of gill, liver and kidney tissue were collected from the same fish sampled for metal content for determination of tissue metallothionein and for histopathological examination. Samples were processed according to protocols identified by Dr. J. Klaverkamp, Department of Fisheries and Oceans, including preservation in standard histopathological solutions (histopathology) and rapid freezing with dry ice (metallothionein). Whole viscera of small fish were also collected and preserved in the same manner, with separate samples of viscera each prepared for metal and metallothionein analyses and whole fish for histopathology. All histopathology and metallothionein samples were maintained in preserved condition and forwarded to Dr. J. Klaverkamp for analysis.

Biological Measurements

Fin rays were utilized as ageing structures for white suckers and walleye, and cleithra for northern pike. The fin rays were first cleaned of remaining tissue using warm water. The clean bones were then embedded in epoxy resin and allowed to harden for 12 hours. Using an isomet saw, sections (300 to 400 μm thick) of the embedded bone were cut perpendicular to the bone and close to the base. At least two of the sections were mounted in glycerine and aged by counting annular rings. The sections were subsequently stored in labelled micro-centrifuge vials.

Egg subsamples collected in the field were gently rinsed into a 180 μm sieve to remove preservative. The volume of egg mass, which was initially determined in the field, was again measured by volume displacement in a 50 mL burette as the volume of preserved eggs was usually substantially higher than that of unpreserved eggs, due to subsequent fluid uptake.

The displacement volumes of three further laboratory subsamples of the egg mass were measured with a 10 mL burette and the number of eggs within each of the three new subsamples were counted. All eggs were then re-preserved and stored.

The volumes and the number of eggs in laboratory subsamples were then used to estimate the number of eggs in the original subsample collected in the field. Fecundity was estimated volumetrically by using the total volume of the ovary (measured in the field) divided by the volume of the original subsample (measured in the field) multiplied by the number of eggs in the original subsample.

Egg size was also determined based on volume of the ovary measured in the field. It was calculated by simply dividing the total displacement volume of the ovary (measured in the field) by the fecundity (i.e., number of eggs per female).

All metallothionein and histopathological measurements were made at the Department of Fisheries and Oceans (Winnipeg) under the direction of Dr. J. Klaverkamp. Methods are as outlined in their report (Appendix E).

Tissue Metals

All tissue samples were analyzed by RPC Laboratories, Fredericton, for metal content. This involved weighing and drying the samples, followed by acid digestion. Analyses were by ICP-MS, with the exception of mercury which was determined by cold vapour atomic absorption spectroscopy. Analytical methods are presented in Appendix E.

Data Analysis - Biological Measurements

Field (raw) data were transcribed from field sheets into an electronic spreadsheet (EXCEL™, Version 5.0). Summary statistics, including means, standard deviations and sample size were then computed using formulae inherent to the spreadsheet software.

Based upon previous experience, the judgement was made that analyses of data sets representing fewer than ten individual fish per area (by sex) would not yield reliable results. Therefore, statistical analyses were completed only for female white sucker. Fewer than ten northern pike, walleye (either sex) or male white sucker could be obtained from the rivière Bousquet reference or exposure sites, or for the rivière Noire exposure site. No single fish type (including female sucker) could be obtained from the rivière Noire in sufficient numbers for statistical analysis. No adult fish or small fish could be captured in the rivière Noire, downstream of the Mine Dumagami discharge. However, a few fish were captured in the mouth of the river, in lac Preissac.

Detailed statistical analysis focused on four main fish response characteristics; age structure, growth, energy storage, and reproductive investment. Some of the variability associated with individual parameters may be dependent on other parameters (covariates). For the purposes of evaluating the data, the four major response characteristics were examined according to the parameter-covariate combinations appearing in Table 8.2.

The data for all combinations were plotted to visually identify those variables showing potential differences between reference and effluent-exposed groups. Plots for all dependent variable and covariate combinations appearing in Table 8.2 are provided in Appendix E together with detailed statistical analysis results.

TABLE 8.2: RESPONSE CHARACTERISTICS AND COVARIATES USED IN THE EVALUATION OF BIOLOGICAL DATA FROM ADULT FEMALE WHITE SUCKER

Response Characteristic	Dependent Variable (y)	Potential Covariates (x)
Age Structure	Mean Age	None
	Age distribution	None
Growth	Body weight	Age
	Length	Age
Energy Storage	Body weight	Length
	Liver weight	Age, adjusted body weight, and length
Reproductive Investment	Gonad weight	Age, adjusted body weight and length
	Egg weight	Age, adjusted body weight, and length
	Egg number (fecundity)	Age, adjusted body weight, and length

Detailed statistical analyses were then performed using SYSTAT™ (Version 5.2.1) and SPSS™ (Version 5.0 for Windows™) on selected parameters representing the four response categories judged to possibly suggest differences between reference and effluent-exposed groups.

Data Analysis - Trace Metals

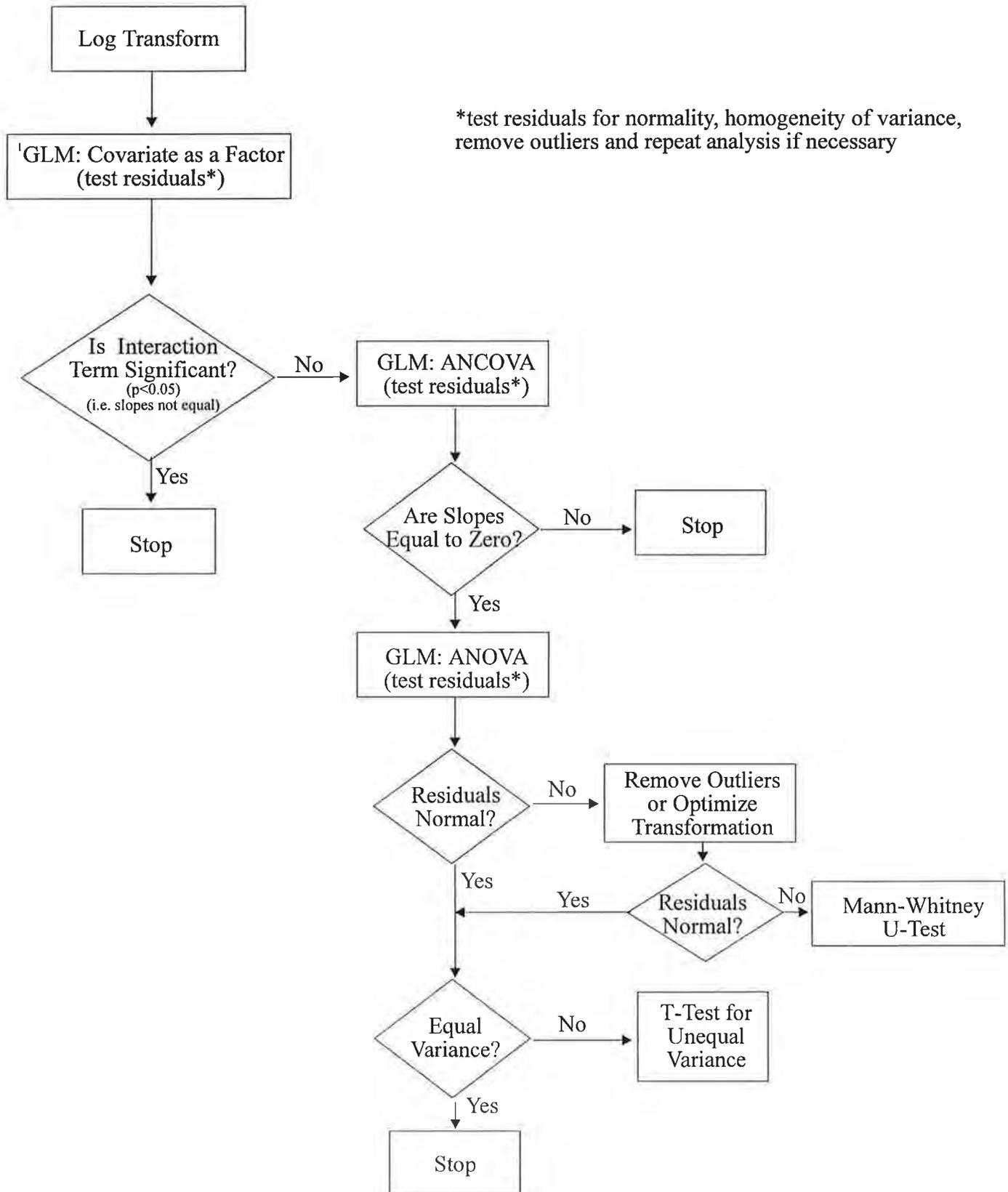
All tissue metal data were provided in electronic format from RPC Laboratories, and were converted into EXCEL™, Version 5.0 format.

Data were initially plotted against age for nine metals (Al, As, Cd, Cr, Cu, Pb, Hg, Ni and Zn) in liver and kidney for all white sucker specimens to identify any visually apparent differences between male and female fish. No obvious differences were observed, so males and females were pooled for all further analyses. The same pooling of male and female metal data was undertaken for northern pike. This pooling of data provided a reasonable minimum of at least ten samples for each species and each of two locations (rivière Bousquet reference and exposure) for statistical analysis. Fewer samples were available for walleye; thus, no statistical analyses of walleye metal data were undertaken.

Plots of tissue metals against age for northern pike and white sucker for Bousquet reference and exposure fish suggested some degree of covariance with age for some metals. Similar plots of metals against other candidate covariates (total length, total weight) appeared less promising and further investigation of these covariates was discontinued. Tissue metal vs. age plots are presented in Appendix E.

Statistical analysis of the metal data proceeded following the general scheme outlined in Figure 8.2 using SPSS (Version 5.0 for Windows) with the exception that data were not log-transformed. Statistical analyses were performed only on tissue types having a sample size of a least ten by site and species, which resulted in exclusion of muscle samples from both northern pike and white sucker, and also exclusion of gill in northern pike. No fish species was caught in sufficient abundance from the mouth of the rivière Noire for statistical analyses of the metal data.

FIGURE 8.2: PROCEDURES FOLLOWED IN DETAILED STATISTICAL ANALYSES OF FISHERIES DATA



¹ General Linear Models

For the purposes of illustration, histograms were also produced for the nine metals listed above and the three adult fish types, three locations and four tissue types. This was considered reasonable for the purpose of illustration, because covariance with age was generally not strong.

Metal levels in viscera of small fish were plotted in histogram form by metal and species for the four sites and nine metals listed above. No specific statistical analyses of the data were carried out, since in most cases the results proved to be quite variable within sites and since there was substantial inconsistency in the species available for comparison among sites.

Regression analysis was used to identify relationships between tissue metal and metallothionein (MT) levels using EXCEL™ (Version 5.0). Data were evaluated for adult white sucker and northern pike (the two most abundant large fish) from all locations. Similar analysis could not be carried out using the small fish data, since MT and metal data were not available for the same fish, and since clear differences among areas were not apparent in terms of visceral MT and metal concentrations. Based on the recommendation of Dr. J. Klaverkamp, the metals considered included Cd, Cu and Zn, three metallothionein-inducing metals. Metallothionein concentration in each tissue was regressed against the sum of the molar concentrations of these three metals. Males and females were initially considered separately; however, differences in relationships were not apparent between sexes, and all analyses were carried out with the data for both sexes and all locations combined.

8.2 Results

8.2.1 Preliminary Reconnaissance

Tissue metals in fish collected in the preliminary survey were analyzed in samples of the same large fish and tissues that were analyzed for metallothionein, and on replicates of the same species for young-of-the-year brown bullhead and northern pike. Results are presented along with MT results in Appendix E.

Although there are some apparent occasional anomalies in the data, the following conclusions are made:

- clear differences in concentrations of heavy metals did not exist between exposure and reference areas for individual fish species and tissue types;
- among gill, liver and kidney, metal concentrations varied substantially among tissues and species, at least for some metals; and
- MT concentrations showed no near clear upstream-downstream differences in the tissues sampled that might be attributed to mining-related metal exposure or to increased metal concentration downstream of the mines. MT levels were generally comparable in reference and exposure fish within tissue types.

8.2.2 Main Survey.

8.2.2.1 Fish Catches

Adult fish were captured in reasonable abundance in reference (lac Bousquet outlet) and exposure (rivière Bousquet mouth) areas (upstream and downstream of Mine Doyon and Complexe Bousquet) (Table 8.3). However, no adult fish could be recovered from the rivière Noire directly, despite intensive sampling efforts, although low numbers of adult fish were netted from the shallow river mouth area in lac Preissac (Table 8.3). These fish are lake-resident fish that occurred only transiently in the rivière Noire mouth area. The general absence of fish (adult and small fish) from the rivière Noire may be attributed to a toxicity problem (e.g., high ammonia levels). The species captured and retained for analysis from all areas included white sucker, northern pike and walleye. Three large fish species were retained rather than the originally intended two, owing to the difficulty encountered in capturing the targeted numbers of males and females (at least ten each) of any two species.

Small fish were obtained by electrofisher and seine in the same three areas sampled for large fish (Table 8.3). In addition, small fish were sampled from lac Joannès and from a reference area in rivière Noire, near Highway 117. Unfortunately, different species were captured in the different areas, and no single species was captured in adequate numbers from all four areas. The species present included northern pike, yellow perch, white sucker

TABLE 8.3: FISH SPECIES CAPTURED AND PROCESSED FOR TISSUE ANALYSIS AND FISHING EFFORT REQUIRED, OCTOBER 1995

Location	Method	Species	Effort	Total
Bousquet Reference	gill net	pike 21, walleye 8, white sucker 46	67,000 ft.hrs.	75
Bousquet Exposure	gill net	pike 10, walleye 31, white sucker 27	76,650 ft.hrs.	68
rivière Noire Exposure	gill net	pike 2, walleye 15, white sucker 5	111,400 ft.hrs.	22
lac Joannès	seine net	yellow perch 42, white sucker 9	6 hrs.	51
Bousquet Reference	seine net	pike 44, yellow perch 68, brown bullhead 15	16 hrs.	127
Bousquet Exposure	seine net	pike 27, yellow perch 49, brown bullhead 28	18 hrs.	104
rivière Noire Reference	electro-fishing*	white sucker 7, stickleback 7	3 hrs.	16
rivière Noire Exposure	electro-fishing*	-	1.5 hrs.	0
Bousquet Exposure	electro-fishing*	yellow perch 1, brown bullhead 2	1 hr.	3

* most electro-fishing was done from a boat

and brown bullhead (all young-of-the-year), and brook stickleback. As observed with large fish, no small fish could be obtained from rivière Noire (or ruisseau Dormenan) downstream of the active and inactive mines, possibly owing to toxicity.

Accordingly, the fish data analyses are based primarily on samples obtained from the rivière Bousquet system where preliminary reconnaissance information showed limited fish effects in terms of metallothionein and tissue metal levels were found. Thus, fish data obtained are from an area of little mining impact, and a second area of major impact where fish survival appears to be compromised (making the measurement of biological or biochemical effects difficult).

8.2.2.2 Biological Effects

The various categories of biological response evaluated in female white sucker from reference and exposure areas in the rivière Bousquet include age, growth, energy storage and reproduction.

Evaluation of the age structure of a fish population may permit identification of age classes potentially affected by environmental stressors (Gibbons *et al.*, 1993). Effects on age distribution may result from changes in recruitment success (reproductive development, spawning success, survival of early life stages) and/or adult mortality (exploitation, predation). Alternately, delayed sexual maturity may occur in fish exposed to some industrial effluents as a result of suppression of sex hormone production.

Growth is a non-specific, integrative measurement of an organism's response to the environment (e.g., Munkittrick and Dixon, 1988, 1989). A stressor may affect growth directly by diverting energy from growth, or indirectly by reducing the availability of food (Munkittrick and Power, 1990). Growth rate is typically described by the relationship of size (length or weight) at age (EC and DFO, 1995).

Liver weight can be associated with both energy storage and exposure to contaminants. Protein-based enzymes within liver tissue are responsible for the detoxification of some contaminants within body fluids and tissues, and contaminant exposure may induce enzyme synthesis which may, in turn, be reflected in increased liver weight (Addison, 1984; Heath,

1987). Increased liver weight may also occur to greater energy storage (e.g., as glycogen), either at the expense of growth or reproduction (Munkittrick *et al.*, 1991) or simply due to greater food availability (e.g., Tyler and Dunn, 1976). Other possible energy storage responses may be associated with weight at length (or condition), which may vary in fish in response to enhanced or reduced food availability.

The existence of a fish population is dependent on successful reproduction and recruitment. A stressor may affect reproduction by altering the availability of energy through food resources or metabolic pathways, biochemical processes related to reproduction, and/or reproductive behaviour (Munkittrick and Power, 1990). These may, in turn, be reflected in suppression in gonad size, egg size or fecundity (number of eggs).

Detailed plots of biological parameters and their covariates from the Rivière Bousquet are presented in Appendix E for reference and exposed female white sucker. As described earlier, inadequate numbers of other adult fish (< 10 per sex) were obtained for meaningful statistical evaluation. The only statistically significant differences ($p < 0.05$) between these two groups of fish were that exposed fish had greater body weight at age, greater gonad weight at total length and greater egg size (Table 8.4). The statistical power associated with these measures was relatively low to moderate, providing little confidence that the differences are true. None of these results appears to be clearly linked to an impact due to mining effects, and none is indicative of a negative effect.

8.2.2.3 Adult Fish Metals

Detailed data on metal levels in each of 225 fish tissues sampled (duplicates excluded) are presented in Appendix E for all metals, along with histogram plots for a subset of nine metals (Al, As, Cd, Cr, Cu, Pb, Hg, Ni, Zn).

Tables 8.5 to 8.7 provide summaries of significant ($p < 0.05$) statistical relationships found in the data. Key results may be summarized as follows:

- several metals varied with age of fish, depending on tissue type, although all of these relationships were weak (R^2 values < 0.5 in all cases; Tables 8.5 and 8.6); and

TABLE 8.4: SUMMARY OF STATISTICAL COMPARISONS OF MEASUREMENTS ON FEMALE WHITE SUCKER WHERE EFFECTS WERE SIGNIFICANT (P < 0.05)

Reference versus Lower rivière Bousquet Fish					
Parameter	Covariate	Procedure	p-value	Power	Comment
Body Weight (Log)	Age (Log)	reduced ANCOVA	0.027	0.610	Bousquet elevated
Gonad Weight (Log)	Total Length (Log)	reduced ANCOVA	0.033	0.578	Bousquet elevated
Egg Size (Log) ¹	none	ANOVA	0.015	0.696	Bousquet elevated

¹ outlier identified - removal did not affect results

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- metals were significantly elevated in some tissues of some exposed fish, and in other cases in some tissues of some reference fish. In nine out of 13 cases (for pike and sucker) where statistically significant results were obtained (ignoring cases where there were significant interaction terms between the two groups with age as a covariate), exposed fish tissues had the higher metal concentration. However, some contradictory results were obtained, with single metals being greater in one tissue type in exposed fish and in another tissue type in reference fish (this occurred for Zn and Cd). In most cases (8 out of 13), statistical power associated with these effects was relatively low (e.g., power <0.8; Tables 8.7 and 8.8).

Examination of tissue concentration histograms for adult fish revealed various trends:

- tissues accumulating the greatest concentrations of metals varied by metal, and were inconsistent among species; variability associated with the data was very high in most cases;
- muscle was rarely the tissue where the greatest level of accumulation occurred; kidney, gill or liver were usually dominant among tissue types in terms of metal concentration;
- the species showing the highest levels of bioaccumulation of metals varied with metal, with white sucker most frequently dominant (Cd, Cu, Zn);
- where fish from one of the three sampling areas appeared to accumulate somewhat greater metal concentration, the concentrations in rivière Noire fish were most often highest; however, given the wide variability associated with the data, this conclusion is tenuous, at best; and
- sample duplicate data showed relatively good reproducibility (relative standard deviations $\leq 20\%$), except where concentrations were close to detection limits where relative standard deviations rose to as high as 80% for liver and 140% for kidney.

TABLE 8.5: LIST OF METALS IN WHITE SUCKER TISSUES SHOWING A STATISTICALLY SIGNIFICANT RELATIONSHIP WITH AGE (P < 0.05)

Parameter	Regression p-value	R² Value for Regression
Aluminum in Liver (outlier(s) removed)	0.012	0.255
Arsenic in Liver	significant interaction	
Cadmium in Liver	0.003	0.252
Cadmium in Liver (outlier(s) removed)	< 0.001	0.521
Cadmium in Kidney	< 0.001	0.396
Chromium in Liver	0.038	0.166
Copper in Liver	0.036	0.171
Copper in Kidney (outlier(s) removed)	0.005	0.239
Lead in Liver	< 0.001	0.414
Lead in Liver (outlier(s) removed)	< 0.001	0.470
Lead in Kidney (outlier(s) removed)	significant interaction	
Mercury in Liver	0.001	0.306
Mercury in Liver (outlier(s) removed)	< 0.001	0.512
Mercury in Kidney	0.001	0.300
Mercury in Kidney (outlier(s) removed)	< 0.001	0.423
Mercury in Gills	0.020	0.179
Mercury in Gills (outlier(s) removed)	0.003	0.376
Nickel in Liver (outlier(s) removed)	0.026	0.185
Nickel in Kidney	0.016	0.203
Nickel in Kidney (outlier(s) removed)	0.001	0.339
Zinc in Liver	0.029	0.146
Zinc in Gills	< 0.001	0.496

TABLE 8.6: LIST OF METALS IN NORTHERN PIKE TISSUES SHOWING A STATISTICALLY SIGNIFICANT RELATIONSHIP WITH AGE (P < 0.05)

Parameter	Regression p-value	R² Value for Regression
Chromium in Liver	0.019	0.296
Lead in Kidney	0.047	0.193
Mercury in Liver	significant interaction	-
Mercury in Kidney	significant interaction	-
Zinc in Liver	0.016	0.346

TABLE 8.7: SUMMARY OF STATISTICAL COMPARISONS OF METALS IN WHITE SUCKER TISSUES WHERE EFFECTS WERE SIGNIFICANT (P < 0.05)

Reference versus Lower rivière Bousquet Fish					
Parameter	Covariate	Procedure	p-value	Power	Comment
Cadmium in Liver	Age	reduced ANCOVA	0.039	0.549	Bousquet ¹ fish elevated
Mercury in Liver (outlier(s) removed)	Age	reduced ANCOVA	0.021	0.655	Bousquet fish elevated
Mercury in Kidney (outlier(s) removed)	Age	reduced ANCOVA	0.031	0.588	Bousquet fish elevated
Mercury in Gills (outlier(s) removed)	Age	reduced ANCOVA	0.003	0.89	Bousquet fish elevated
Zinc in Gills	Age	reduced ANCOVA	0.001	0.933	Reference fish elevated
Cadmium in Gills	none	ANOVA	< 0.001	1.000	Reference fish elevated
Chromium in Gills	none	ANOVA	0.004	0.844	Reference fish elevated
Nickel in Gills	none	ANOVA	0.011	0.739	Bousquet fish elevated

¹ Bousquet fish = exposure fish

**TABLE 8.8: SUMMARY OF STATISTICAL COMPARISONS OF METALS IN
NORTHERN PIKE TISSUES WHERE EFFECTS WERE SIGNIFICANT (P < 0.05)**

Reference versus Lower rivière Bousquet Fish					
Parameter	Covariate	Procedure	p-value	Power	Comment
Mercury in Liver	Age	ANCOVA	significant interaction		
Mercury in Kidney	Age	ANCOVA	significant interaction		
Zinc in Liver	Age	reduced ANCOVA	0.019	0.682	Bousquet ¹ fish elevated
Arsenic in Liver	none	ANOVA	0.036	0.57	Bousquet fish elevated
Arsenic in Kidney	none	ANOVA	0.018	0.69	Bousquet fish elevated
Cadmium in Kidney	none	ANOVA	0.003	0.897	Reference fish elevated
Zinc in Kidney	none	ANOVA	0.015	0.711	Bousquet fish elevated

¹ Bousquet fish = exposure fish

8.2.2.4 Small Fish Metals

Metal data in viscera of 65 different small fish samples (duplicates excluded) are presented in Appendix E. These include data for:

- rivière Bousquet reference area - northern pike, yellow perch, brown bullhead
- rivière Bousquet exposure site - northern pike, yellow perch
- lac Joannès - yellow perch, white sucker
- rivière Noire reference - white sucker, stickleback

As observed with adult fish, metal data for small fish were highly variable in terms of differences within and among metals, species and locations (refer to Appendix E histograms). Apparent differences were observed in terms of:

- greater Cd and Cu in lac Joannès sucker relative to the reference site on rivière Noire; and
- greater Zn concentration and lower Ni, Al and Pb in northern pike than in yellow perch and brown bullhead.

As shown by relative standard deviation plots of sample duplicates, small fish data were generally less reproducible than data from large fish. Reasons for this greater analytical variation are unclear.

8.2.2.5 Metallothionein

Metallothionein (MT) results for the main survey are presented in Appendix E in histogram form by site and species, with all MT data tabulated along with corresponding zinc, copper and cadmium concentrations in the same tissues for adult white sucker and northern pike.

Of the 23 possible tissue-sample area comparisons made, only one (MT in gill from rivière Noire adult fish versus rivière Bousquet reference fish) showed statistical significance. These results are perhaps not surprising, given the inconsistent and variable results obtained for fish tissue metals, the relative lack of impact in terms of environmental metal levels between the major sites for comparison (reference and exposure in the rivière Bousquet),

and the dubious exposure history of the few fish collected from the mouth of the rivière Noire.

Regression analysis of the metal and MT data showed significant and positive relationships between metals and MT in liver of adult white sucker and northern pike, although none was found in other tissue types (Table 8.9 and Appendix E). These results appear somewhat unexpectedly, since MT concentrations generally do not vary by location, and since area differences in metal concentrations are relatively few and inconsistent. The observed correlations are interpreted as indicating the covariance of tissue metals and MT over all sampling areas. Results are consistent with the understanding that MT is induced by metal exposure. The absence of meaningful MT-metal relationships in other tissues indicates that liver may be the most useful tissue for MT monitoring. However, because MT induction is known to occur in a wide range of tissues (Palmiter, 1987), further evidence should be obtained from the field sites before a conclusion is made that effort should be focused in future only on hepatic tissues for MT and metals analysis in monitoring programs at Canadian mine sites.

8.2.2.6 Histology

A detailed report on the results of histological examination of fish tissues collected in this study, as provided by Evans and Klaverkamp (1996), is presented in Appendix E. The following is taken largely from the summary of that report.

Histological analyses of gills demonstrated no serious impairments in any of the fish examined, and no gill lesions were observed that could be unequivocally related to the sampling site. A clubbing of secondary lamellae was observed in gills from some of the walleye collected from the rivière Noire mouth; however, this lesion was not observed in any walleye from the rivière Bousquet-reference and rivière Bousquet-exposure sites.

Although few fish could be collected at the rivière Noire site, preliminary evidence in white sucker and walleye indicated the presence of posterior kidney damage. No serious impairments to kidney function were observed in white sucker and northern pike from the Bousquet-reference and Bousquet-exposure sites. DFO was unable to assess renal tubular

Table 8.9: Regression Analysis of Metallothionein and Metal Concentrations in Fish Tissues

Species	Tissue	R ²	Probability	Equation
White Sucker	Liver	0.7526	<0.001	y = 230.66x - 182.34
	Kidney	0.1239	0.041	y = 147.55x - 8.0086
	Gills	0.0317	0.314	y = 56.107x + 20.256
Northern Pike	Liver	0.6638	<0.001	y = 88.898x + 16.314
	Kidney	0.0057	0.737	y = 2.2106x + 199.61

y = metallothionein concentration in µg/g
x = [Cd + Cu + Zn] in µmole/g

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structure and make conclusions on kidney function in walleye from rivière Bousquet-reference and exposure sites.

Histological analyses of liver from white sucker and walleye from rivière Noire mouth demonstrated some apparently adaptive responses. These included larger hepatocyte nuclear diameter and greater hepatocyte cell areas. These enlargements could reflect enhanced synthesis of proteins. Northern pike from the rivière Bousquet exposure site had considerably less glycogen than liver from rivière Bousquet reference pike, possibly suggesting inferior feeding or some other stress. This result, however, is not coincident with any differences in liver weight between these two groups (Subsection 8.2.2.2).

Information was also obtained on vitellogenesis (egg development) in female white sucker, northern pike and walleye. No differences were observed between rivière Bousquet reference and exposure sites in egg development of white sucker and northern pike as measured by gonadosomatic indices (GSIs), relative fecundities (eggs/gram of fish), egg diameters and weights, and percent of clutch oocytes. These results appear inconsistent with the results of biological measurements made by BEAK on the same fish (Table 8.4), which showed greater gonad weight at length and egg size in rivière Bousquet exposure sucker. This inconsistency may, in part, be attributed to the fact that the measurements made by BEAK and DFO were different (i.e., GSI versus gonad weight at length) and egg size as measured by calliper (DFO) and by volume displacement (BEAK).

Histological analyses were conducted on livers from 95 small white suckers, northern pike, yellow perch, brown bullheads and sticklebacks ranging in length from 4.3 cm to 12.8 cm. No overt lesions were observed in liver from any of these fish. The nuclear diameter in livers of yellow perch from lac Joannes were larger than those observed in yellow perch from the rivière Bousquet reference area. Livers of yellow perch from the rivière Bousquet reference site also had larger cell sizes than liver of rivière Bousquet exposure perch, due to higher glycogen content, possibly reflecting better feeding conditions at the reference site. Similar to large northern pike, glycogen content in livers from small northern pike was highest in fish from the rivière Bousquet reference site, again possibly indicating better feeding conditions at that site.

8.3 Costs

Costs associated with fish tissue analysis include collection cost, sample preparation cost and analytical cost.

In terms of sample collection and preparation, costs will vary depending on fish abundance (catch-per-unit-effort). In this case, fish capture consumed 12 ten-hour days each for two trained field technicians (total 240 person-hours). Much of this effort was associated with substantial vehicle and boat travel among the various capture sites. Sample processing and maintenance of samples (maintenance of dry ice supply, record-keeping, etc.) consumed slightly less effort (about 200 person-hours for four trained technicians). Sample processing in the field, including all biological measurements and the collection and preservative of tissues for metallothionein, histopathology and metal determination required about 20 minutes per adult fish plus set-up and clean-up time for each set of dissections (about one hour total) for a four-person crew (included in the 200 hours above). Young fish could be processed much more quickly (e.g., two to three minutes per fish). It should be noted that the field crew used here was extremely proficient in fish dissection for biological measurements and dissections (age, size, fecundity, etc.) gained from extensive experience during 1995 Environmental Effects Monitoring studies for pulp and paper operations across Canada. At an assumed average (relatively low) hourly rate of \$35.00 for this work, field capture and sample processing consumed about \$15,400. Additional field costs would be associated with subsistence (about \$3,800), supplies and equipment (about \$1,600) and project management/supervision in the field (a high level of supervision and participation by the project manager was necessary (about \$6,800 including subsistence costs). Thus, the total cost, exclusive of travel, etc., for field collection and preparation of samples was about \$28,000. Additional costs would be associated with travel, shipping, etc. It should be noted that, if metallothionein and histopathology analyses were not carried out, these costs would have been reduced only by a relatively small fraction (perhaps 25%). Note that all of the above excludes the cost of fish collection and processing during the preliminary reconnaissance, which was carried out by senior project staff. Laboratory processing costs for biological measurements (age, egg size, fecundity) were relatively low, totalling about \$30.00 per fish in technician time, or \$1,950 for 65 adult fish captured in the main survey.

Costs for metal analyses were \$68.50 per sample, or \$19,865 for the 290 tissue samples collected during the main survey. This cost could be reduced if the number of parameters could be reduced to ≤ 5 or 6 (as discussed in Section 5.0), and if the number of species, fish or tissue types could be reduced. Unfortunately, because none of the fish were collected from clear metal exposure gradients, it is not possible from this data set to demonstrate effective means of sample reduction. Per sample costs are relatively low (in comparison with sediment and water analysis by ICP-MS) owing to the higher sample volume discounts achieved.

Costs for metallothionein and histopathological determinations are unknown, as they were carried out by a government laboratory. BEAK has obtained histopathological determinations and MT measurements in fish in other instances through other laboratories, but the prices paid for these services was generally \$40 for metallothionein analysis and \$175/fish for a full pathological examination.

8.4 Evaluation of Methods

Overall, it is difficult to evaluate the cost-effectiveness and sensitivity of the various fish measurements carried out in this study to identify mining-related effects, as fish could not be obtained from an area with a clear gradient in metal exposure and tissue contaminant levels. However, a few observations and general conclusions may be made:

- metals may be readily measured at detectable levels in all tissue types sampled; there is probably some inherent redundancy in the analysis of four tissue types in adult fish;
- hepatic MT levels covaried with the sum of hepatic cadmium, zinc and copper; monitoring of MT in liver may be more effective than monitoring of MT in gill or kidney;
- field crews were able to collect and secure adequate samples for analysis of MT, although in some cases (remote locations) obtaining an adequate supply of dry ice will be difficult and quite costly;
- histological work indicated some possible differences in fish tissues among sites, suggesting that such measurements may be of some value as measures of biological response; and

- young fish are easier to collect and maintain in viable condition than adult fish; field costs could be substantially reduced if small fish viscera could be monitored rather than organs of large fish.

In terms of the biological measurements made with adult fish (growth, fecundity, age, etc.), AETE program participants should be aware that such measurements have been collected broadly in reference and exposure fish from more than 135 pulp and paper mills across Canada, with reports submitted to Environment Canada on 01 April 1996. The effectiveness of these measurements in distinguishing effluent exposure effects at pulp mills, along with the problems encountered in distinguishing meaningful effects, should be considered before these measurements are included in the aquatic effects monitoring technologies for mine sites.

It is our recommendation that, based on this pilot study, further measurements of fish response be made in future AETE field evaluations at locations where fish are known to occur in metal concentration gradients.

9.0 REFERENCES

- Addison, R.F. 1984. Hepatic mixed function oxidase (MFO) induction in fish as a possible biological monitoring system. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.), Contaminant Effects on Fisheries. Adv. Envir. Sci. Technol. 16:51-60.
- EC and DFO (Environment Canada and Department of Fisheries and Oceans). 1995. Further guidance for the adult fish survey for aquatic environmental effects monitoring related to federal *Fisheries Act* requirements. EEM 1. 72 pp.
- Aquatic Effects Technical Evaluation (AETE) Committee. 1995. 1995 Pilot Field Site: Field Trip Report and Recommendations.
- Evans, R.E. and J.F. Klaverkamp. 1996. Histological analyses of gills, posterior, kidneys, livers and ovaries of fish from the Bousquet and Noire rivers of Quebec. Dept. of Fisheries and Oceans - Freshwater Institute, Winnipeg.
- Gibbons, W. *et al.* 1993. Interpretive Review of the Adult Fish Survey Used for Aquatic Environmental Effects Monitoring. Draft, January 1993.
- Heath, A.G. 1987. Water pollution and fish physiology. CRC Press, Boca Raton, Florida. 245 pp.
- Lortie, D. 1995. Impacts des activités Minières dans le bassin hydrographique du lac Preissac.
- Munkittrick, K.R. and D.G. Dixon. 1988. Growth, fecundity and energy stores of white sucker (*Catostomus commersoni*) from lakes containing elevated levels of copper and zinc. Can. J. Fish. Aquat. Sci. 45:1355-1365.
- Munkittrick, K.R., C.B. Portt, G.J. Van Der Kraak, I.R. Smith and D.A. Rokosh. 1991. Impact of bleached kraft mill effluent on population characteristics, liver MFO activity and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. Can. J. Fish. Aquat. Sci. 48:1371-1380.
- Munkittrick, K.R. and D.G. Dixon. 1989. Use of white sucker (*Catostomus commersoni*) populations to assess the health of aquatic ecosystems exposed to low-level contaminant stress. Can. J. Fish. Aquat. Sci. 46:1455-1462.
- Munkittrick, K.R. and E.A. Power. 1990. Literature review for biological monitoring of heavy metals in aquatic environments. Prepared for the British Columbia Acid Mine Drainage Task Force by EVS Consultants Ltd. 127 pp.

- Palmiter, R.D. 1987. Molecular biology of metallothionein gene expression. Exp. Suppl. (Basel) 52: 25-61.
- Phaneuf, S. 1995. Literature review on the techniques of sonar profiling and grid sampling, using a grab sampler for the identification and mapping of lake sediment facies for environmental effects monitoring. Report to Aquatic Effects Technical Evaluation (AETE) Committee.
- Tyler, A.V. and R.S. Dunn. 1976. Ration, growth, and measures of somatic organ condition in relation to meal frequency in winter flounder, *Pseudopleuronectes americanus*, with hypothesis regarding population homeostasis. Journal of the Fisheries Research Board of Canada 33:63-75.