AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM

Field Evaluation of Aquatic Effects Monitoring

1997 Study Design

AETE Project 4.1.2

1996 Field Evaluation

AQUATIC EFFECTS MONITORING

1997 STUDY DESIGN

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AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

Notice to Readers

Aquatic Effects Monitoring
1996 Preliminary Field Surveys

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of provincial governments; it is coordinated by the Canada Centre for Mineral and Energy Technology (CANMET). The program is designed to be of direct benefit to the industry, and to government. Through technical evaluations and field evaluations, it will identify cost-effective technologies to meet environmental monitoring requirements. The program includes three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring. The program includes literature-based technical evaluations and a comprehensive three year field program.

The program has the mandate to do a field evaluation of water, sediment and biological monitoring technologies to be used by the mining industry and regulatory agencies in assessing the impacts of mine effluents on the aquatic environment; and to provide guidance and to recommend specific methods or groups of methods that will permit accurate characterization of environmental impacts in the receiving waters in as cost-effective a manner as possible. A pilot field study was conducted in 1995 to fine-tune the study design.

A phased approach has been adopted to complete the field evaluation of selected monitoring methods as follows:

Phase I: 1996- Preliminary surveys at seven candidate mine sites, selection of sites for further work and preparation of study designs for detailed field evaluations.

Phase II: 1997-Detailed field and laboratory studies at selected sites.

Phase III: 1998- Data interpretation and comparative assessment of the monitoring methods: report preparation.

Phase I is the focus of this report. The overall objective of this project is to conduct a preliminary field/laboratory sampling to identify a short-list of mines suitable for further detailed monitoring, and recommend study designs. The objective is NOT to determine the detailed environmental effects of a particular contaminant or extent and magnitude of effects of mining at the sites.

In Phase I, the AETE Technical Committee has selected seven candidates mine sites for the 1996 field surveys:

1) Myra Falls, Westmin Resources (British Columbia)
2) Sullivan, Cominco (British Columbia)
3) Lupin, Contwoyto Lake, Echo Bay (Northwest Territories)
4) Levack/Onaping, Inco and Falconbridge (Ontario)
5) Dome, Placer Dome Canada (Ontario)
6) Gaspé Division, Noranda Mining and Exploration Inc. (Québec)
7) Heath Steele Division, Noranda Mining and Exploration Inc. (New-Brunswick)

Study designs were developed for four sites that were deemed to be most suitable for Phase II of the field evaluation of monitoring methods (Myra Falls, Dome, Heath Steele, Lupin). Lupin was subsequently dropped based on additional reconnaissance data collected in 1997. Mattabi Mine, (Ontario) was selected as a substitute site to complete the 1997 field surveys.

For more information on the monitoring techniques, the results from their field application and the final recommendations from the program, please consult the *AETE Synthesis Report* to be published in September 1998.

Any comments regarding the content of this report should be directed to:

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PROGRAMME D'ÉVALUATION DES TECHNIQUES DE MESURE D'IMPACTS EN MILIEU AQUATIQUE

Avis aux lecteurs

Surveillance des effets sur le milieu aquatique
Études préliminaires de terrain - 1996

Le Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ÉTIMA) vise à évaluer les différentes méthodes de surveillance des effets des effluents miniers sur les écosystèmes aquatiques. Il est le fruit d'une collaboration entre l'industrie minière du Canada, plusieurs ministères fédéraux et un certain nombre de ministères provinciaux. Sa coordination relève du Centre canadien de la technologie des minéraux et de l'énergie (CANMET). Le programme est conçu pour bénéficier directement aux entreprises minières ainsi qu'aux gouvernements. Par des évaluations techniques et des études de terrain, il permettra d'évaluer et de déterminer, dans une perspective coût-efficacité, les techniques qui permettent de respecter les exigences en matière de surveillance de l'environnement. Le programme comporte les trois grands volets suivants : évaluation de la toxicité aiguë et sublétale, surveillance des effets biologiques des effluents miniers en eaux réceptrices, et surveillance de la qualité de l'eau et des sédiments. Le programme prévoit également la réalisation d'une série d'évaluations techniques fondées sur la littérature et d'évaluation globale sur le terrain.

Le Programme ÉTIMA a pour mandat d'évaluer sur le terrain les techniques de surveillance de la qualité de l'eau et des sédiments et des effets biologiques qui sont susceptibles d'être utilisées par l'industrie minière et les organismes de réglementation aux fins de l'évaluation des impacts des effluents miniers sur les écosystèmes aquatiques; de fournir des conseils et de recommander des méthodes ou des ensembles de méthodes permettant, dans une perspective coût-efficacité, de caractériser de façon précise les effets environnementaux des activités minières en eaux réceptrices. Une étude-pilote réalisée sur le terrain en 1995 a permis d'affiner le plan de l'étude.

L'évaluation sur le terrain des méthodes de surveillance choisies s'est déroulée en trois étapes:

Étape I 1996 - Évaluation préliminaire sur le terrain des sept sites miniers candidats, sélection des sites où se poursuivront les évaluations et préparation des plans d'étude pour les évaluations sur le terrain.

Étape II 1997- Réalisation des travaux en laboratoire et sur le terrain aux sites choisis

Étape III 1998 -Interprétation des données, évaluation comparative des méthodes de surveillance; rédaction du rapport.

Ce rapport vise seulement les résultats de l'étape I. L'objectif du projet consiste à réaliser des échantillonnages préliminaires sur le terrain et en laboratoire afin d'identifier les sites présentant les caractéristiques nécessaires pour mener les évaluations globales des méthodes de surveillance en 1997 et de développer des plans d'études. Son objectif N'EST PAS de déterminer de façon détaillée les effets d'un contaminant particulier, ni l'étendue ou l'ampleur des effets des effluents miniers dans les sites.

À l'étape I, le comité technique ÉTIMA a sélectionné sept sites miniers candidats aux fins des évaluations sur le terrain:

1) Myra Falls, Westmin Resources (Colombie-Britannique)
2) Sullivan, Cominco (Colombie-Britannique)
3) Lupin, lac Contwoyto, Echo Bay (Territoires du Nord-Ouest)
4) Levack/Onaping, Inco et Falconbridge (Ontario)
5) Dome, Placer Dome Mine (Ontario)
6) Division Gaspé, Noranda Mining and Exploration Inc.(Québec)
7) Division Heath Steele Mine, Noranda Mining and Exploration Inc.(Nouveau-Brunswick)

Des plans d'études ont été élaborés pour les quatres sites présentant les caractéristiques les plus appropriées pour les travaux prévus d'évaluation des méthodes de surveillance dans le cadre de l'étape II (Myra Falls, Dome, Heath Steele, Lupin). Toutefois, une étude de reconnaissance supplémentaire au site minier de Lupin a révélé que ce site ne présentait pas les meilleures possibilités. Le site minier de Mattabi (Ontario) a été choisi comme site substitut pour compléter les évaluations de terrain en 1997.

Pour des renseignements sur l'ensemble des outils de surveillance, les résultats de leur application sur le terrain et les recommandations finales du programme, veuillez consulter le *Rapport de synthèse ÉTIMA* qui sera publié en septembre 1998.

Les personnes intéressées à faire des commentaires sur le contenu de ce rapport sont invitées à communiquer avec M^{me} Diane E. Campbell à l'adresse suivante :

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1.0
INTRODUCTION

The Aquatic Effects Technology Evaluation (AETE) Program was established to conduct field and laboratory evaluation and comparison of selected environmental effects monitoring technologies for assessing impacts of mine effluents on the aquatic environment. The focus of the Program is on robustness, costs, and the suitability of monitoring technologies.

Building upon previous work, which includes literature reviews, technical evaluations, and pilot field studies, the AETE Program sponsored, in 1996, preliminary evaluations of aquatic effects monitoring at seven candidate mine sites. Separate reports have been provided detailing the findings of studies conducted in 1996 at each of the seven sites (EVS, ESP and JWEL, 1996a-g). Final recommendations regarding selection of sites for 1997 work have also been provided (EVS, ESP and JWEL, 1996h). The present report provides our recommended study design for the 1997 studies, beginning with a generic study design (Section 3.0) and continuing to specific study designs for three sites (Section 4.0).

1.1 OBJECTIVES

The overall goal of this document is to provide a study design focused on endpoints and which recommends the most appropriate methods to monitor biological characteristics and water and sediment quality in selected receiving environments exposed to mining effluents, relative to hypotheses which the AETE Program wants to test in 1997. These hypotheses are listed in Table 3-1. The specific objectives of the present document are to provide:

1. A detailed, generic study design for testing all of the AETE hypotheses.

2. Detailed, site-specific study designs for those sites selected for testing the AETE hypotheses in 1997.

3. Estimated costs for 1997 studies.

1.2 BACKGROUND

The AETE Program was established to review appropriate technologies (i.e., methods) for assessing impacts of mine effluents on the aquatic environment. The results of this Program are expected to be applicable to programs including environmental effects monitoring (EEM, which the AETE Program does not replace), impact assessment, and baseline monitoring.

The AETE Program has three main areas: toxicity (acute and sublethal), receiving water biology, and water and sediment monitoring. Its major goal is:

> "to evaluate and identify technologies to meet environmental monitoring requirements, at the lowest cost."

Note that lowest cost does not necessarily mean the cheapest method, since the cost for making wrong decision(s) can be large. Rather, lowest cost means the most cost-effective approach to making the right decision(s).

The overall goal of the AETE field programs is to conduct field/laboratory evaluation and comparison of selected aquatic environmental effects monitoring technologies. This goal does not include determining the environmental effects of mining at the sites; such are the purview of EEM. The AETE Program consists of field studies, together with a variety of literature reviews and technical evaluations, many of which have influenced the field program design proposed herein (e.g., Taylor, 1996; BAR, 1996).

The AETE conducted a pilot field study in 1995 (Beak, 1996a) and then began, in 1996, a comprehensive field program for evaluation of selected monitoring methods. The present report and previous reports by EVS, ESP and JWEL (1996a-h) document the first phase of this comprehensive field program, involving preliminary field evaluations at seven candidate mine sites, and selection of some of these sites for further work in 1997. Studies at selected sites in 1997, based on the design provided herein, form the second phase of this comprehensive field program. The third phase will involve data interpretation and comparative assessment of monitoring methods.

Following completion of all three phases of this comprehensive field program:

- Appropriate methods will have been comprehensively tested in the field.

- Different tools (i.e., methods) will have been assessed to determine their sensitivity in detecting differences or relationships in the case of slight to moderate levels of impact.

- Recommendations will be possible for a monitoring program for the mining industry in Canada. In other words, based on the totality of work conducted under the AETE Program, a technically sound and logistically possible basis will have been established for an EEM Program for mining in Canada (cf. Figure 2-1; Section 2.2).

2.0
APPROACH

2.1 BASIS FOR DESIGN

Our design for the 1997 field studies is based on the:

- 1995 pilot study (Beak, 1996a)
- 1996 preliminary site surveys (EVS, ESP and JWEL, 1996a-g)
- 1997 site selection (EVS, ESP and JWEL, 1996h)
- draft plan for the 1997 studies (Table 1, Addendum 1 of the Request for Proposal for 1996 field studies)
- final hypotheses provided by the AETE Technical Committee (Table 3-1).

2.2 GUIDANCE QUESTIONS

Out study design is also based on AETE's four guidance questions:

1. Are contaminants entering the system?

This question deals solely with exposure, i.e., the presence and concentrations of any contaminants. If contaminants are not present, there is no chance of adverse effects on the biota related to contaminants. However, effects on biota can occur for other reasons, for instance physical habitat changes. Answering this question requires measurements of contaminant concentrations in effluent, water and sediment chemistry. It also requires measurements of other factors which influence the biota, such as habitat. Both types of measurements are included in the 1997 study design.

2. Are contaminants bioavailable?

This is a key question, since only if contaminants are bioavailable can they possibly produce an adverse effect on the biota. If contaminants are not bioavailable, they are not of concern. Information to answer this question will be obtained in 1997 by analyses of resident biota (e.g., tissue body burdens, metallothionein analysis) and by laboratory toxicity testing (e.g., of effluent, water, sediments).

3. Is there a measurable response?

This question applies if both the previous two questions (exposure and bioavailability) are answered in the affirmative. Answering this question will require both field studies (e.g.,

changes in resident benthos, fish communities) and laboratory studies (e.g., toxicity testing). These are included in the 1997 study design.

4. Are the contaminants causing this response?

This question applies if all previous questions are answered in the affirmative, must consider other stressors, and is best determined experimentally. This question is not addressed in the 1997 field studies directly, though correlations and measurements of modifying factors which are part of the 1997 field studies, will provide some information relevant to this question.

The role of the above questions in the AETE Program is illustrated in Figure 2-1, and results in decisions regarding essential monitoring and tools which are expected to be applied in a tiered testing strategy. As noted above, only Guidance Questions 1-3 are included in this figure.

2.3 DEFINITION OF AN EFFECT

We note that the AETE definition of an aquatic environmental effect caused by mining activity is presently:

> *"A measurable difference in an environmental variable (chemical, physical or biological) between a point downstream (or exposed to mining) in the receiving environment and an adequate reference point (either spatial or temporal)"*

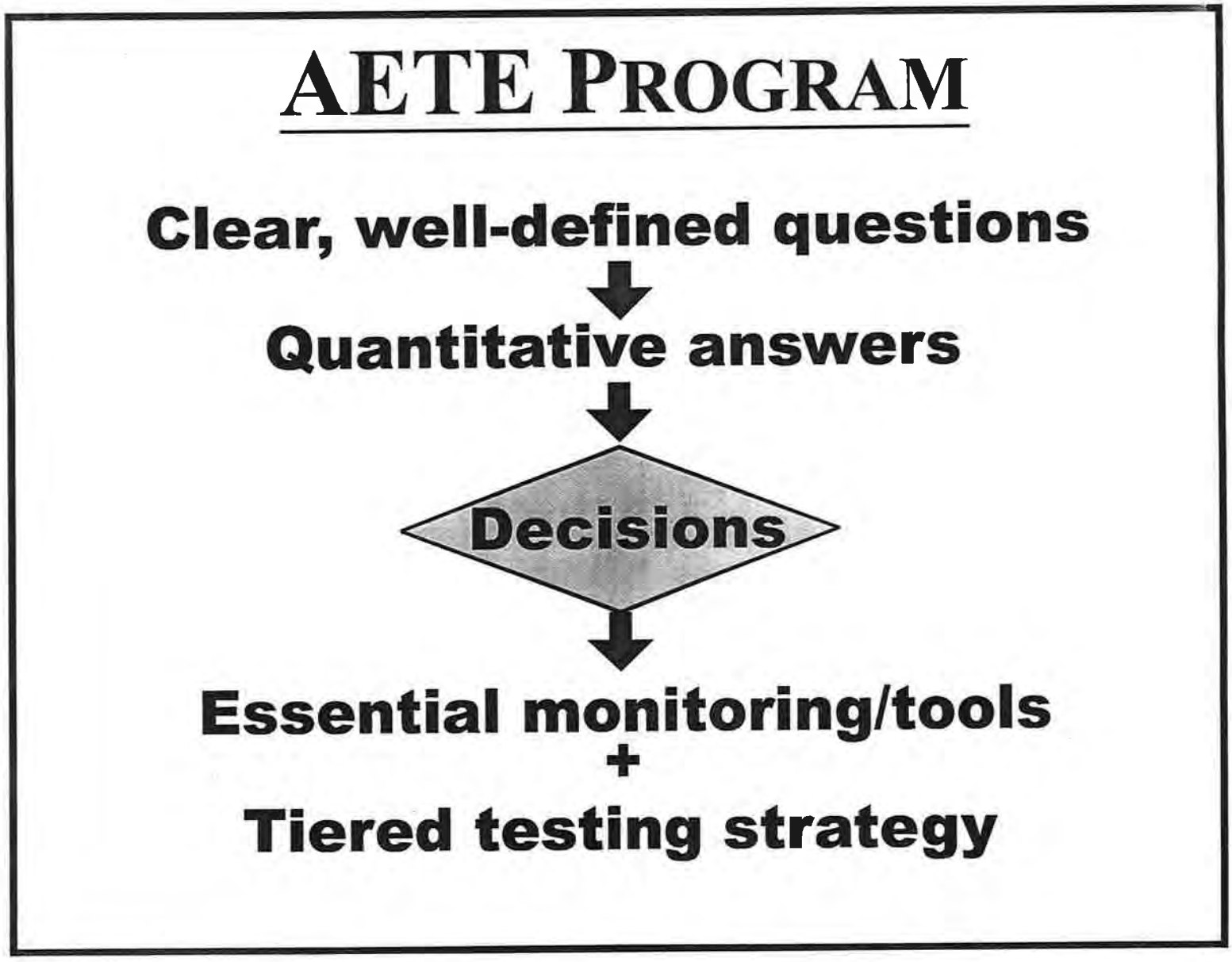
We have previously noted to the AETE Committee that this definition is technically incorrect, since only biological changes such as significant toxicity and alterations in benthic community structure are effects. Chemical changes deal solely with exposure (cf. Section 2.2., Question 1).

We have proposed that the above definition be revised as follows:

> *"A measurable difference in a biological variable between a point downstream (or exposed to mining) in the receiving environment and an adequate reference point (either spatial or temporal)."*

The AETE Committee decided (their meeting of January 14, 1997) not to revise the generic definition at this time but rather to provide clarification in a further AETE report. However, our interpretation of this definition was used in preparing the 1997 study design.

Figure 2-1. Role of guidance questions in AETE program.



2.4 DESIGN COMPONENTS PROVIDED

The key design components for the 1997 field studies are:

- Statistical design
- Sampling plan
- Field/Laboratory analysis plan
- QA/QC

These are provided generically in Section 3.0 and specifically for selected sites (EVS, ESP and JWEL, 1996h) in Section 4.0. Estimated costs are provided, as requested by the AETE Committee, in an appendix (Appendix D).

3.0
GENERIC STUDY DESIGN

The design for 1996 studies involved: use of readily available historical data; five different toxicity tests on single effluent samples; determination of any differences between exposure and reference areas (habitat characterized and kept as uniform as possible; *t*-test statistical comparisons between reference and exposure areas); measurements of benthos, water and sediment chemistry at six stations in each area; fish collections from reference and exposure areas where possible/appropriate.

3.1 HYPOTHESES

There are thirteen hypotheses to be tested in the 1997 field study (Table 3-1), which incorporate recommended revisions to the original hypotheses (cf. Appendix A). Specific statistical designs are provided for each hypothesis and for groupings of hypotheses.

Table 3-1. Hypotheses to be tested in 1997.

Sediment Monitoring 1. Sediment Toxicity: H: *The strength of the relationships between sediment toxicity responses and any exposure indicator is not influenced by the use of different sediment toxicity tests or combinations of toxicity tests.*
Biological Monitoring - Fish 2. Metals in fish tissues (bioavailability of metals): H: *There is no difference in metal concentrations observed in fish liver, kidney, gills, muscle or viscera (or whole fish).*
3. Metallothionein in fish tissues: H: *There is no difference in metallothionein concentrations observed in fish liver, kidney, gills or viscera (or whole fish).*
4. Metals vs. metallothionein in fish tissues: H: *The choice of metallothionein concentration vs. metal concentrations in fish tissues does not influence the ability to detect environmental exposure in fish to metals.*
5. Fish - CPUE: H: *There is no environmental effect in observed CPUE (catch per unit effort) of fish.*
6. Fish - Community: H: *There is no environmental effect in observed fish community structure.*
7. Fish - Growth: H: *There is no environmental effect in observed fish growth.*
8. Fish - Organ/Fish Size: H: *There is no environmental effect in observed organ size (or fish size, etc.).*
Integration of Tools 9. Relationship between water quality and biological components: H: *The strength of the relationship between biological variables and metal chemistry in water is not influenced by the choice of total vs. dissolved analysis of metals concentrations.*
10. Relationship between sediment chemistry and biological responses: H: *The strength of the relationship between biological variables and sediment characteristics is not influenced by the analysis of total metals in sediments vs. either metals associated with iron and manganese oxyhydroxides or with acid volatile sulphides.*

11. Relationship between sediment toxicity and benthic invertebrates: H: *The strength of the relationship between sediment toxicity responses and in situ benthic macroinvertebrate community characteristics is not influenced by the use of different sediment toxicity tests, or combinations of toxicity tests.*
12. Metals or metallothionein vs. chemistry (receiving water and sediment): H: *The strength of the relationship between the concentration of metals in the environment (water and sediment chemistry) and metal concentration in fish tissues is not different from the relationship between metal concentration in the environment and metallothionein concentration in fish tissues.*
13. Chronic Toxicity - Linkage with Fish and Benthos monitoring results: H: *The suite of sublethal toxicity tests cannot predict environmental effects to resident fish performance indicators or benthic macroinvertebrate community structure.*

3.2 STATISTICAL DESIGN

In determining the statistical design we first examined the questions addressed by each hypothesis and divided the hypotheses into groups. Next we determined which statistical design (gradient or control-impact [CI]) was required to test each hypothesis. Then we determined which design should be emphasized in 1997. Finally, we examined the candidate mine sites to determine which could provide a suitable environment to apply a gradient and/or CI design. Thus we determined, in a systematic way, which hypotheses could be tested at which site (cf. EVS, ESP and JWEL, 1996h).

The questions addressed by the hypotheses (designated H*n* - see Table 3-1 for full listing of all hypotheses) can be divided into three basic types:

- tests for environmental effects, defined as differences between exposed and reference areas (H5-H8)
- comparisons of tools (e.g., methods, variables, target tissues, etc.) (H1-H4)
- comparisons of correlations (r) between chemistry, toxicity and biological variables (H9-H13; H13 could also be considered a test of the null hypothesis that one or more r=0)

H1 could also be rephrased and tested as comparisons of correlations. Each type of hypothesis requires a different statistical model and analyses, briefly described in Section 3.2.1. Appendix A provides the rationale for selecting the models, and details of interest to

statisticians and to field personnel planning or conducting the studies. Section 3.2.2 provides an overall design for the 1997 field studies; Section 3.2.3 provides a brief summary and discussion of statistical power and recommended sample sizes.

This report assumes that the 1997 studies will emphasize tests of integrative hypotheses H9-H13. These hypotheses and others form the basis of the sediment quality triad, in which correlations within and among chemistry (*C*), toxicity (*T*) and biological (*B*) variables are examined (Green et al., 1993). The Triad approach can be extended to water quality.

3.2.1 Statistical Designs and Analyses

Tests for Environmental Effects:

Basic Design: Control-Impact (CI) design, with reference and exposed areas

Statistical Analyses: *t*-test or one-way ANOVA or ANCOVA

Units of Replication: Fishing locations (H5, H6)
Individual fish (H7, H8)

Sampling Allocation: Equal numbers of replicates in reference and exposed areas

Key Issues:

Units of replication for fish variables do not match those for other variables. Statistical power for analyses of fish catch and community variables (H5, H6) will be limited.

Comparisons of Tools:

Basic Design: Control-Impact (CI) design, with reference and exposed areas

Statistical Analyses: Split-plot ANOVA or multivariate analogs

Units of Replication: Stations (H1)
Individual fish (H2-H4)

Sampling Allocation: Equal numbers of replicates in reference and exposed areas

Key Issues:

Exposure gradients ideally comprise a ≥10-fold range in metal concentrations in water and sediment. Lesser gradients will reduce the power of tests of integrative hypotheses.

Differences between weakly correlated variables (i.e., tools) may be the most important or interesting but are the most difficult to detect. Given equal sample sizes, tests for differences of differences (i.e., comparisons of tools) are more powerful than simple tests of differences between areas (i.e., tests of environmental effects) for the same variables when $r^2>0.5$ ($r>0.7$), but less powerful when $r^2<0.5$ ($r\leq 0.7$). H1 should be tested using a comparison of correlations.

Comparisons of Correlations (Integrative Hypotheses):

Basic Design: Gradient design, with a strong exposure gradient in the exposed area

Statistical Analyses: Bivariate and partial correlation analyses; multivariate analyses

Units of Replication: Stations (H9 - H11)

Sampling Allocation: Many replicates from exposed area; few from reference area

Key Issues:

H12 and H13 are statistically untestable, but are qualitatively testable; fish variables cannot be included in a gradient design but benthos can be (cf. Appendix A, Section A3.1). The problem is that units of replication (individual fish or fishing locations) differ from those used for other variables (stations) (cf. Appendix A). Formal tests for comparing correlations of two or more variables with a third (i.e., specific tests of H9 - H11) can be difficult to construct and/or interpret (cf. Marcus and MacDonald, 1992). However, the general approach recommended in Appendix A should allow an exploratory assessment of those hypotheses, and many others concerning the integration of monitoring tools. Examples of "weight of evidence" approaches are provided in Chapman (1996), Green and Montana (In Press) and Menzie et al. (1996).

3.2.2 Overall Study Design

Two different basic designs, a CI design (including split-plot) and a gradient design, are required to test the hypotheses the AETE Committee wishes to address in 1997 field studies. The CI design is primarily useful for testing hypotheses related to fish variables; the gradient design is primarily useful for assessing relationships among various *C*, *T* and *B* variables. The 1997 program is not restricted by the need to conduct sediment toxicity tests and benthos sampling where CI designs are used, nor restricted to sampling fish where gradient analyses are conducted. This approach was adopted for the 1997 field study plans, since it would optimize the suitability of a site to test hypotheses and it would be difficult or impossible to reconcile the different sample allocations required for the two designs. Both designs could then be used at the same site, where possible. Metal concentrations in water or sediment

would be the only two variables common to both designs; at sites where both designs are used, additional reference samples are required to meet the demands of the CI design.

The first step in considering which hypotheses were to be tested at specific sites (Section 4.0) was to decide if a gradient design could be used. If so, the next step was to decide which integrative hypotheses could be tested at that site, without encountering logistical and other difficulties. If the site was also suitable for a CI design, then the specific CI hypotheses which could practically be tested were also identified, and the subset of hypotheses which could be tested there was identified. Sites not suitable for gradient analyses should be suitable for CI designs. The net result is that the 1997 studies will focus on testing each hypothesis at the most suitable sites, and using the most suitable statistical design. Note EVS, ESP and JWEL (1996h) only considered whether the variables necessary to test the hypothesis could be measured at the site; this report adds the restriction that the site must also be suitable for the specific designs used to test the hypothesis.

3.2.3 Statistical Power and Sample Sizes

Based on Section 3.2 and Appendix A, recommended sample sizes are summarized in Table 3-2. These recommendations are based on practical as well as statistical considerations. The sample sizes for stations and individual fish are adequate to detect differences of one standard deviation (SD) in CI designs, and correlations ≥ 0.50 or 0.60 in gradient designs, which we consider reasonable target effect sizes. Increased sample sizes, if possible (i.e., are there enough fish or stations?) and affordable, would be welcome but will produce diminishing returns in the sizes of differences or correlations which can be detected. Smaller sample sizes would reduce statistical power, and compromise the robustness of any statistical tests. Therefore, we question whether tests of H5 and H6 (i.e., CPUE and fish community studies) are realistic if maximum achievable sample sizes are 5-10 fishing locations per area.

Final reports for the 1997 studies must report SD and absolute differences for CI designs; these data allow readers to make their own *a posteriori* power analyses. For example, differences between areas, expressed as % increases in exposed areas relative to reference, and their confidence limits, could be reported (Paine et al., 1996). Correlation matrices, or correlations between *C*, *T* and *B* variables and principal components or other composite variables derived from them, are routinely provided in Triad studies (e.g., Chapman et al., 1992; SeaConsult and EVS, 1996). A table of selected correlations and their confidence limits, based on the sample sizes used, would be a useful addition to Triad studies.

Table 3-2. Sample sizes (*n*) recommended for 1997 studies.

DESIGN	HYPOTHESES TESTED	UNIT OF REPLICATION	RECOMMENDED *N*	
			EXPOSED AREA (N_E)	REFERENCE AREA (N_R)
Gradient	H1, H9 - H11	Stations	≥15	5
CI	H2-4; H7, H8	Individual fish	≥20 per sex*	≥20 per sex*
	H5, H6	Fishing locations	5-10	5-10

* cf: Section 3.3.7.

Given limited costs, the important issues for finalization of the 1997 field plans and sample sizes will be:

- the total budget available
- assuming limited budgets, the importance of each hypothesis relative to the costs of testing that hypothesis (= cost allocation among hypotheses)

Some assumptions about cost allocation have been made in this report, but the ultimate decisions must be made by the client. However, we agree with Green et al. (1993):

> *When cost limits the number of variables and/or replicates which can be used, and compromises must be made, the number of variables (which could mean the number of hypotheses in this project) should be reduced rather than the number of replicates.*

For that reason, EVS, ESP and JWEL (1996h) includes an evaluation of variables as well as sites.

Regardless of the sample sizes finally selected, power can be increased by manipulating the other variables which influence power (i.e., α and SD). Major recommendations made in this section and elsewhere include:

- use one-tail rather than two-tail tests when appropriate
- composite water, sediment and benthos samples within stations whenever the composites can be analyzed for the same cost as single samples
- pool several catches within fishing locations if additional catches can be collected and counted with minimal effort
- use composite variables (e.g., community metrics based on many species; principal components based on concentrations of all metals) when possible.

Multiple mine sites are another form of increased replication, and in some cases, formal tests of hypotheses can be made using results from more than one site (Appendix A).

Finally, we were required to address the question of whether replication within stations should be used. The short answer is no; it will almost always be more effective to add more stations rather than to replicate within stations.

3.3 FIELD SAMPLING PLAN

3.3.1 Overview

The 1997 survey program builds upon information and data collected during the 1996 preliminary surveys. Generic methods for sampling each parameter or component are described in this section. Site-specific details and information are provided in subsequent sections. It should be noted that in some respects the 1997 survey will require additional field reconnaissance before sampling begins. For example, in 1997 it might be necessary for the contractor to conduct a study to determine the exposure area in order to establish a gradient (i.e., from high exposure to low exposure). This may require measuring conductivity downstream from the effluent discharge point, as conductivity is generally correlated with metals (NDM, 1996). In addition, it will be necessary to undertake some habitat characterization and field water quality measurements (i.e., conductivity) to assist with the sample station selection process.

Field program components:

The field program components to be undertaken in 1997 are:

Confirmation of reference and exposure areas
- (determination of an exposure gradient using field conductivity measurements and finalization of site-specific designs)

Habitat characterization and selection of sampling locations

Receiving Water
- Sample collection
- Chemical analysis

Sediments
- Sample collection
- Chemical analysis
- Toxicity characterization

Benthos
- Collection
- Identification and enumeration

Fisheries
- Collection
- Data and catch analysis
- Tissue analysis (metals and metallothionein)

Effluent
- Sample collection
- Toxicity characterization
- Chemical characterization

Sampling Schedules:

General recommended timing for events is outlined below. This may be modified for site specific considerations (cf. Section 4.0). Detailed generic descriptions of the various field components are provided in the following subsections.

3.3.2 Selection of Multiple Exposure and Reference Areas

The original study design for the 1997 program suggested that approximately 8 - 10 reference areas and 3 - 5 exposure areas be sampled. However, optimal sample allocation is not the same for each of the study designs recommended in Section 3.2. To optimize sample allocation in a control-impact (C-I) design, equal replication within each area is advised.

3.3.3 Habitat Characterization and Classification

Characterization of habitat and substrate should be conducted at stations coincident with water and sediment chemistry and benthos. If there are large differences in habitat type among stations (e.g., pool habitat at one station vs. riffle at another), we recommend that habitat characterization be conducted 25 meters upstream and 25 meters downstream at each station. If, however, there is little variation in habitat, then less time should be devoted to this task. Information on habitat characterization collected during 1996 should be verified to ensure that habitat changes have not occurred due to spring flood conditions, construction activities, etc.

Characterization of receiving environments should be done using the Department of Fisheries and Oceans (DFO) and the New Brunswick Department of Natural Resources and Energy (NBDNRE) habitat characterization form (EVS, ESP and JWEL, 1996a-g). Based on the substrate types identified in the habitat characterization, the study area should be classified into constituent habitats based on the habitat classification scheme of Cowardin et al. (1979) developed for the U.S. Fish and Wildlife Service. Full details are provided in Appendices B and C.

Deliverables:

Detailed habitat maps should be produced at a scale of approximately 1:2,000. Example habitat maps are provided in EVS, ESP and JWEL (1996a-g). Similar formats and legends should be followed for consistency.

Timing:

Habitat maps should be prepared when aquatic and riparian vegetation is clearly developed. This would generally be from early summer to mid autumn, which would also allow for habitat characterization of streams and rivers affected by snowmelt. Very early spring should be avoided if possible because of high-discharge events, but there are no other major timing constraints for habitat mapping.

3.3.4 Water Chemistry

Composite grab water samples should be collected from reference and exposure stations. The number of stations will depend on the hypotheses being tested (Section 3.2). Historical water sampling stations should be used wherever possible.

The following paragraphs describe collection, filtration and preservation of surface water samples for metals analyses, based on procedures used in 1996. Note that AETE is presently contracting with the Geological Survey of Canada (GSC) to review and possibly update these procedures. This information should be available prior to commencement of the 1997 field studies.

In shallow receiving environments (<2 m), one grab sample should be collected at the surface from each station with bottles prepared by the analytical laboratory. Clean techniques must be used at all times to minimize sources of contamination. Samples should be collected in bottles which have been triplicate rinsed in sample media, by submerging the container, removing the cap below the surface to avoid any surface contamination, and completely filling. In deeper receiving environments (>2 m), sub-surface (i.e., either mid-depth or at 1 m from bottom and 1 m from surface) grab samples should be collected using a Van Dorn-type sampler.

Separate samples should be collected for total and dissolved (i.e., operationally defined as water filtered through a 0.45 μm filter) metals. The dissolved sample should be field-filtered according to standard methods (APHA, 1995 -Section 3030B). We strongly recommend (cf. Section 3.5.1) that the laboratory conducting chemical analysis provide all contractors with the same filtering apparatus for collection of the dissolved filtrate. This would include the same type of filter pump and pre-treated (soaked in HNO_3) filters that are individually

wrapped. In addition, sample bottles should not be pre-loaded with acid preservative. Instead the laboratory should provide individual ampules of preservative that are added to sample bottles. Such precautionary procedures are necessary to limit variability of dissolved metals analyses. Both metals samples (total and dissolved) need to be acidified with ultrapure HNO_3 (provided by the analytical laboratory) to pH <2. All samples should be cooled as rapidly as possible and shipped on ice, with a minimum exposure to light, to the laboratory for analysis.

Field measurements of temperature, conductivity, dissolved oxygen, and pH should be taken at each station sampled using, for example, a Hydrolab H20 multiprobe or YSI meters. Before sample collection, conductivity measurements should be taken at individual mine sites to characterize mixing zones and exposure zones, and to identify other possible sources of contaminants to the receiving environment. This analysis is important in determining whether there is a gradient of exposure in the receiving environment, as there is a strong correlation between conductivity and metal levels (NDM, 1996). For example, at the Sullivan mine site conductivity was positively and strongly correlated with Cu (r^2= 0.61) and Zn (r^2=0.62) metal levels (EVS, ESP and JWEL, 1996b). At the Myra Falls mine site, conductivity was strongly and positively (r^2 = 0.84) with strontium (EVS, ESP and JWEL, 1996a). Therefore, this approach potentially offers an inexpensive, but accurate means to delineate the exposure area. All field instruments should be calibrated prior to use and values recorded manually in the field. In addition, back-up probes and extra supplies (e.g., membranes, reference solutions) should be available at each field site.

QA/QC:

At each mine site QA/QC of receiving water chemistry should include collection and analysis of transport or trip blanks, filter blanks and field replicates (collected at the exposure station closest to the effluent discharge). If sub-surface samples are collected using a Van Dorn-type sampler, then sampler blanks should also be collected. We also recommend the following additional control procedures: (1) Split samples from the exposure area sent to at least one independent laboratory to verify accuracy of the project laboratory, (2) de-ionized, distilled water samples spiked with reference toxicant sent to the project laboratory and at least one independent laboratory, and (3) analysis conducted on distilled water, filters, and acids used for sample preservation.

The transport blank and filter blank water should be provided by the analytical laboratory. Detailed QA/QC procedures are provided in Appendix B.

3.3.5 Sediment Chemistry

When possible, sediment samples should be collected in conjunction with water chemistry samples and only if a station has an extensive area of depositional habitat. The substrate in this depositional habitat will be composed of fine-grained particles as this material

preferentially binds metals. In erosional ecosystems where fine-grained sediment are rare, we suggest that periphyton or macrophytes be analyzed for metals as an alternative, as this material may be a significant source of metals for aquatic insects. For example, we observed substantial growths of green algae at the St. Mary River in the reference and exposure area, whereas we observed very few areas that contained fine-grained sediment (EVS, ESP and JWEL, 1996b). Two approaches for collection of algae are possible and in both cases composites are recommended: (1) collect scrapings of periphyton from natural substrate using a template such as a disc, or (2) collect scrapings from artificial substrates, such as glass slides or glazed-ceramic tiles. This material should be analyzed for inorganic and organic matter dry weight and for metals. We do not recommended the use of artificial substrates unless two sampling trips are planned, because their use would require two trips (one to place them in the stream, one to collect them). For collection of macrophytes, whole plants of the same species should be collected from the reference and exposure areas. The important point here is that collection of plant material in erosional ecosystems will allow the contractor in 1997 to integrate measures (e.g., Is there a relationship between metal levels in plant tissue and benthic invertebrate community structure?) which could not be done otherwise in the absence of sediment. Note that this alternative would require more development for use in the 1997 field studies and is not included in the estimated costs (Appendix D).

The sampling device to be used for sediment collection will depend on the nature of the substrate (e.g., pole-mounted Eckman in shallow, soft substrate; Petite Ponar in deep, compact substrates). Sampling devices should be of stainless steel construction. Detailed notes should be made on the most suitable sediment sampler to be used at each site. Only the upper approximately 2 cm (i.e., oxic sediments) of the sediment column should be used and the desired sampler penetration should be to at least 4-5 cm depth to ensure the upper 2 cm is not disturbed. One composite sediment sample should be collected per station, consisting of five replicate grabs. The upper 2 cm of substrate from each of the five grabs should be the portion of the grab targeted for collection. This surficial layer from each grab should be placed in a glass mixing bowl and homogenized with a plastic spoon.

Samples for AVS analyses should be handled as follows (Landis Hare, INRS, pers. comm.). Place sediments in a labelled Whirlpack bag, flatten and roll up to eliminate all air, then place the bag in a larger plastic bag filled with previously collected anoxic sediment. Many Whirlpack bags can be placed in the same larger bag. Store the larger bag at 4°C if analysis will be reasonably rapid. If not, freeze the larger bag. The large volume of anoxic sediment serves to preserve sediment samples in their Whirlpack bags.

Sample jars provided by the laboratory (i.e., pre-cleaned glass with Teflon-lined lids) should be filled to the top to minimize air space. Duplicate jars should be collected at all stations in case of breakage.

Mixing bowls and plastic utensils should be cleaned between sampling stations using the following protocol: a) water rinse, b) phosphate-free soap wash, c) deionized water rinse, d) 5% HNO_3 rinse, and e) deionized water rinse. The following guidelines should be used to determine the acceptability of a grab sample: a) the sampler is not over-filled, b) overlying water is present indicating minimal leakage, c) overlying water is not excessively turbid indicating minimal disturbance, d) the desired penetration depth is achieved (i.e., at least 4-5 cm for a 2 cm deep surficial sample). If all the above criteria are not met, the sample should be rejected.

All samples should be cooled as rapidly as possible and shipped on ice, with a minimum exposure to light, to the laboratory for analyses. Each sample should be analysed for site specific metals, total organic carbon, particle size and loss on ignition.

QA/QC:

Samplers should be cleaned between sampling stations using a phosphate-free detergent wash and a rinse with de-ionized water. A swipe blank should be collected to determine the effectiveness of field decontamination procedures (e.g., an acid-wetted, ashless filter paper should be used to wipe down any sampler and mixing bowl/spoon surfaces likely to contact sample media). Similar to water sample collection, field samples should be split as well as spiking sediment collected from a reference area with a reference toxicant, and these samples sent to at least one independent laboratory for analysis. The use of powdered latex gloves should be avoided since the powder contains zinc and other contaminants. Full details of QA/QC procedures are provided in Appendix B.

3.3.6 Benthos

When possible, benthic samples should be collected from reference and exposure areas in conjunction with water chemistry and sediment samples. The same person should collect benthic samples at each mine site to ensure consistency.

As noted in Appendix A, pooling of several small samples is recommended to obtain:

- sufficient organisms per sample from soft sediments to analyze statistically
- a better estimate of the mean abundances from riffle stations by pooling several small, spatially separated samples from within each station.

The number of replicate benthic samples and the number of sampling stations will depend on site-specific study designs. Samples from each station should be collected from similar habitat types, as per Section 3.3.3, using a quantitative sampler (e.g., Hess) with a 250 μm mesh net (Taylor, 1996). In general it is better to collect more, smaller samples, rather than

fewer, larger samples (Taylor, 1996). Substrate within the area of the sampler should be disturbed to a depth of 5 cm and each rock within the sampler area thoroughly scrubbed clean of invertebrates. In deeper habitats, an Eckman/Ponar grab sampler should be used for sample collection, with these samples passed through 250 μm mesh sieve. Every effort should be made to minimize the amount of inorganic and organic detritus placed into a sample jar. This will allow for a shorter processing time by the benthic taxonomist(s) which, in turn, will minimize costs.

All benthic samples should be placed into plastic containers and preserved in 10% buffered formalin. Invertebrates in each sample should be counted and identified to the lowest practical level. For most situations this means to the genus level. Identification to species may be necessary for some chironomids to differentiate between exposure and reference areas.

QA/QC:

QA/QC for benthic invertebrate sample analyses should include: a) 10% resort of samples to confirm 95% sorting efficiency, b) 10% of sub-sampled sample for determination of sub-sampling error, c) separation of sorted and sorted fractions, and d) development of a voucher collection. Details of the QA/QC procedures to be followed are provided in Appendix B.

Timing:

The biomass and diversity of the benthic invertebrate community is generally highest in early spring before emergence has occurred, or in late autumn prior to freeze up.

3.3.7 Fisheries

One to two sentinel fish species should be selected at each mine site based upon the available historical data and results of the 1996 preliminary survey. One species will reduce the processing time but more than one species may be required to ensure collection of a sensitive species or good indicator. The sentinel species should be abundant at both reference and exposure areas.

The sentinel fish species may consist of a forage species including minnows. These are suitable for determination of growth rates, age to maturity, etc. Greater precision in measuring standard variables (e.g., length, weights, organ size) will be required if using a small forage fish as one of the indicator species. Benthic species may be more suitable sentinel species than pelagic ones at sites that receive intermittent effluent discharge (i.e., Lupin) or where sediment is the most important route of metal exposure, because at these sites metal accumulation via prey items (benthic invertebrates) is probably more important than accumulation via water exposure.

Nets should be used in deep water habitats while a backpack electroshocker and barrier nets should be used where appropriate in shallow streams. In large streams where appropriate, we recommend that multiple shockers be used to increase sampling efficiency. Specifically, a line of shockers should simultaneously sweep a stream section with a line of netters immediately behind them to collect stunned fish. Based on 1996 fish capture success, the 1997 contractors will need to place considerable effort (time) into collecting enough fish for analysis. It is quite possible that fish sampling will require several days to collect fish. The techniques used should catch all size and age classes of the target species, as this is not just an adult fish survey. Capture of all size and age classes will permit more definitive measures of growth (size at age) rates and age at maturity.

For biological characteristics (e.g. length, weights, ages - H7, H8) a minimum of 20 fish of each sex should be captured from each area. Data collection details are provided in Appendix B (Section B4.2.6). Fish can be kept alive in aerated buckets for immediate processing. If delays occur, fish can be held in nets at their collection site. Frequent inspection is recommended to ensure protection from predators.

For metal and metallothionein analysis a minimum of 10 fish should be sampled from each area. Fish for metallothionein analysis must be captured alive, and processed after being freshly killed. It is imperative that tissues be placed on dry ice immediately and kept frozen until delivery to the laboratory. Dry ice evaporates quickly so each cooler must have enough ice to maintain frozen fish until arrival at the analytical laboratory. In 1996 substantial quantities of dry ice (100-150 lbs) were used due to rapid sublimation.

When possible equal numbers of females and males of each species should be collected. If the fish are large enough (e.g., >15 cm), tissue samples should be split for metals and metallothionein analyses. Smaller fish should be frozen whole. Samples for metallothionein analyses should be shipped on dry ice to Dr. J. F. Klaverkamp, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, R3T 2N6. Before and during dissection, fish health characteristics should be noted for only the fish sacrificed for metals analysis using methods outlined in Adams et al. (1993).

Livers and gonads should be dissected and removed from at least 20 males and 20 females for accurate weight measurements. These values are used for calculation of Gonadal Somatic Index (GSI) and Liver Somatic Index (LSI). A balance accurate to at least three decimal places is required. In addition, aging structures (scales, fin rays, otoliths) should be collected for analyses.

Timing:

Many potential sentinel fish species are spring spawners. Species such as lake trout, brook trout and lake whitefish are fall spawners. If gonad measurements are required, then it is

necessary to sample the fish either prior to spawning (very early spring) or in very late fall when the gonads are developing for the following year. There may be some "ethical" concerns regarding the capture of ripe fish in spawning condition particularly for sportfish species (e.g., pickerel, northern pike), but these concerns may not be expressed by local agencies for forage or coarse fish species (e.g., suckers).

It would be useful if fish could be sampled on two occasions during the season, one corresponding to sampling for tissues (e.g., gonads) and at one other time. The two samplings would provide a more reliable indicator of species availability and relative abundance through catch per unit effort (CPUE) measures than a single survey. The use of various habitats by fish can vary substantially throughout the season and sampling on separate occasions would address some of this variability. However, because of the additional costs, the site specific study designs (Section 4.0) have been restricted to a single sampling.

QA/QC:

Approximately 10% of the aging structures should be sent out and verified by independent sources. Additional considerations are provided in Appendix B.

3.3.8 Effluent Samples

The following sublethal toxicity tests should be performed in 1997:

Lemna minor growth inhibition
Ceriodaphnia dubia survival and reproduction,
Fathead minnow survival and growth inhibition
Selenastrum capricornutum microplate growth inhibition test.

The four toxicity tests are recommended for the following reasons: (1) they represent a test battery, which concept (at least 3 different tests) is recommended by regulatory agencies including Environment Canada and throughout the scientific literature; (2) they include all key taxa: fish (fathead minnow), invertebrates (*C. dubia*), algae (*Selenastrum*) and plants (*Lemna*); (3) three of the tests are arguably the most widely used, standardized, "validated", and accepted regulatory toxicity tests in North America (fathead minnow, *C. dubia*, *Selenastrum*), the other is seeing increasing use and promise for this purpose (*Lemna* - Petersen et al., 1996); (4) all of these tests are cost-effective (i.e., do not require a great deal of sample and are relatively short-term; (5) in addition to measures of mortality, these tests measure bioenergetics responses in the form of growth and survival - information on survival, growth and reproduction of individuals is necessary to extrapolate to persistence and viability of populations: (6) all of these tests showed some response to at least some of the effluents tested in 1996; (7) none of these tests showed an inordinate level of test failures

in 1996. The rainbow trout embryo tests is not recommended because: (1) fish responses are already measured by fathead minnows; (2) the test continues to show a surprising level of control failures with no certainty that this will be alleviated in 1997 resulting in useful data; (3) the test as presently recommended by Environment Canada requires inordinate amounts of sample.

Receiving water samples should be collected from reference stations prior to commencement of the field program. Samples should be collected after the "spring flood" has dissipated to avoid either concentrations of toxins or samples that have been highly diluted by melt water (e.g., for Dome acceptable timing is after June 01). Such samples are necessary to determine if receiving waters cause toxicity to either *Ceriodaphnia dubia* or fathead minnow and, if so, to acclimate these organisms to the water before toxicity evaluation.

Upon commencement of the field programs, receiving water samples should be collected from the reference sites and shipped cooled to the biological testing laboratory for use as dilution water during the actual toxicity tests.

Effluent samples should be collected end-of-pipe if possible and must be shipped within 72 hours of sampling to the biological testing laboratory. All sample containers, chain of custody forms should be provided by the biological testing facility.

Timing:

To account for possible seasonal or temporal variation in effluent quality it is recommended that effluent be collected on three occasions (early spring, summer, autumn) in 1997 and characterized for toxicity and chemistry. Note that the quality of effluent held during the winter may vary substantially compared to that of effluent at the end of summer.

Collection of effluent requires close coordination with mine personnel. Even mines with continuous discharge may stop discharging under certain circumstances.

3.4 LABORATORY ANALYSIS PLAN

3.4.1 Physical Measurements and Water Chemistry

Water chemistry samples should be analysed for general chemistry and total and dissolved metals. General chemistry parameters to be analysed should be determined on a site specific basis (Section 4.0), and may include cations, anions, nutrients, hardness, alkalinity, dissolved organic and inorganic carbon, turbidity, total suspended solids, pH and conductivity. Total and dissolved metals to be analysed should also be determined on a site specific basis and

will include the most prevalent metals (as determined by historical results and results of the 1996 field surveys). Target detection limits should be 1/10 of the CCME guidelines for protection of the aquatic environment, where appropriate.

QA/QC samples (i.e., trip blanks, filter blanks, field replicates and sampler blanks) should be analysed for site specific general chemistry and total and dissolved metals parameters as described above. Results of the QC samples should be reviewed by the QCO (Quality Control Officer) to ensure DQO (Data Quality Objectives) are met (Appendix B).

3.4.2 Sediments

Sediment Chemistry:

Sediment chemistry samples should be analysed for site specific metals (Section 4.0), total organic carbon, particle size distribution and loss on ignition. Sediment metals should be normalized, which can be to percent fines as per the following equation:

$$Metal_{NF} = Metals / Fines$$

where: $Metal_{NF}$ = Metal concentration normalized to fines
Metal = Reported sediment metal concentration
Fines = Proportion (i.e., 80% = 0.8) of fines (i.e., silt + clay fractions) in sediment.

An example of this normalization for reported copper concentrations of 45 mg/kg dw with grain size of 65% silt and 5% clay is as follows:

$$Metal_{NF} = 45 \text{ mg Cu/kg Sed dw} / 0.7 \text{ Fines/Sed dw} = 64.3 \text{ mg Cu/kg Fines}$$

A regression-based (i.e., ANCOVA) approach is however, more useful (cf. Hebert and Keenleyside, 1995; Appendix A 1.1).

Transformation of metals data (if needed) should occur after normalization. Swipe blanks should be analyzed for site specific metals to determine the effectiveness of field decontamination procedures.

Sediment Toxicity:

Sediment samples should be collected for toxicity testing where possible and appropriate (cf. Section 4.0). Details of sample collection are outlined in Section 3.3. The location for sample collection at each mine site is specified in the site specific study designs (Section 4.0). The following sediment toxicity tests should be conducted at each mine site using individual

replicate samples of the upper 2 cm of sediment from the same five replicate grabs used for sediment:

- *Hyalella azteca* (14 day amphipod test);
- *Tubifex tubifex* (28 day oligochaete reproductive test); and
- *Chironomus riparius* (10 day survival and growth test).

The method employed for each test should follow accepted, published protocols with specific Standard Operating Procedures (SOPs) specified by the selected testing laboratory.

3.4.3 Benthic Invertebrates

Benthic invertebrate samples should be sent to the same taxonomist to ensure consistent sample processing, enumeration and quality control. When samples arrive at the laboratory, they should be checked for adequate preservation and proper labelling before being logged and kept secure. All benthic samples should be sorted with the use of a stereo microscope (10X) and keyed to the generic level. To expedite sorting, all samples may be stained with a protein dye that is absorbed by aquatic organisms but not by organic material such as detritus and algae. Samples should be washed through a 250 μm sieve and sorted entirely except in the following instances: those samples with large amounts of loose organic matter, and samples with high densities (>100) of major taxa. In these cases, samples should first be washed through a large mesh size sieve (e.g., 3.36 mm) to remove all coarse detritus, leaves, and rocks. Large organisms such as leeches, crayfish, and molluscs, retained in the sieve should be removed from the associated debris. The remaining sample fraction should be subsampled before sorting (cf. Appendix B). For those samples that are subsampled, sorted and unsorted fractions should be represerved separately. Sorted organisms should be placed in 1 oz. glass bottles and represerved in 80% ethanol. Each bottle should be labelled externally and internally with survey name, date, station and replicate number, and sorter's name.

Chironomids and oligochaetes should be mounted on glass slides in a clearing medium prior to identification. In samples with large numbers of oligochaetes and chironomids, a random sample of no less than 20% of the picked individuals from each group should be removed from the sample for identification, up to a maximum of 100 individuals. Following identification and enumeration, a detailed species list should be prepared for each station and replicate, summarizing the total organism density and total number of taxa. The species list should be in a standard spreadsheet format and of a high quality, ready for submission in final reports.

Prior to commencement of the field programs, the QCO should discuss the objectives of the benthic invertebrate sampling program, field sampling and sieving protocols (SOPs), analyses protocols and DQO with the analytical laboratory. The quality control officer must

ensure that the required level of taxonomic resolution is made known to the analytical laboratory well in advance. Quality control protocols should include:

- Use of the most updated and widely used taxonomic keys for all taxonomy.
- Confirmation of sorting efficiency. Ten percent of processed samples should be resorted by a second person to confirm 95% recovery of all organisms.
- Estimation of subsampling error in a minimum of 20% of samples subjected to subsampling. This should be accomplished by entirely sorting 20% of the samples that were subjected to subsampling.
- Verification of taxonomy by an independent expert.
- Retention of all unsorted and sorted fractions of samples until taxonomy and sorting efficiency are confirmed.
- Preparation of a voucher collection of identified organisms for both reference and exposure stations.
- Review of all tabulated benthic data to ensure there have been no data entry errors or incorrect spelling of scientific nomenclature.

Note that the AETE is conducting a technical evaluation of QA/QC for benthic invertebrates. This will be completed by April 1997.

3.4.4 Fish

Fish Population:

Fish age should be determined by the appropriate structure (scales, otoliths, pectoral spines) following established protocols. The following quality control protocols will be followed for the fisheries assessment:

- All aging structures should be sent to the same laboratory for analyses to ensure consistency and quality control;

- Aging structures should be archived for future reference - 10% of the structures from each mine site should be verified for age by an independent expert; and

- A sample numbering system should be designed to facilitate tracking of age structures and tissue samples taken from the same fish.

Fish Tissue:

Tissue processing protocols and laboratory analyses of fish tissues for metals and metallothionein should follow procedures used in the 1996 preliminary site surveys (EVS, ESP and JWEL, 1996a-g) as directed by Dr. J.F. Klaverkamp of the Freshwater Institute,

Winnipeg, Manitoba. For quality control a sample numbering system should be designed to facilitate tracking of all tissue sub-samples taken from the same fish.

3.4.5 Effluent Chemistry and Sublethal Toxicity

Chemistry:

Samples of effluent and receiving water for chemical analyses should be collected synoptically with collection of samples for sublethal toxicity testing. Samples for both general chemistry and for total and dissolved metals should be collected. General chemistry parameters to be analysed will be determined on a site specific basis (Section 4.0), and may include cations, anions, nutrients, hardness, alkalinity, dissolved organic and inorganic carbon, turbidity, total suspended solids, pH and conductivity. Total and dissolved metals to be analysed will also be determined on a site specific basis and will include the most prevalent metals (as determined by historical results and results of the 1996 field surveys). Target detection limits should be 1/10 of the CCME guidelines for protection of the aquatic environment, where appropriate.

Sublethal Toxicity Testing:

Samples of effluent and receiving water should be collected for sublethal toxicity testing. The frequency of sample collection and the volume of media required is outlined in Section 3.3.8. The location for sample collection at each mine site is specified in the site specific study designs (Section 4.0).

3.5 QA/QC

Full details of QA/QC are provided in Appendix B. The purpose of this section is not to repeat information provided elsewhere, but rather to highlight specific issues arising from the 1996 field studies that need to be considered in 1997.

3.5.1 Laboratories

General Issues:

Due to the highly integrated nature of the AETE Program, it is essential that all laboratories subcontracted to provide analytical services (e.g., chemistry, toxicity, benthos, fish aging, metallothionein) be familiar with the scope and objectives of the overall AETE Program and the specific field projects. It is recommended for the 1997 field program that:

- Initiation meetings or conference calls be held between the project manager(s) and contact personnel for analytical laboratories providing services. The scope of such interactions should include field schedules, field and laboratory protocols, QA/QC, deliverables, and any problems with recommended solutions.

- A single contact should be identified for each analytical laboratory to ensure a one-window, coordinated approach to communication and problem resolution.

Chemical Analytical Laboratory:

One of the key issues in any field study is the choice of chemical analytical laboratory. The 1996 preliminary field surveys proved to be a challenge as regards maintaining a high standard of quality control with respect to the laboratory chosen for this work. Despite the challenges, we recommend that a single chemical analytical laboratory also be used in 1997. However, for the 1997 studies we also recommend that:

- The contract with any analytical laboratory include a performance clause with penalties for not meeting QA/QC requirements.

- The performance of the analytical laboratory be checked by means including, at a minimum, blanks and duplicates sent to at least one other laboratory.

- All field sampling equipment connected with the analytical laboratory be supplied and certified by that laboratory (e.g., sample containers and distilled water; if field filtration is done, all necessary items including preservatives, filters and filter holders).

In addition, we recommend that sample screening be instituted as outlined below relative to the issue of dissolved versus total metal concentrations. Specifically, the raw data from the 1996 monitoring program (EVS, ESP and JWEL, 1996a-g) show that, in some cases, dissolved metals concentrations were reported at levels greater than the total recoverable metals concentrations from the same samples. The reason(s) for this difference are unknown. This apparent inconsistency could result from factors such as contamination of the dissolved sample during field filtering or improper laboratory procedures, or it could result from the overlap of analytical tolerances associated with the total recoverable and dissolved laboratory analyses. Because of the latter factor, dissolved levels can in fact be reported at levels higher than total recoverable levels in some instances. However, there is a limit to this difference.

All laboratory analyses have tolerance(s) associated with the reported values. Thus, the actual concentration is within the range of the reported value plus or minus the associated tolerance. The lower the reported concentration relative to the laboratory reporting limit, the greater the impact of the analytical accuracy. For example, the stated accuracy for a value at the

laboratory reporting limit should be approximately in the range of plus or minus 25 to 50 percent, whereas the stated accuracy for a value an order of magnitude higher should be approximately plus or minus 15 percent.

Data near the laboratory reporting limit represent the worst-case for possible reporting of dissolved metals concentrations greater than total metals concentrations. At concentrations near the laboratory reporting limit, a dissolved constituent could be under-reported by as much as 50 percent. To illustrate this situation, consider a metal which is present completely in a dissolved form, at 1.0 concentration units, with analytical error at the maximum tolerance limits:

Actual Total and Dissolved Concentrations	=	1.0
Reported Total Recoverable Concentration	=	0.5
Reported Dissolved Concentration	=	1.5

Ratio of Reported Dissolved to Total Recoverable Concentrations = $1.5 \div 0.5 = 3.0$

Thus, reported dissolved metal concentrations could exceed the total recoverable metal concentration by a factor of up to 3.0, and still be within the tolerance range of the analyses. Dissolved to total recoverable metal ratios in excess of 3.0, however, would be invalid in any case. Therefore, we recommend that the database produced in 1997 be screened to identify all cases where dissolved metals levels exceeded total recoverable metals concentrations by a factor exceeding 3.0. Note that this screening criterion is very conservative and only addresses a stated laboratory accuracy of 50 percent.

The dissolved metals values identified by this screening should be removed from the database. The total recoverable metals concentrations should be left as part of the database for subsequent statistical analysis.

3.5.2 Couriers

Courier pickups and deliveries are always problematic from remote locations, especially where samples are time-limited. Such problems can never be eliminated but can be minimized by:

- Shipping samples as early as possible in field programs to allow for recollection and resending in case of courier failures.

- Maintaining a one-window approach for communications between couriers and field crews. The field team leader should be in direct contact with couriers and should provide all necessary information on the scope of the project and the necessity for guaranteed delivery to ensure that sample submission time frames are met.

4.0
SITE-SPECIFIC STUDY DESIGNS

4.1 MYRA FALLS, BC

4.1.1 Hypotheses

The Myra Falls mine was selected for the 1997 AETE Program because of its apparent suitability to test many of the hypotheses listed in Table 3-1 (EVS, ESP and JWEL, 1996h). Previous success with fisheries studies indicate that many of the hypotheses are testable at this site.

Table 4-1 summarizes the rationale for selection of hypotheses for the 1997 AETE Program. Decisions regarding specific hypotheses were based on the following:

- Study design options and statistical considerations (as presented in Section 3.2).
- Data and field observations from the 1996 AETE Program - Myra Falls mine (EVS, ESP and JWEL, 1996a).
- Data and field descriptions from historical monitoring programs (reviewed in EVS, ESP and JWEL, 1996a).

4.1.2 Study Design

General Considerations:

We recommend that the monitoring program at the Myra Creek site focus on Buttle Lake and Upper Campbell Lake rather than the immediate receiving environment in Myra Creek. Factors influencing this recommendation include:

- The importance of fish populations in Buttle Lake.
- The presence of a physical barrier which prevents fish movements into Myra Creek.
- The absence of barriers to movement within Myra Creek and the potential for free movements of resident fish populations between areas above and below the mine.

Table 4-1. Hypotheses for 1997 AETE Program - Myra Falls Mine.

No	Hypothesis	Study Design (CI or Gradient)	Testable? (Y,N, or Partial)	Comments
H2	Metals in fish tissues	CI	Y	H2 - H4: Fishing success in 1996 was limited due to restriction of the fish collection licence; we recommend the use of rainbow trout followed by cutthroat and Dolly Varden as sentinel species based on the 1996 study and previous studies.
H3	Metallothionein in fish tissues	CI	Y	
H4	Metals vs. metallothionein in fish tissues	CI	Y	
H5	Fish - CPUE	CI	P	H5/H6: Increased fishing effort should allow these hypotheses to be addressed.
H6	Fish - community	CI	P	
H7	Fish - growth	CI	P	Same as H2 - H4.
H8	Fish - organ/fish size	CI	P	Same as H2 - H4.
H9	Water quality and biology	Gradient*	Y	We recommend that zooplankton be used rather than benthos as locating habitat/substrate similar between the reference and exposure areas was not possible in 1996 and is assumed to be difficult in 1997.
H12	Metals or metallothionein vs. chemistry	CI*	Y	A gradient design may be better to test this hypothesis. However, fish populations may overlap gradients so likely only a CI design testable. Zooplankton (for metals vs. chemistry) could be considered if enough biomass could be collected. Likely only qualitatively testable.
H13	Chronic toxicity	CI	Y	May be difficult for fish populations, may be easier for zooplankton populations.

* Hypothesis can be analyzed using CI or gradient design; design selected to match site/parameter

Sample areas for control/impact and gradient designs in Buttle Lake and Upper Campbell Lake are given in Figure 4-1. While the study design is largely hypothesis-specific, there are important factors which must be considered in determining the overall design to ensure a fully-integrated and cost-effective study. For the Myra Falls Mine, these factors are as follows:

- An exposure gradient may be difficult to detect as the ranges of water chemistry parameters are great and overlap between historical exposure and reference areas (EVS, ESP and JWEL, 1996a). For example, a station near the mine discharge showed a distinct decrease over time for many metals and these ranges overlap with those at the northern end of Buttle Lake and southern end of Upper Campbell Lake.

- Integration of hypotheses into common designs: flexibility in design type (i.e., choice of CI or gradient) for certain hypotheses (e.g., for H9) allows a more complete integration of various study components.

- Reference areas: there was some difficulty interpreting results of the 1996 AETE Program (i.e., it was not clear whether differences were related to exposure or simply because other environmental factors are different between reference and exposure areas); this issue may be resolved by adding an additional reference area (i.e., CI design with two "control" areas) and/or by sampling more stations on a continuum (i.e., gradient approach).

- Timing of investigations: appropriate window(s) need to be identified for all study components.

- Additional survey requirements: in 1996 samples were only collected in a relatively small area of Buttle Lake north of the mouth of Myra Creek. Plume dispersion should be assessed using conductivity as an indicator; stations should be located across a gradient of conductivities.

Existence of an exposure gradient

Section 3.2 presents study design options for each of the 1997 hypotheses. The designs essentially fall into two categories: control/impact (CI) or gradient. The presence of an environmental quality gradient in a medium is required before selecting any gradient study design. The presence of a strong gradient in Buttle Lake is uncertain, as historical and 1996 station locations do not begin near the mouth of Myra Creek. Historical stations begin near Station E1 (from the 1996 study), two stations cover the middle of the lake and one occurs in the north end. Although the ranges over various study years overlap for some metals in the water column, we believe that a gradient does exist in the water column as one does exist for

Figure 4-1a. Site location map for Myra Falls Mine: Control/Impact Design

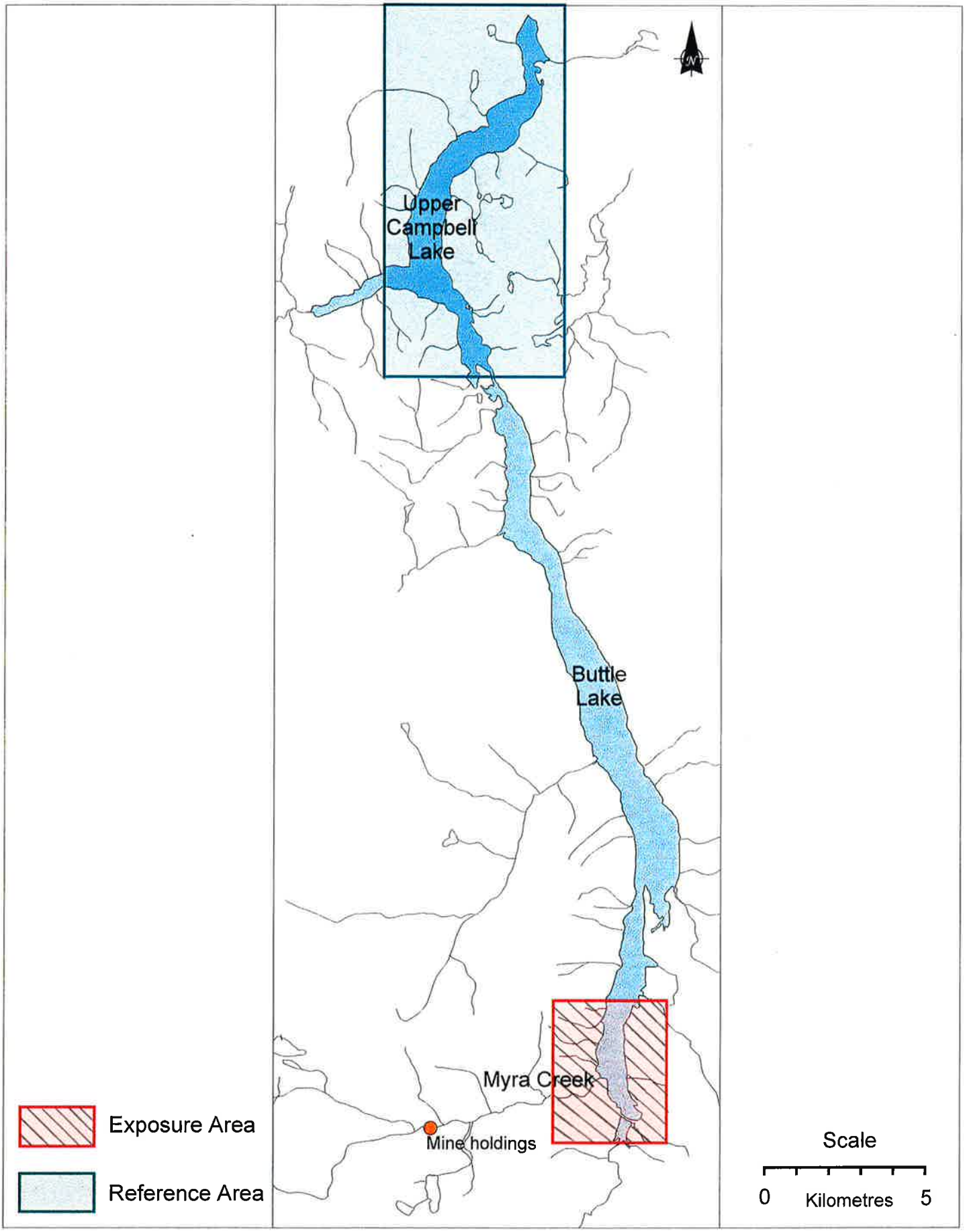
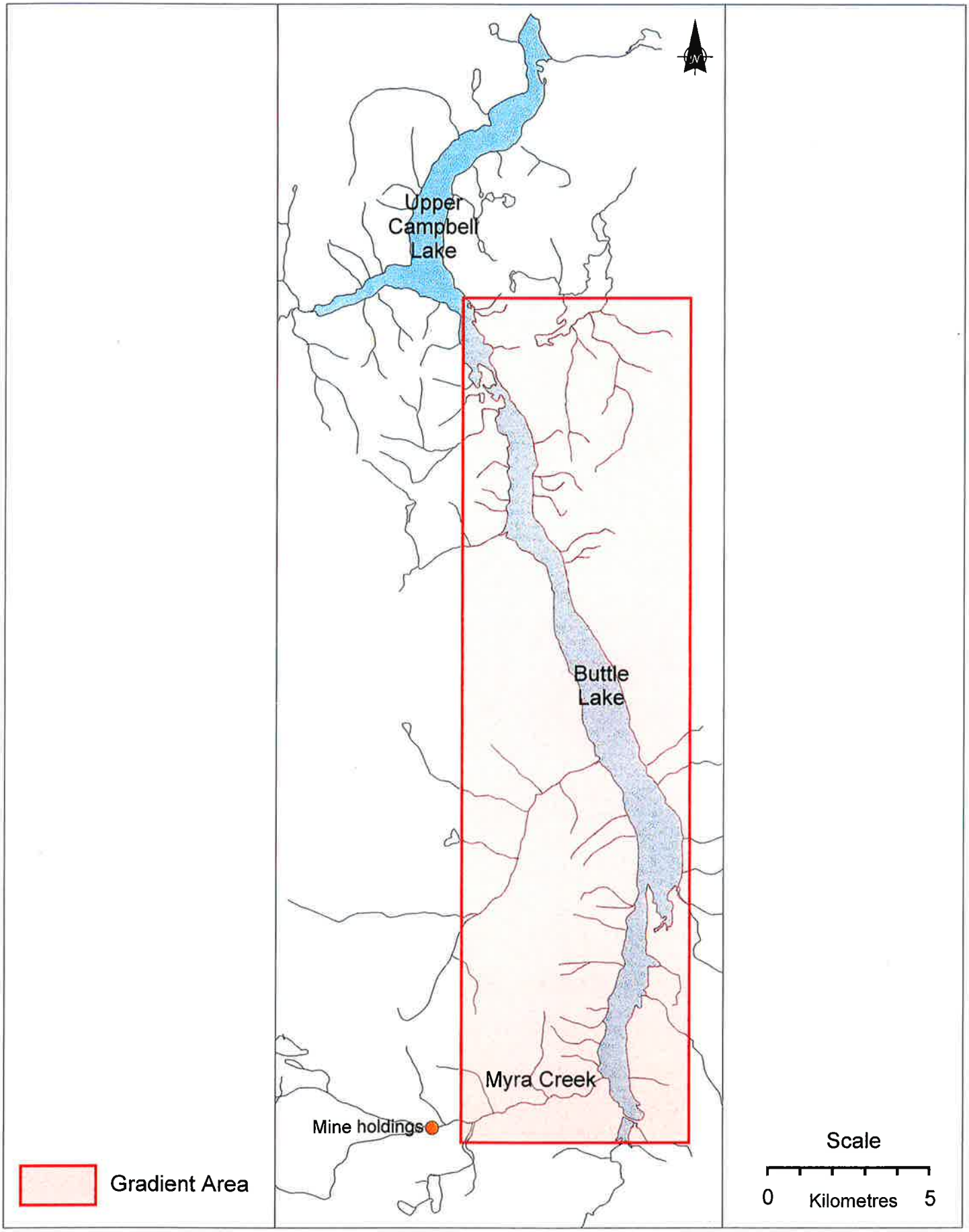


Figure 4-1b. Site location map for Myra Falls Mine: Gradient Design



the sediments (historically) in Buttle Lake (EVS, ESP and JWEL, 1996a). However, the range and strength of this gradient are not known and should be investigated in the field (using conductivity as a surrogate) prior to final station selection in the 1997 field program.

Integration of hypotheses into common designs

The hypotheses selected for analysis at the Myra Falls mine will drive the development of an integrated study design. Section 3.2 recommends that all hypotheses relating to fish (i.e., H2-H8, H12) use the CI design, which is consistent with the lack of barriers to fish in the study area (i.e., movement of fish would make a gradient approach difficult). If preliminary field measurements in 1997 support the existence of a gradient in water quality, H9 could be addressed using a gradient design. This would be appropriate if the zooplankton community were used as the biological component. However, if fish are used, or if there is no gradient, a CI design will have to be used. Note that if both CI and gradient designs are used in the study area to address different hypotheses, water sampling would have to be conducted at all stations.

Reference areas

The 1996 AETE Program at Myra Falls showed concentrations to be higher in the reference and lower in the exposure stations for Al, Pb, Mg, and Zn and the reverse for Mn, Sr, Ca and Na (EVS, ESP and JWEL, 1996a). Historical data have shown elevations for some parameters at the northern end of Buttle Lake; therefore, it may be useful to sample further down the system (i.e., northern end of Upper Campbell Lake) to ensure that the reference area has lower concentrations than the exposure area (i.e., sample an additional reference area to the 1996 reference area).

Timing of investigation

As the Buttle Lake system is part of a reservoir system for BC Hydro, the field survey should be timed for when a substantial change in water level is not likely to occur. For example, during the 1996 study the lake level dropped over a 30-45 cm. Fluctuating water levels may affect sample collection such that water depths may differ from one site to the next should water levels be fluctuating.

Hypothesis-Specific Designs:

Table 4-1 summarizes the 1997 hypotheses (cf. Table 3-1) which will be addressed in the 1997 AETE Program at Myra Falls mine. To facilitate explanation of study designs, specific hypotheses have been integrated into designs related to the main study components as follows:

- Fisheries: the CI design will be emphasized, with rainbow trout selected as the sentinel species.

- Effluent/receiving water quality: the gradient design will be emphasized, with enough stations to support it and the CI hypotheses for fisheries

Fisheries - H2, H3, H4, H5, H6, H7, H8, H12

The focus of the fisheries component should be to address exposure of fish to metals-contaminated water/sediments. There is no sense attempting to sample fish synoptically with the water chemistry gradient because fish live at a different spatial scale than invertebrates (i.e., the range of fish is likely to overlap more than one triad station); a CI study design should be used for hypotheses addressing exposure/effects and fish. Upper Campbell Lake and possibly an additional reference area (to be determined in the field) should be used as reference areas for the CI design.

A demersal fish is the recommended sentinel species for addressing exposure to sediment-associated metals. However, in this study system we anticipate the exposure pathway to fish may be as much from the water column (e.g., water, plankton) as from the sediments. We recommend using rainbow trout (*Oncorhychus mykiss*), which was found in both the exposure and reference areas, as the sentinel fish species for Myra Falls mine. In the event that insufficient numbers are collected to test the hypotheses, cutthroat trout or Dolly Varden should be used as the sentinel species, although these are less ideal than rainbow trout (EVS, ESP and JWEL, 1996a). However, the 1996 study as well as previous studies suggest that there should be sufficient numbers of rainbow trout to meet 1997 study requirements with an acceptable level of effort.

Fish should be collected by gill net (or other suitable method) at several locations (e.g., 5 - 10) within exposure and reference areas (Figure 4-1). Individual fish (or composites of several fish) are the replication units for testing H2, H3, H4, H7 and H8; we recommend collecting at least 20 fish of each sex in the exposure and reference areas (at least 10 of each sex from each reference area = 20 total). Fish should be analyzed for both metals and metallothionein.

Hypotheses addressing catch per unit effort (CPUE; H5) and community composition (H6) should be addressed in the same sampling effort described above; however, the unit of replication will be sampling location (i.e., 5 or 10 locations within each area). Again, sampling areas should be allocated evenly between reference areas (i.e., if sample size is 6 locations, each reference area should contain 3 locations). All fish caught should be enumerated and identified.

H12 cannot be tested in a fully quantitative manner at this site, as there are no barriers to fish migration and the presence of a very strong gradient is uncertain as the 1996 data are based on a CI design and would have to be verified in the field (as noted above). This hypothesis can, however, be tested qualitatively.

Receiving water quality - H9

The principal biological parameter to measure is the plankton community. The benthic community will be difficult to measure as sediment is difficult to locate in the reference area. It is possible to sample the rocky shores (littoral zone) by methods other than grab (e.g., Hess sampler, kick net); however, this is not a feasible option due to the sharp increase in water depth in this area. Previous studies have shown that zooplankton are good indicators of mine-related effects, with abundance and richness decreasing with increasing metal concentrations (e.g., Roch et al., 1985). Zooplankton can be collected synoptically with water samples. Fish do not lend themselves to discrete relationships in the field as there are no barriers along the gradient or between reference and exposure areas.

- Receiving water (effluent dispersion): field measurements of a water quality variable (e.g., conductivity or another easy-to-measure variable that can be linked back to chemistry) should be made to estimate plume dilution and extent in the receiving environment. This information should be used to delineate the sampling area within the receiving environment and supplement receiving water chemistry for gradient stations.

- Receiving water (chemistry): samples should be collected for the parameters listed in Table 4-2 in a gradient from the mouth of Myra Creek, through the southern end of Buttle Lake and possibly include the middle and northern areas of Buttle Lake and southern area of Upper Campbell Lake should the field measurements indicate this is appropriate. The full extent of sampling areas should be determined by the aforementioned measurements while in the field. Water samples should be collected at surface, mid-depth and bottom, as determined by the conductivity survey, to account for incomplete mixing in the exposure zone, using a Van Dorn type water sampler.

- Receiving environment (biota): samples should be collected synoptically (i.e., at the same station and time) with chemistry samples. Zooplankton samples taken to determine productivity should be collected by vertical tow using a conical net of 64 µm mesh size and a 20 cm mouth diameter.

Table 4-2. Effluent physico-chemical parameters to be measured at Myra Falls in 1997.

METALS (TOTAL AND DISSOLVED)	GENERAL CHEMISTRY
Aluminum[1]	Alkalinity
Copper[2]	Hardness
Cadmium[2]	Anions
Lead[1]	Cations
Manganese[13]	Ion Balance
Nickel[1]	pH
Strontium[1,3]	Conductivity
Zinc[2]	Turbidity
	Dissolved Organic Carbon
	Total Dissolved Solids
	Nutrients

1 detected in effluent and receiving water in 1996
2 historical contaminant of concern
3 elevated in exposure compared with reference stations in 1996

Effluent/receiving water quality - H13

The Myra Falls mine effluent was found to be toxic, with fathead minnows being the least sensitive and *Selenastrum* being the most sensitive in 1996.

- Effluent chemistry: as effluent quality may vary, three sets of samples should be collected and analyzed for conventionals (including ammonia and sulphides) and metals (total and dissolved). We recommend that samples be collected quarterly to account for seasonal variation in effluent quality with one sampling event coinciding with the field monitoring program at the Myra Falls mine site. Presumably the mine already conducts chemistry analysis coincident with their regular toxicity testing; if so, there is no need to repeat these measurements.

- Effluent toxicity (four tests): samples should be collected synoptically with chemistry samples; note that effluent samples for screening tests (fathead and *Ceriodaphnia* only) and receiving water samples for acclimation (fathead only) will need to be collected prior to the field program to allow full preparation by the testing laboratory.

Effluent chemistry/toxicity data will be used in conjunction with the biological data collected for H2-8 (fish) and H9 (zooplankton) to assess this hypothesis.

4.2 LUPIN, NT

4.2.1 Hypotheses

The Lupin Mine was selected for the 1997 AETE Program because of its apparent suitability to test most of the hypotheses listed in Table 3-1 (EVS, ESP and JWEL, 1996h). The presence of substantial depositional areas was of particular importance for testing hypotheses related integrated approaches such as the Sediment Quality Triad (Green et al., 1993).

Table 4-3 summarizes the rationale for selection of hypotheses for the 1997 AETE Program. Decisions regarding specific hypotheses were based on the following:

- Study design options and statistical considerations (as presented in Section 3.2).
- Data and field observations from the 1996 AETE Program - Lupin Mine (EVS, ESP and JWEL, 1996c).
- Data and field descriptions from historical monitoring programs (reviewed in EVS, ESP and JWEL, 1996c).

Note that the AETE Committee made the decision, for cost reasons, not to conduct fisheries studies at the Lupin Mine site. Hence, such work is not included in this study design.

4.2.2 Study Design

General Considerations:

While the study design is largely hypothesis-specific, there are important aspects of the overall design that require consideration to ensure a fully-integrated and cost-effective study. For the Lupin Mine, these factors are as follows:

- Existence of an exposure gradient: many of the hypotheses assume that an exposure gradient exists.
- Integration of hypotheses into common designs: flexibility in design type (i.e., choice of CI or gradient) for certain hypotheses (e.g., for H1) allows a more complete integration of various study components.

Table 4-3. Hypotheses for 1997 AETE Program - Lupin Mine.

No	Hypothesis	Study Design (CI or Gradient)	Testable? (Y,N, or Partial)	Comments
H1	Sediment toxicity	Gradient*	Y	H1: Existing pattern of contamination appears to increase through Inner Sun Bay to a maximum at southern Outer Sun Bay; this gradient is tenuous but based on available data should be expected to decrease through Outer Sun Bay and into the main portion of Contwoyto Lake.
H2	Metals in fish tissues	CI	Y	H2 - H4: Historical As contamination in fish tissues. Fishing success during 1996 was limited; we recommend the use of demersal fish (e.g., burbot) due to close association with sediment; however, only one individual was caught in both the exposure and reference area. If capture of burbot is low, then we recommend using lake trout as abundances are high in both areas. However, lake trout is not an ideal sentinel species for this location. As recommended by the AETE Committee, this hypothesis will not be tested in 1997 at this site.
H3	Metallothionein in fish tissues	CI	Y	
H4	Metals vs. metallothionein in fish tissues	CI	Y	
H5	Fish - CPUE	CI	Y	H5/H6: Increased fishing effort could allow these hypotheses to be addressed; the key issue would be to find suitable locations to set gill nets in each area. As recommended by the AETE Committee, this hypothesis will not be tested in 1997 at this site.
H6	Fish - community	CI	Y	
H7	Fish - growth	CI	Y	Same as H2 - H4. As recommended by the AETE Committee, this hypothesis will not be tested in 1997 at this site.
H8	Fish - organ/fish size	CI	Y	Same as H2 - H4. As recommended by the AETE Committee, this hypothesis will not be tested in 1997 at this site.
H9	Water quality and biology	NA	P	Short exposure duration due to limited effluent discharge limits our ability to link receiving water quality to biological responses. We recommend addressing this issue indirectly by testing effluent toxicity and extrapolating results to the receiving environment.

Table 4-3. Continued

No	Hypothesis	Study Design (CI or Gradient)	Testable? (Y,N, or Partial)	Comments
H10	Sediment chemistry and biology	Gradient	Y	Can be tested as part of sediment quality triad.
H11	Sediment toxicity and benthos	Gradient	Y	Can be tested as part of sediment quality triad.
H12	Metals or metallothionein vs. chemistry	CI	P	Limited effluent discharge makes this hypothesis difficult to test using receiving water chemistry; we recommend using sediment quality and demersal fish.
H13	Chronic toxicity	Gradient	P	Can be tested with benthos qualitatively, but not to fish since not sampled synoptically.

* Hypothesis can be analyzed using CI or gradient design; design selected to match site

- Reference areas: there was some difficulty interpreting results of the 1996 AETE Program (i.e., it was not clear whether differences were related to exposure or simply because other environmental factors are different between reference and exposure areas); this issue does not need to be resolved in 1997 since fisheries work will not be conducted and the other hypotheses will be tested via a gradient design.

- Timing of investigations: appropriate window(s) need to be identified for all study components.

- Stations: some pre-survey work will be required to finalize station selection.

Existence of an exposure gradient

Section 3.2 presents study design options for each of the 1997 hypotheses. The designs essentially fall into two categories: control/impact (CI) or gradient. Section 3.2 recommends that all hypotheses relating to fish use the CI design. However, a number of hypotheses can be tested using either a CI design or gradient design. Given the intermittent nature of effluent discharge (i.e., an annual two-week discharge event), we do not recommend using a gradient approach on hypotheses related to receiving water. Based on available data, we believe that sediments are a metal sink and thus serve to integrate the influences of annual effluent discharge events over time. Consequently, gradient-type hypotheses for the Lupin Mine should focus on sediments, provided that an appropriate exposure gradient exists.

The presence of a gradient in Contwoyto Lake is expected but it may not be as strong a gradient as desirable. In 1996, there was less than a ten fold difference between the highest and lowest arsenic sediment concentrations at the exposure stations. Arsenic was 13.9, 45.1 and 27 mg/kg in Inner Sun Bay and 15.4, 33.3 and 58.9 in Outer Sun Bay. Historically, arsenic also tended to be higher in Outer Sun Bay than Inner Sun Bay. Although the gradient is assumed not to be unidirectional from the mouth of Seep Creek due to sediment transport issues, the presence of a gradient that includes a several fold difference from lowest to highest concentrations is expected.

To evaluate as fully as possible the exposure gradient in sediments at Inner and Outer Sun Bay of Contwoyto Lake, sediment chemistry data from the 1996 AETE Program and from the 1985 and 1990 historical sediment investigations (Porter et al., 1991) were examined. Based on these data, we have the following conclusions regarding the presence of an appropriate exposure gradient:

- A comparison of sediment metal concentrations in the 1996 AETE Program show up to approximately five-fold differences (higher in exposure area) between exposure

and reference areas. Conclusion: Metals concentrations are considerably elevated in the exposure area.

- While not examined directly in the 1996 report (EVS, ESP, JWEL, 1996c), there appears to be a good correlation between metal concentration and the fine sediment fraction (i.e., stations with high silt/clay have higher metals). Conclusion: As expected, metals appear to be associated with the fine fraction of sediments.

- Based on available data, we believe water flow through Inner Sun Bay (i.e., from the Concession Lake watershed and Seep Creek) transports sediment-bound contaminants to Outer Sun Bay where primary deposition occurs. Because of these hydrodynamic patterns, metal concentrations are higher at stations farther from the effluent discharge point. Sediment grain size results at the exposure stations in the 1996 AETE Program (EVS, ESP and JWEL, 1996c) corroborate the hydrodynamically-induced contaminant gradient (i.e., stations closest to Seep Creek generally contained more sand than stations in Outer Sun Bay). Conclusion: Metals concentrations are highest in Outer Sun Bay.

- The 1990 study (Porter et al., 1991) had one station to the northwest of Outer Sun Bay (the objectives of the 1996 study did not warrant stations outside the predicted main exposure area). This station generally contained lower metals concentrations than stations in Outer Sun Bay. Conclusion: Sediment metals concentrations appear to decrease with distance away from the southern portion of Outer Sun Bay.

Thus, while there is some uncertainty as to the northern extent of an exposure gradient in the Sun Bay area, we assume (i.e., best professional judgement considering the limited data available and general contaminant fate and transport behaviour) that such a gradient exists.

Integration of hypotheses into common designs

Given the assumption of a sediment exposure gradient, all hypotheses dealing with sediments should be based on a gradient design. The goal is to integrate related hypotheses in such a way that they can be addressed within a traditional triad approach.

Reference areas

While the 1996 AETE Program at Lupin clearly showed differences in metal concentrations between exposure and reference areas, benthos results were sometimes difficult to interpret. There appear to be no adverse effects from metal exposure (i.e., total richness and abundance were not significantly different between areas; EVS, ESP and JWEL, 1996c); however, examination of individual taxa (see raw benthos data in EVS, ESP and JWEL, 1996c) show that there are some differences between areas. The significance of these differences is

difficult to interpret without data for other reference areas. We recommend sampling an additional reference for the 1997 CI study designs.

Timing of investigation

Effluent discharge at Lupin generally occurs in late July/early August. The 1997 AETE Program at Lupin should be timed to overlap with the approximately two-week effluent discharge period in the summer. This period also coincides with the best weather window for the low arctic.

Hypothesis-Specific Designs:

Table 4-3 summarizes which of the 1997 hypotheses (cf. Table 3-1) will be addressed in the 1997 AETE Program at Lupin Mine. To facilitate explanation of study designs, specific hypotheses have been integrated into designs related to main study components as follows. Given the intermittent discharge of effluent and the availability of depositional areas, the sediment quality triad component is the primary focus for 1997.

Sediment quality triad - H1, H10, H11

The sediment quality triad (triad) consists of sediment chemistry, toxicity and benthos. The triad is designed to cover the predicted exposure gradient extending northwest and northeast from Outer Sun Bay (see Figure 4-2). Between 20-40 stations should be sampled; due to the lack of substrate type information for most of this area, the final station locations should be decided in the field following habitat characterization. Conventional sonar techniques supplemented by grab-sampling would cost-effectively identify depositional areas (Beak, 1996a). At each triad station, the following samples should be collected:

- Sediment chemistry (metals, iron and manganese oxyhydroxides, SEM, AVS, grain size, total organic carbon [cf. Table 4-4]): single composite sample of upper 2 cm of sediment from 5 replicate grabs with Petit Ponar.

- Sediment toxicity (freshwater amphipod [*Hyalella azteca*], freshwater midge [*Chironomus spp.*] and oligochaete [*Tubifex tubifex*]): individual replicate samples of upper 2 cm of sediment from the same 5 replicate grabs used for sediment chemistry.

- Benthos (taxa identified to lowest practical level): single composite sample of 3-5 replicate grabs (whole grab; minimum penetration depth of 4 - 5 cm) per station.

Figure 4-2. Station selection for sediment collection.

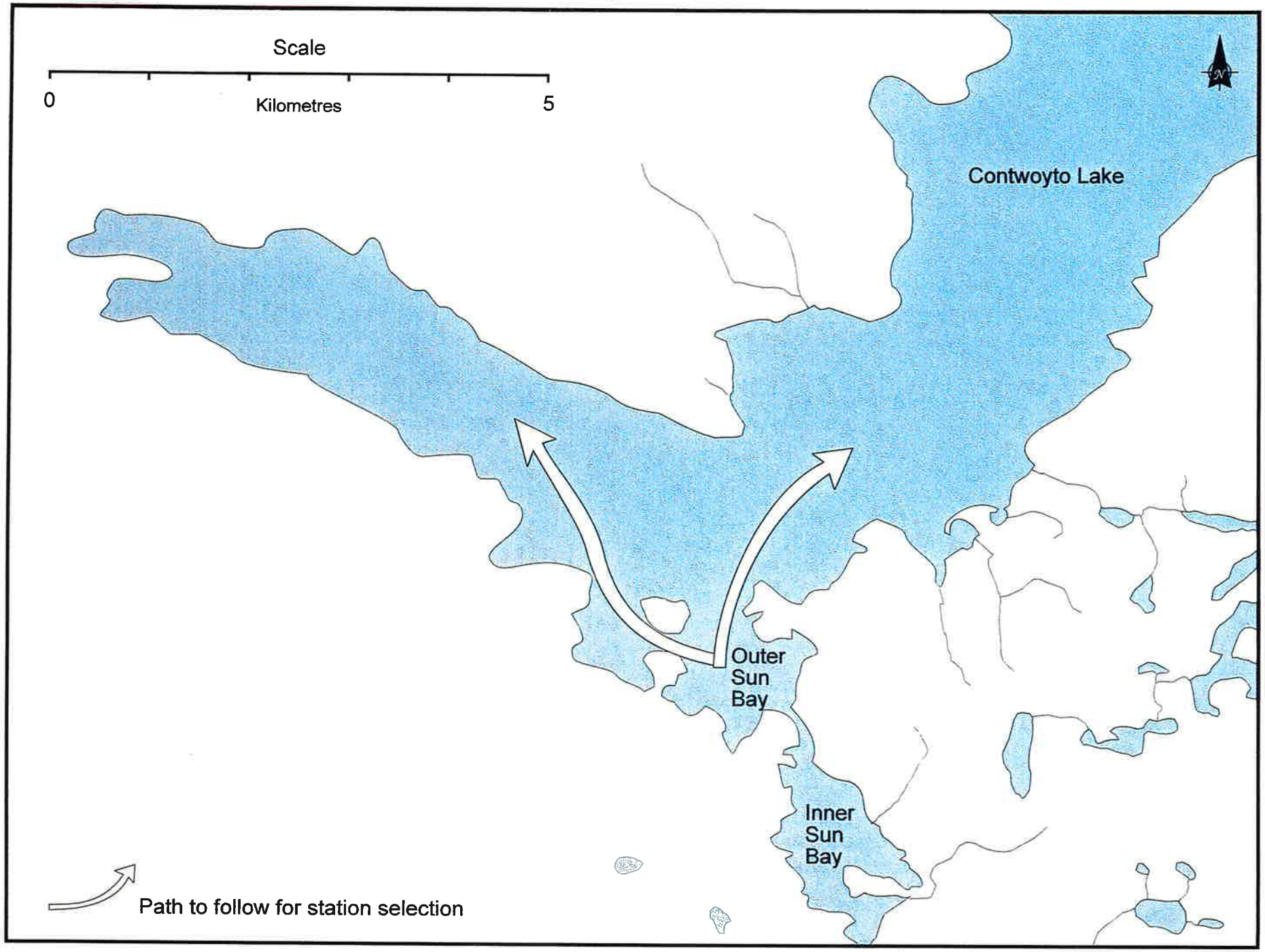


Table 4-4. Basic sediment physico-chemical parameters to be measured at Lupin in 1997.

METALS	CONVENTIONALS
Arsenic[1]	Cyanide, total
Chromium[1]	Total Organic Carbon
Cobalt[1]	Particle Size
Copper[1]	Percent Moisture
Lead[1]	Loss on Ignition
Nickel[1]	
Vanadium[1]	
Zinc[1]	

1 elevated in sediments in 1996

Depth is a potentially confounding factor for benthos; if possible, stations should be positioned to minimize variability in depth among stations (e.g., place stations in 8 to 12 m of water). If this is not possible, the influence of depth can be accounted for by using it as a covariable in an analysis of covariance.

Effluent/receiving water quality - Indirect assessment of H9

Given the presence of an exposure gradient in sediments and the short duration of exposure in receiving water, hypotheses linking exposure to responses are addressed via the sediments. However, water quality remains an important issue at Lupin during the discharge period. We recommend an effluent-focused assessment that measures chemistry and toxicity and relies on dilution estimates to extrapolate exposure and toxicity to the field. As such, the basic design is neither CI nor gradient. The proposed approach is as follows (physico-chemical parameters are provided in Table 4-5):

- Effluent chemistry: samples should be collected at the beginning, middle, and end of the discharge period and analyzed for conventionals (including ammonia and sulphides) and metals (total and dissolved).

- Effluent toxicity (four tests): samples should be collected synoptically with chemistry samples; note that effluent samples (from the tailings pond near the decant area prior to discharge) for screening tests (fathead and *Ceriodaphnia*) and receiving water samples for acclimation (fathead only) will need to be collected prior to the field program to allow full preparation by the testing laboratory.

Table 4-5. Effluent physico-chemical parameters to be measured at Lupin in 1997.

[Note: No effluent was discharging in 1996 therefore water chemistry data were not relevant to effluent discharges. In fact, many parameters were statistically different, with the reference elevated compared with the exposure stations. Information from historical data and accumulations in sediments were used to assess water chemistry data]

METALS (TOTAL AND DISSOLVED)	GENERAL CHEMISTRY
Aluminum[1]	Cyanide, total[1]
Arsenic[2]	Alkalinity
Chromium[2]	Hardness
Copper[1,2]	Anions
Iron[1]	Cations
Lead[2]	Ion Balance
Nickel[1,2]	pH
Selenium[1]	Conductivity
Vanadium[2]	Turbidity
Zinc[2]	Dissolved Organic Carbon
	Total Dissolved Solids

[1] detected in receiving water in 1996
[2] elevated in sediments in 1996

- Receiving water (effluent dispersion): field measurements of a water quality variable (e.g., conductivity or another easy-to-measure variable that can be linked back to chemistry) should be made at the beginning, middle, and end of the effluent discharge period to estimate plume dilution and extent in the receiving environment. This information should be used to extrapolate effluent toxicity and chemistry results to the receiving environment.

4.3 DOME, ON

Detailed information on this site is provided in EVS, ESP and JWEL (1996d). Rationale for choice of this site for 1997 studies is provided in EVS, ESP and JWEL (1996h).

4.3.1 Hypotheses

Based on information presently available, most of the hypotheses can be tested at this site (Table 4-6; for complete descriptions of hypotheses, see Table 3-1):

Table 4-6. Hypotheses for 1997 AETE Program - Dome Mine.

No.	Hypothesis	Statistical Design (CI or Gradient)	Testable (Y/N or Partial)	Comments
H1	Sediment toxicity	CI and/or Gradient	Y	Sediments are readily available. Clear metal concentration differences between Reference and Exposure areas
H2	Metals in fish tissues	CI	Y	H2 - H4: Fish are present but increased fishing effort is required. 1996 results indicate higher metal levels in forage species from Exposure Area. 1996 results for metallothionein levels in forage species not conclusive. Yellow perch recommended as an additional sentinel species in 1997
H3	Metallothionein in fish tissues	CI	P	
H4	Metals vs. metallothionein in fish tissues	CI	Y	
H5	Fish - CPUE	CI	Y	H5 - H6: Increased fishing effort recommended in 1997 using a variety of fishing techniques in lakes and rivers
H6	Fish - community	CI	Y	
H7	Fish - growth	CI	Y	H7 - H8: Increased fishing effort recommended in 1997. A minimum of 20 of each sex of yellow perch and pearl dace is recommended.
H8	Fish - organ/fish size	CI	Y	
H9	Water quality and Biology	Gradient	P	We recommend using the benthic community to test this hypothesis; cannot be assured of discrete fish samples along a well-defined gradient. Recommend the collection of composite benthic samples at 20 different river stations, evenly distributed among the different river areas (one reference and three stream exposure areas)
H10	Sediment chemistry and biology	Gradient	Y	Can be tested as part of sediment quality triad.
H11	Sediment toxicity and benthos	Gradient	Y	Can be tested as part of sediment quality triad

Table 4-6. Continued

No.	Hypothesis	Statistical Design (CI or Gradient)	Testable (Y/N or Partial)	Comments
H12	Metals or metallothionein vs. chemistry	Gradient	N	Cannot be assured of discrete fish samples along well-defined gradient, no clear barrier to fish migration exists at this site.
H13	Chronic toxicity	Gradient	Y	Results of sublethal toxicity can be qualitatively compared to fish and benthic indicators.

Many of the hypotheses are related. For example, if we are able to obtain suitable sediments, then hypotheses 1, 10 and 11 can be tested within the limitation and design constraints (e.g., Control vs. Impact and Gradient designs) as described in Section 3.2. Since we are confident that we can capture adequate sample sizes of fish, hypotheses 2,3,4,5,6,7, and 8 should be testable.

4.3.2 Study Design

General Considerations:

Full discussion of the rationale for the generic statistical design is provided in Section 3.2. Variables to be measured in water and sediment are indicated in Table 4-7. Testing of the different hypotheses requires different statistical models and approaches. For example, hypotheses 1 to 4 are testing of tools. H2 - H4 can be tested with Control - Impact designs; in other words, comparison between a clear Reference and Exposure area are adequate. Similarly, H5 - H8 can be tested between a Reference and Exposure area. Hypotheses 9, 10, 11 require gradients of exposure. Due to uncertainty related to fish migration and discrete levels of exposure gradients, H12 is not recommended for testing at this site. This hypothesis would seem better suited to testing under controlled laboratory conditions.

Table 4-7. Variables to be measured in water and sediment at the Dome site.

WATER	SEDIMENTS
arsenic	arsenic
barium	
beryllium	
cobalt	cobalt
copper	copper
manganese	
molybdenum	molybdenum
nickel	nickel
	silver
magnesium	
strontium	
zinc	
alkalinity	
conductivity	
sulphate	
chloride	
sodium	
total cyanide	
ammonia	TKN
calcium	Particle size
potassium	Loss on Ignition
	Total Organic Carbon

All variables except zinc were significantly different between the Reference and Exposure areas.

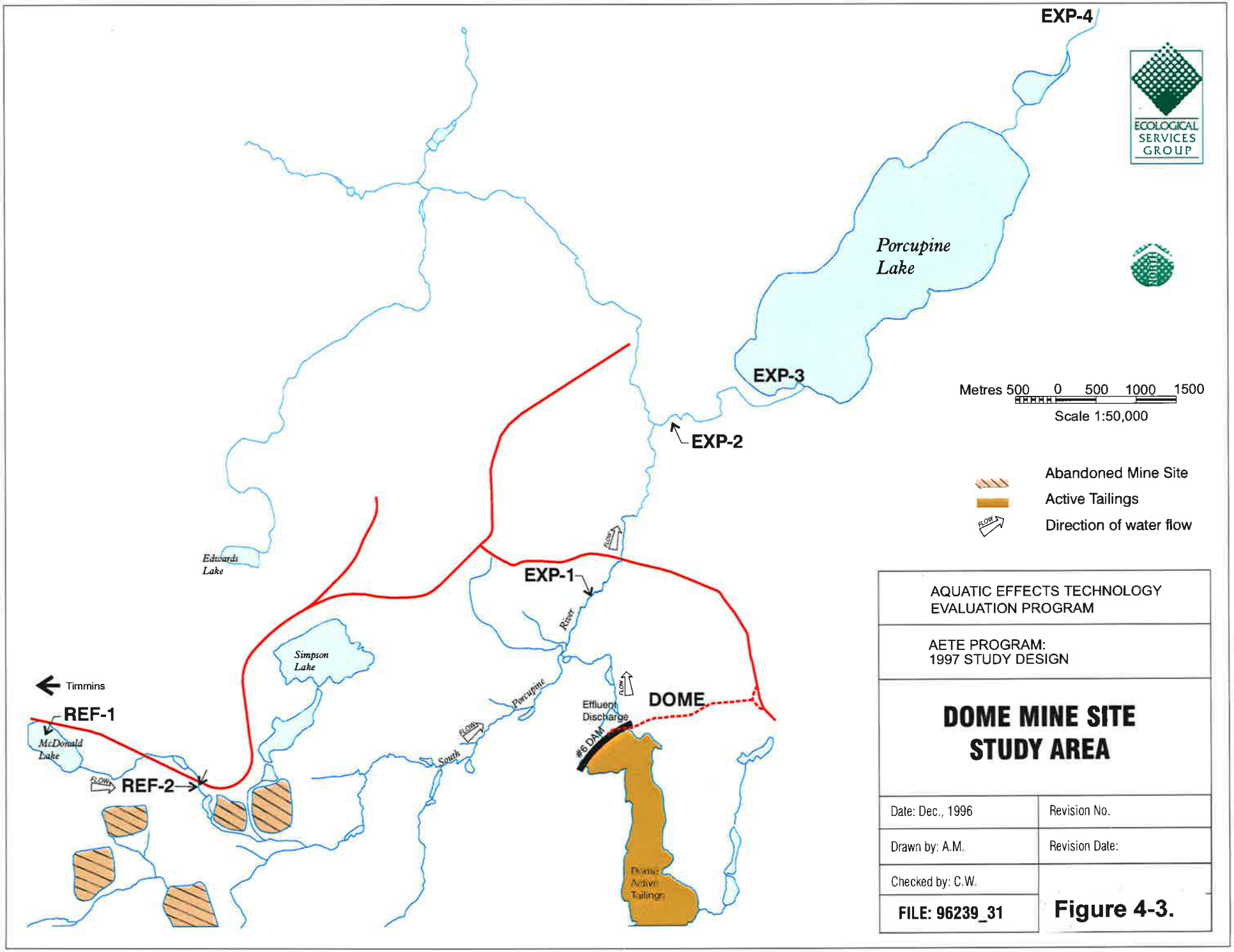
The 1997 survey is based on sampling some variables on repeat visits to the study site to obtain a measure of temporal variability. Other variables do not lend themselves to repeat sampling or the robustness of the data are not increased by repeat sampling. The frequency of sampling for the different variables is as follows:

Effluent:	3 times (Note that Ron Connell, Dome Mine, pers. comm. suggests drawing effluent directly from the tailings pond prior to effluent treatment, to ensure toxicity)
Water:	once
Sediment:	once
Benthos:	once
Fish:	once - with appropriate fishing effort

For the 1997 survey we recommend 2 reference areas (Ref-1, Ref-2) and four exposure (Exp-1, Exp-2, Exp.-3, Exp-4) areas (Figure 4-3). Multiple stations and repeated measurements for some variables will be undertaken to establish a gradient within the exposure areas. The four different exposure areas represent where gradients in exposure are expected to occur (e.g., due to incoming tributaries). Also, fish will essentially be collected from within a finite area (e.g., Exposure 1) and not along a complete gradient. It is important to have more than one reference area to measure within-area variability. A limitation at the Dome site, however, is not having the same habitat types for multiple reference areas, e.g., Ref-1 is a lake, and Ref-2 is a stream. It is not possible at this location to obtain multiple reference areas of the same habitat type within the same watershed. Due to this limitation the Dome study design does vary somewhat from the generic study design (Appendix A).

Three river exposure areas are recommended to obtain a measure of exposure gradient away from the point of effluent discharge. Conditions at this site are somewhat complicated by other nonpoint source discharges (e.g., old tailings areas) into the South Porcupine River and other active mines within the watershed.

The available information suggests that a gradient of exposure to metals can be expected along the river. We do not have site specific data for Exposure area 2 but it is below the confluence with the north branch of the Porcupine River so considerable dilution of the Dome effluent can be expected due to this influx of water. We do have some sediment chemistry data for sites near Exposure area 4 (4b is about 1.5 km downstream of 4a) which indicate lower concentrations of metals at this area compared with Exposure area 1 (ESP unpublished data). For example (concentrations in mg/kg):



ECOLOGICAL SERVICES GROUP

AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

AETE PROGRAM:
1997 STUDY DESIGN

DOME MINE SITE STUDY AREA

Date: Dec., 1996	Revision No.
Drawn by: A.M.	Revision Date:
Checked by: C.W.	
FILE: 96239_31	Figure 4-3.

	REFERENCE 2	EXPOSURE 1	EXP. 4A	EXP. 4B
Copper	238	1056	652	306
Cobalt	17	73	22	20
Nickel	45	433	171	103
Arsenic	182	430	28	17

Benthos should only be sampled in the stream areas (Ref-2, Exp-1,2,4) for similarity of habitat type.

Fish should be sampled at Ref. 1,2 and Exp. 1,3. The location of Exp-3 in Porcupine Lake is intended to match up with Ref-1 (McDonald Lake). Large populations of fish are known to exist in both these lakes and should provide large sample sizes for fish tissue analysis. The recommended sentinel species in the lakes is either yellow perch or white sucker. The 1996 studies did not show a difference in MT levels in small forage fish (Pearl dace) at Exposure 1 and Ref. 1. This should be repeated in 1997 with larger sample sizes to again test if small forage fish are suitable for metal and MT analysis. The results should be compared with larger fish captured from the lake ecosystems for similar analyses. The results will provide useful data relative to the utility of forage fish in this type of aquatic monitoring program.

It is recommended that fish be sampled in very early spring shortly after ice-out and prior to spawning. Gonad size will be maximized for easier measurement and there will be a better chance to detect population differences. Benthos can be sampled in the early spring, or autumn if direct comparisons with the 1996 data are required. Collection of as much data in spring as possible is recommended to maximize the available time for sample analyses and proper data interpretation prior to reporting.

All sampling locations are readily accessible by road. There is a boat ramp at MacDonald Lake on Moneta Road out of Timmins. Exposure Area 1 is within Dome property immediately above an internal road. Access must be arranged with mine personnel. Exposure Area 2 is accessible from Evan Street in South Porcupine. There are a number of access points to Porcupine Lake. Exposure Area 4 is below Porcupine Lake and accessible from the road to the sewage treatment plant. Exposure areas 2, 3 and 4 were not surveyed in 1996 so some site reconnaissance will be required.

Note that the terminology for Exposure Area (e.g., 1, 2, 3, 4) refers to the general areas, only. Gradients of stations will be established within these areas and the data analyzed as total linear gradient wherever possible.

Hypothesis-Specific Designs:

The suitability of the Dome site for each hypothesis and rationale are briefly summarized below, together with comments where appropriate. The proposed sampling design is summarized in Table 4-8.

H1. Sediment toxicity

Sediments collected immediately downstream of the Dome effluent which are known to contain elevated metal concentrations should be subject to four different sediment toxicity tests for both acute (survival) and sublethal (growth) effects. It is recommended that tests be performed on 20 sediment samples along a gradient in the exposure area and 5 samples from Reference area 2.

H2. Metals in fish tissues

A minimum of 20 fish samples from the sentinel species should be collected from the Reference and Exposure areas. The suggested sentinel species are yellow perch or white suckers from Ref. 1 and Exp. 3, and Pearl dace from Ref. 2 and Exp. 1. Only yellow perch should be used to test differences between tissues. The tissues should be dissected out and analyzed for metal concentrations. The relative proportioning between tissues will be metal specific. We know from experience and the literature that certain essential metals (e.g., zinc, copper) will tend to accumulate in liver and kidney. Mercury tends to accumulate in muscle tissue while lead accumulates in bone or gill tissues. Whole, composite samples of Pearl dace should be collected to test for different metal levels between Reference and Exposure areas. This will provide further information on the suitability of forage fish as a monitoring tool.

There are no data from 1996 on which to base expectations, but fish are present, and there are differences in environmental concentrations of metals between the Reference and Exposure areas so the hypothesis can be tested. The design leans towards Control-Impact more than a gradient approach.

H3. Metallothionein (MT) in fish tissue

Tissues for MT analysis should be collected from yellow perch from Ref-1 and Exp-3 as for H2 above.

H4. Metals vs. metallothionein in fish tissues

Metal and metallothionein results from H2 and H3 above should be compared with water and sediment concentrations from the collection areas.

Table 4-8. Summary of Proposed Sampling Design for Dome

SUMMARY OF PROPOSED SAMPLING DESIGN: PLACER DOME							
	RESPONSE		#AREAS	AREAS ARE:	# STATIONS / AREA	#REPLICATES / STATION	SAMPLE SIZE
Benthos	abundance or integrative measure		4	Ref 2, E 1, 2, 4	5	0	20
Sediment Quality	a suite of sediment quality variables		6	Ref 1, 2, E 1, 2, 3, 4	5		30
Water Quality	a suite of water quality variables		6	Ref 1, 2, E 1, 2, 3, 4	4	1	24
Fish	metal in liver, kidney, gills, muscle	Yellow Perch	2	Ref 1, E 3		20	40
Fish	MT in liver, kidney gills CPUE, community, growth, organ size	Pearl Dace	4	Ref 2, E 1, 2, 4		20	80
Effluent Toxicity Tests[1]	survival, reproduction, growth inhibition		1				4 species x 3 times
Sediment Toxicity[2]	mortality and reproduction?		4	Ref 2, E 1, 2, 4	5	1	20

1 Effluent tests are: Fathead minnow survival and growth inhibition, *Ceriodaphnia dubia* survival and reproduction, *Lemna minor* growth inhibition and *Selanastrum* growth inhibition

2 Sediment bioassays are: *Hyallella azteca* and *Chironomus* spp., 28 day *Tubifex*

H5. Fish-CPUE

Fishing effort between the Reference and Exposure areas should be standardized and the resultant catch compared. This will largely be a qualitative comparison. Different fishing techniques are recommended for the lake and stream habitats (cf. Section 3.3; Appendix B).

A variety of netting methods (e.g., gill, trap, seine) should be used to sample perch in the lake environments. The fish community in the river environment may best be sampled with a boat electrofishing unit, although other techniques may be successful.

H6. Fish-community

During the fishing surveys for H5 above, all fish captured should be recorded. This will provide a measure of relative abundance of fish representing different trophic levels and ecological niches. Also, the presence/absence of indicator species should be recorded at the Reference or Exposure areas. Some species may be pollution sensitive (e.g., salmonids, brook stickleback) whereas other species are known to be pollution tolerant (white suckers, yellow perch).

H7. Fish-growth

Length, weight and age of a minimum of 20 fish of each sex from each of the reference and exposure areas should be accurately documented and growth curves compared by Analysis of Covariance. Growth curves should first be examined to ensure that the slopes are parallel; if not, no further analysis can be undertaken. Separate growth curves should be prepared for each sex where possible. Yellow perch or white sucker are recommended from R2, E3, and Pearl Dace from at least R2, E1, E2 and E4.

H8. Fish-organ/tissue size

Liver and gonads should be removed from a minimum of 20 fish (yellow perch, Pearl dace) of each sex and accurately weighed. Organ weight is proportional to fish size, so body weight would be used as the covariate in analysis of covariance (ANCOVA). The liver somatic index (LSI) and gonadal somatic index should also be measured. Since these indices are expressed as ratios (e.g., LSI = liver weight/body weight) they cannot be statistically compared, only qualitatively viewed.

H9. Water quality and biology

The principal biological parameter to measure is benthic community structure. Fish do not lend themselves to such discrete relationships in the field. To test this relationship, benthos will be sampled along a gradient of exposure conditions within the South Porcupine River.

Composite benthic samples should be collected at 20 different river stations. Sampling should be evenly distributed among the different river areas (one reference and three stream exposure areas).

Water samples should be field filtered and analyzed for both total and dissolved metal concentrations.

H10. Sediment chemistry and biology

Benthos and sediments should be collected from 20 stream stations along a gradient. Sediments should be analyzed for total metals, particle size, total organic carbon, AVS and SEM. The relationship between these parameters should be statistically examined using appropriate regression or multivariate techniques.

H11. Sediment toxicity and benthos

The *in-situ* benthic community at Ref.-2 and from along a gradient from E1, E2 and E4 should be compared with the results of the sediment toxicity tests.

H12. Metals or metallothionein vs. chemistry

The hypotheses cannot be adequately tested at this site. There is no clear barrier to fish migration. Moreover, a very clear gradient of metal concentrations in the environment is required to test this hypothesis, which suggests it is better suited to a laboratory experiment where exposure can be controlled, and clear dose-response relationships formulated. The 1996 data are based on Control-Impact design, so actual gradients are not well known.

H13. Chronic toxicity

Results of the 1996 sublethal toxicity tests at this site were not clear. The test results for the Rainbow trout embryo test were considered invalid. Two tests showed no effects (Fathead minnow and *Ceriodaphnia*) while the *Lemna* and *Selenastrum* tests indicated an effluent effect. Previous toxicity tests have shown the Dome effluent to be acutely toxic (EVS, ESP and JWEL, 1996d). The Dome effluent should be sampled for toxicity as early in the spring of 1997 as is possible. Potential toxicity of effluent water may be at its highest after sitting for the winter with little degradation of toxic compounds (e.g., ammonia, cyanide). If there is no toxicity on this sample, further toxicity testing is questionable.

Effluent from the Dome site should be collected on three occasions (early spring, mid summer and fall) and subject to four sublethal toxicity tests (Fathead minnow, *Ceriodaphnia dubia*, *Lemna* and *Selenastrum*). These results should be qualitatively compared with the fish

and benthic data collected in the exposure and reference area. It may be appropriate to compare results among study sites (e.g. mine) to examine the strength of the relationship.

Effluent chemistry should be measured with each sampling. Dilution water for the toxicity tests should also be collected and analyzed on these three occasions.

4.4 HEATH STEELE, NB

4.4.1 Hypotheses

The Heath Steele Mine was selected for the 1997 AETE Program because of its apparent suitability to test many of the hypotheses listed in Table 3-1 (EVS, ESP and JWEL, 1996h). The presence of a strong exposure gradient in an exposure area was of particular importance for testing hypotheses requiring a gradient statistical design. The Heath Steele Mine site is suitable for testing the hypotheses listed in Table 4-9 including those requiring a control-impact (CI) statistical design and those requiring a gradient statistical design. Hypotheses 1 (sediment toxicity), 10 (sediment chemistry and biology) and 11 (sediment toxicity and benthos) cannot be tested at Heath Steele because suitable, depositional sediments are unavailable at this site. Hypothesis 6 (fish community) is also likely untestable because only two species of fish were found in significant numbers in the exposure and reference sites. Hypothesis 12 (metals or metallothionein vs. chemistry) can also not be tested at Heath Steele because an estimation of the relationship between fish tissue metal or MT, levels and aqueous metal levels requires some variability in the aqueous metal concentrations to which fish are exposed. At the Heath Steele site, fish are not found in the only location where a gradient in aqueous metal levels exists (South Branch Tomogonops River). Consequently, no relationship between fish tissue metal or MT, levels and aqueous metal levels is possible. Therefore, this hypothesis is not testable at this site.

Hypotheses 2 (metals in fish tissue), 3 (metallothionein in fish tissue) and 4 (metals vs. metallothionein) can only be tested on whole fish or fish viscera (rather than on specific fish tissues) because the fish are too small for dissection. These hypotheses have therefore been listed as partially testable in Table 4-9. Hypothesis 8 (effects on fish organs or fish size) can be tested on length or weight but not on individual organs (e.g., liver) because the fish are too small for dissection. This hypothesis was therefore considered partially testable. Hypothesis 9 (fish/benthos and water quality) is testable only on the benthic parameters because of the scarcity of fish in the South Branch Tomogonops. Testing of Hypothesis 13 (chronic toxicity) at the Heath Steele site will require collection of two "effluents" as there are two different potential sources of toxicity. Testing of H13 is described in more detail in the hypothesis specific design which follows.

Table 4-9. Summary of Hypotheses to be tested at Heath Steele in 1997.

No.	Hypothesis	Statistical Design (CI or Gradient)	Testable (Y/N or Partial)	Comments
H2	Metals in fish tissues	CI	P	Sentinel species too small for effective dissection of various tissues. Whole fish measurements or measurements on viscera only are possible.
H3	Metallothionein in fish tissues	CI	P	As per H2
H4	Metals vs metallothionein in fish tissues	CI	P	As per H2
H5	Fish - CPUE	CI	Y	Would require fishing at multiple sites in both the exposure and reference area.
H7	Fish - growth	CI	Y	Can be tested but the population of the sentinel species is composed only of juveniles (0-3y); i.e., the range of the data is very narrow..
H8	Fish - organ/fish size	CI	P	Fish too small for dissection of organs. Comparison of fish size possible but results will be similar to those produced for H7.
H9	Water quality and biology	Gradient	P	Requires gradient in exposure which can be found in the South Branch Tomogonops River. Can only be tested on benthic parameters; the sentinel fish species is not found in sufficient numbers here.
H13	Chronic toxicity	Gradient	P	Hypothesis can be tested with fish in Tomogonops River and benthos in the South Branch of the Tomogonops River. Testing will require collection of two "effluents" as described below in the hypothesis specific study design.

4.4.2 Study Design

General Considerations:

Control-Impact Design

Hypotheses 2, 3, 4, 5, 7, and 8 can be tested at the Heath Steele mine site using a control-impact statistical design. These hypotheses should be tested in the reference and exposure areas identified in the 1996 preliminary field survey. The reference area is located on the Northwest Miramichi River upstream and downstream of Payne's Bridge on Highway 430 (Figure 4-4). This area has been used as a reference station historically (HS-21) and is a station sampled routinely by Heath Steele Mines as a component of its regulatory monitoring requirements.

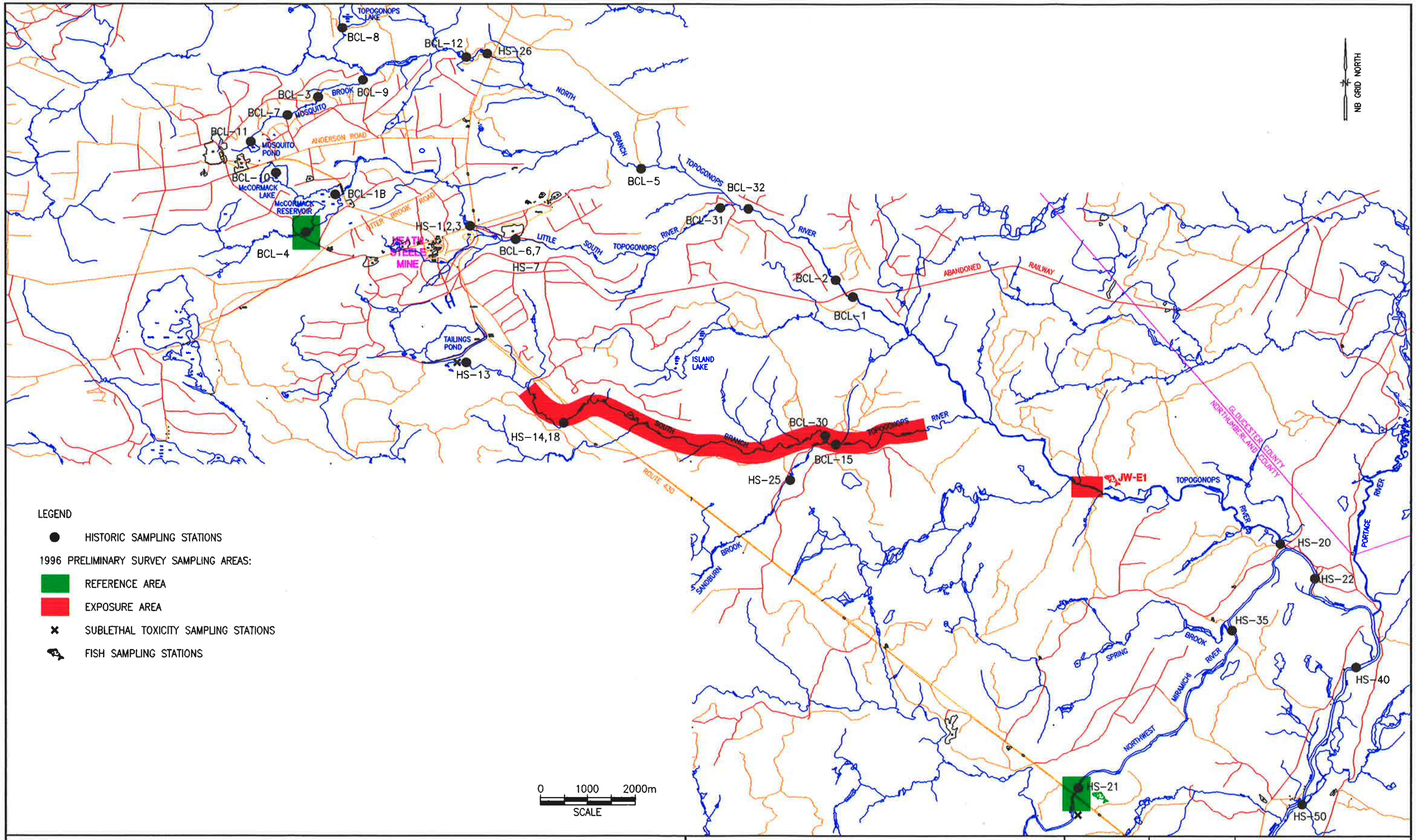
An additional reference station should be located on the Big Sevogle River (HS-70) for testing H2, H3, H4, H7 and H8 with juvenile Atlantic salmon. This additional reference site is deemed necessary to reduce the possibility of migration of juvenile Atlantic salmon between reference and exposure areas.

The exposure area should be located in the Tomogonops River downstream of the confluence of the North and South Branches of the Tomogonops River. This exposure area is approximately 4.3 km upstream of the confluence of the Tomogonops River with the Northwest Miramichi River. This site is affected by both point source (effluent discharge from the tailings pond into the South Branch Tomogonops) and non-point source (seepage into the North Branch and Little South Branch Tomogonops) discharges from Heath Steele Mines.

Gradient Design

The hypothesis requiring the gradient statistical design (H9) should be tested at a second set of reference and exposure areas. The reference area should be located on McCormack Brook upstream and downstream of historical Station BCL-4. This station has been the reference station for the North Branch and Little South Branch Tomogonops Rivers since 1994 and is comparable in habitat to exposure stations located on the Little South Branch and South Branch upstream of their confluence with the North Branch Tomogonops.

The exposure area should be located on the South Branch Tomogonops River and should include Stations HS-14 and BCL-15. This area is located downstream of the effluent discharge from the tailings pond and clear gradients have been observed in water chemistry and benthic invertebrate community structure based on historical data. Water chemistry data collected in 1995 (Beak, 1996b) are presented in Table 4-10 to illustrate the presence of a gradient in water chemistry in the South Branch Tomogonops River. In addition, previous studies have shown that water chemistry and benthic invertebrate community structure in this exposure area differ significantly from the reference area.



FILE: HEATH

SITE LOCATION MAP
HEATH STEELE MINE
1997 STUDY DESIGN

Date:	Scale:
97 01 22	1 : 75 000
Job No.: 8128	Fig. No.: 4.4

Jacques Whitford Environment Limited

Table 4-10. Historical water chemistry data showing gradient of exposure in the South Branch Tomogonops River (Beak, 1996b)

PARAMETER	REFERENCE SITE (HS-35)	EXPOSURE SITE	
		HS-14/18	BCL-15
pH (pH units)	7.9	4.63	7
Conductivity (μmhos/cm)	22	900	580
Dissolved Cu (mg/L)	0.004	0.017	0.013
Dissolved Fe (mg/L)	<0.02	0.52	<0.02
Dissolved Pb (mg/L)	0.0001	0.0476	0.0203
Dissolved Zn (mg/L)	0.004	0.26	0.242

The exposure area on the South Branch Tomogonops River was not sampled during the 1996 field season but has been sampled extensively in previous studies. It will be necessary to establish a number of new stations in this area for testing hypotheses requiring an exposure gradient. Conductivity measurements should be taken in order to confirm the existence of the gradient and determine the degree of exposure at each new station.

Habitat Characterization

A habitat characterization should be conducted at each benthic and fish sampling station in both the reference and exposure areas (cf. Appendices B and C). Such a habitat assessment will ensure uniformity in habitat features between stations both within and between areas.

Water Chemistry

Water chemistry samples should be collected in the reference and exposure areas at those sites where water chemistry parameters are required to test specific hypotheses (see below). Water chemistry is the only program component to be measured in both the CI statistical design and the gradient statistical design.

Water chemistry samples should also be taken of receiving waters and "effluents" on the three occasions when effluent samples are collected for sublethal toxicity testing.

These samples should be analyzed for the parameters listed in Table 4-11. These are parameters which showed statistically significant differences between reference and exposure areas in the 1996 preliminary field surveys or are parameters which can provide valuable information on the receiving environment at a negligible cost.

Table 4-11. Water (and Effluent) Chemistry Parameters to be Measured From Samples Collected at Reference and Exposure Stations at the Heath Steele Mine Site

METALS (TOTAL AND DISSOLVED)	GENERAL CHEMISTRY
Aluminum	*Alkalinity
Barium	Chloride
Calcium	*Nitrate
Copper	Sulphate
Lead	Anion Sum
Magnesium	*Bicarbonate
Manganese	Cation Sum
*Nickel	*Colour
*Potassium	Conductivity
*Selenium	Hardness
Sodium	*Ion Balance
Strontium	*pH
*Uranium	*Turbidity
Zinc	Dissolved organic carbon
	Total dissolved solids
	*Kjeldahl nitrogen

* Samples were detectable in the 1996 Preliminary Field Survey but not statistically different between reference and exposure areas.

In addition to the water chemistry samples above, conductivity, dissolved oxygen, temperature and pH should be measured in the field at each benthic and fish sampling station to confirm exposure (or non-exposure) to mine effluent. Conductivity, in particular, should be used to establish the stations in the South Branch Tomogonops for the hypotheses requiring a gradient for testing.

Benthic Invertebrates

Benthic invertebrates should be sampled to test Hypotheses H9 and H13. Reference and exposure areas at the Heath Steele Mine site consist of erosional habitats. Therefore, a Surber or Hess sampler (250 μm mesh) should be used to collect all benthic samples. Following

Beak (1996b), each benthic sample collected at each station should consist of a three to five sample composite.

Sentinel Fish Species

The sentinel species chosen for testing hypotheses H2, H3, H4, H5, H7 and H8 are juvenile Atlantic salmon and blacknose dace. Juvenile Atlantic salmon are the most abundant species in the study areas and have been the subject of at least five years of previous studies conducted by the mine. Blacknose dace are the most abundant forage species in the study areas based upon historical data.

Timing

Since the effluent is discharged continuously and both fish species are year-round residents of the rivers, timing for field work is not a critical factor.

Requirements for Finalization of the Study Design in 1997

Prior to commencement of sample collection for the 1997 field program, sampling station locations must be finalized. To test H2, H3, H4, H7 and H8 for juvenile Atlantic salmon, it is recommended that a second reference site be located on the Big Sevogle River (a tributary to the Northwest Miramichi River) to reduce the possibility of fish mobility between reference and exposure areas. As this site was not sampled in 1996, it is important that the collection site be comparable (e.g., habitat, water quality, geology) to the first reference site (Northwest Miramichi River) and the exposure site (Tomogonops River).

The exposure area on the South Branch Tomogonops River has been sampled historically but was not sampled during the 1996 field season. It will be necessary to establish a number of new stations in this area for testing hypotheses requiring an exposure gradient. Conductivity measurements should be taken in order to confirm the existence of the gradient and determine the degree of exposure at each new station.

Protocols for collection and preservation of total and dissolved metals samples will be determined and finalized in a separate AETE study. These will have to be incorporated into the 1997 study design and implemented in the 1997 field program.

Protocols for collection of fish tissue for metals and MT analyses, specifically the required sample amounts for tissue analyses, will also have to be finalized. In the 1996 program, juvenile Atlantic salmon sampled for metals and MT analyses were composited in the laboratory to achieve adequate sample amounts. This reduced sample replication significantly. Although a minimum of 10 fish, of each sentinel species, in each area is recommended in 1997 (see H2, H3 and H4 below), these sample sizes may have to increase to achieve adequate sample amounts.

Hypothesis Specific Designs:

Hypotheses 2 and 3

These hypotheses should be tested in a control-impact field design. Juvenile Atlantic salmon and blacknose dace should be used as the sentinel species. A minimum of 10 juvenile Atlantic salmon and 10 blacknose dace should be collected by electrofishing in the reference area of the Northwest Miramichi River at historic station HS-21. If possible, 5 fish of each sex for each species would be preferred. A minimum of 10 juvenile Atlantic salmon and 10 blacknose dace should also be collected by electrofishing in the exposure area of the Tomogonops River at station JW-E1.

Mobility of juvenile Atlantic salmon between reference and exposure areas is a potentially confounding factor for result interpretation. As a result, a second reference site should be located on the Big Sevogle River (HS-70) if it is determined to be suitable. A minimum of 10 juvenile Atlantic salmon should be collected at this site for metals and MT analyses.

Each fish captured should be weighed on an electronic balance to the nearest 0.01 g, measured for fork length and immediately frozen on dry ice. The fish should then be sent off for analysis of metals and metallothionein. The metals analyzed should include those indicated in Table 4-11.

Hypothesis 4

Metal and MT results from H2 and H3 described above, should be compared with metal concentrations from water chemistry samples collected in the reference and exposure areas.

Five water chemistry samples should be collected in each of the reference (Northwest Miramichi and Big Sevogle Rivers) and exposure (Tomogonops River) areas resulting in a total of 15 samples. Samples should be collected from the surface using triplicate rinsed bottles as described in Section 3.3.4 and Appendix B. Samples should be analyzed for the parameters indicated in Table 4-11. Samples for QA/QC of water chemistry samples should also be collected. One field replicate (collected at exposure station JW-E1), one trip blank and one filter blank should be collected.

Hypothesis 5

Five stations should be established in the reference area (NW Miramichi River only, upstream and downstream of HS-21) and exposure area (Tomogonops River upstream and downstream of JW-E1). One of the five stations in the reference area should be located at HS-21. One of the five stations in the exposure area should be located at station JW-E1.

Juvenile Atlantic salmon and blacknose dace should be collected quantitatively by electrofishing at five stations in both the reference (Northwest Miramichi River only) and exposure areas. Each station must be sufficiently separated spatially within each area to

ensure that catches are independent. In both the reference and exposure areas, stations must be selected in sections of the river containing all major habitat/substrate types to ensure both comparability between areas and full representation of all salmon size classes. At each station, a minimum area of 250 m^2 should be isolated by barrier net and sampled with at least five sweeps of the electroshocker. The time for each sweep should be recorded and electroshocking sweeps pooled within each station to obtain an estimate of CPUE.

Hypotheses 7 and 8

For testing of these hypotheses, all juvenile Atlantic salmon and blacknose dace caught at each station in the reference (Northwest Miramichi and Big Sevogle Rivers) and exposure (Tomogonops River) area, as described above under Hypothesis 5, should be measured for fork length and weight to the nearest 0.1 cm and 0.01 g, respectively. Scale samples should be taken from each fish for aging. Length, weight and age of a minimum of 20 fish, of each species, from the reference and exposure areas should be accurately documented and growth curves compared by ANCOVA. Although a minimum sample size of 20 fish per species is suggested, higher sample sizes are recommended if time and budgets allow.

Hypothesis 9

This hypothesis should be tested by measuring water chemistry and benthos using a gradient field design. Five stations should be established in the reference area (McCormack Brook, BCL-4) and 20 in the exposure area (South Branch Tomogonops River). The 20 stations in the exposure area should be chosen based on conductivity measurements that will ensure that the stations fall into a chemical gradient. It is anticipated that discrete areas of exposure (effluent dilution) will be identified on the basis of conductivity. HS-14 (HS-18) and BCL-15, historic sampling sites used in previous studies, should be included as stations.

At each station, a habitat assessment should be conducted to ensure that the habitats and substrate between stations and among areas are similar.

Water chemistry samples should be taken at each station resulting in a total of 25 samples (5 from the reference area and 20 from the exposure area). Samples should be collected from the surface using triplicate rinsed bottles as described in Section 3.3.4 and Appendix B. Samples should be analyzed for the parameters indicated in Table 4-8. QA/QC samples should also be collected.

A benthic sample should be collected at each station using a Surber or Hess sampler with a 250 μm mesh resulting in a total of 25 samples (5 samples from reference area and 20 from exposure area). Each sample should consist of a three to five sample composite. Samples should be split and subsampled if required as described in Appendix B.

Hypothesis 13

This hypothesis states that the suite of sublethal toxicity tests cannot predict environmental effects to resident fish performance indicators and benthic community structure. In order to make comparisons between effluent toxicity tests versus benthos and fish requires that the effluent (s) which affect these populations/communities be sampled. At the Heath Steele site there are two potential sources of toxicity. Effluent from the tailings pond affects the South Branch Tomogonops River and AMD affects the Little South and North Branches of the Tomogonops River. The benthic invertebrate survey will be conducted in the South Branch Tomogonops River along a chemical gradient which is above the influence of the non-point sources and where fish are not available. To test H13 for benthos, effluent should be sampled at HS-13 at the tailings pond east overflow.

To test H13 for fish, a water sample should be collected as the "effluent" for toxicity testing from below the confluence of the North and South Branches of the Tomogonops River at JW-E1. The advantage of choosing this site as a measure of "effluent" is that it integrates the two potential sources of toxicity without making any assumptions and also reduces the effects of environmental fate processes affecting the effluent. The fate process at this site that is of particular concern, is dilution due to Sandburn Brook and groundwater diffusion due to the distance between the effluent sources and the fish habitat (roughly 10 km). The major disadvantage of this approach is that the water sample is not a true effluent sample.

"Effluent" (refers to HS-13 and JW-E1 sample sites) should be sampled three times at each site during the field season (early spring, mid-summer and fall) and subject to four sublethal toxicity tests (fathead minnow survival and growth inhibition, *Ceriodaphnia dubia* survival and reproduction, *Lemna minor* growth inhibition and *Selenastum capricornutum* growth inhibition).

Receiving water samples (for dilution and control) should be taken from the Northwest Miramichi at Station HS-21. Samples of receiving water should be screened for toxicity to *Ceriodaphnia dubia* and fathead minnow before the sublethal tests are conducted on the effluent. Arrangements should be made with the contract laboratory to set the volumes of receiving water and effluent required. Where possible, arrangements should be made with the mine to collect and ship out the water and effluent samples in order to minimize field costs.

Samples of the receiving water (Northwest Miramichi River) and effluent (HS-13 and JW-E1) should be collected on each sampling event (spring, mid-summer and fall) for chemical analyses resulting in a total of 9 sets of samples. Analyses should be conducted for those parameters listed in Table 4-11.

5.0
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APPENDIX A

Detailed Statistical Design

[*Note: section numbers refer to main text, not sections in this appendix*]

This appendix provides the rationale for the statistical models and sample sizes recommended for the 1997 field studies (Section 3.2). Methods for statistical analyses, and potential problems with interpretation, are discussed. References providing detailed methods are given; whenever possible, these references consider similar types of studies or approaches. Some details on field sampling are provided, since the statistical designs require some departures from traditional sampling methods.

This appendix is intended for readers familiar with statistical analyses. The appendix could be useful as an addendum to a Request for Proposals (RFP) to conduct the 1997 studies. However, the AETE Committee should allow the consultants conducting the 1997 studies some freedom to suggest or use alternative procedures, especially for analyses of data from the gradient (Triad) design.

The hypotheses to be tested in 1997 are provided in Table 3-1. As noted in Section 3.2, the hypotheses can be divided into three types:

- tests for environmental effects
- comparisons of tools
- comparisons of correlations

Each type of hypothesis requires a different statistical model and analyses.

A1.0 TESTS FOR ENVIRONMENTAL EFFECTS

A1.1 Statistical Model and Analyses

Hypotheses H5-H8 can be tested using a CI (Control vs Impact) design, in which an exposed area is compared with one or more reference areas. In a CI design, variables (Y) are compared among areas using either a t-test or one-way ANOVA, with area as a factor. Analysis of covariance (ANCOVA) is used when Y is expressed relative to some other variable or covariate X (e.g., growth or body size relative to age {H7}; organ size relative to body size {H8}). ANCOVA should also be used to compare exposure among areas, when the exposure indicators are metal concentrations normalized to some modifying factor (e.g., grain size or AVS in sediments; lipid levels or wet weight content of tissue). Comparing regressions of metal concentrations on the normalizing factor(s) are preferred to comparisons of ratios of the concentrations to the normalizing factors (Hebert and Keenleyside, 1995). Multivariate approaches, similar to those used for analyses of benthic community data, may be used for

analyses of fish community data. When multiple reference and/or exposed areas are used, orthogonal contrasts can be used to address hypotheses of interest (Sokal and Rohlf {1981, pp. 232-242}; Chapman et al. {1996} and Paine et al. {1996} provide examples).

Replicates for testing H5-H8 are either individual fish (H7, H8) or spatially separated fishing locations within areas (H5, H6). *The fishing locations must be sufficiently separated spatially to ensure that catches are independent.* However, more than one seine haul, gill net set or electroshocking run could be pooled within each of several well separated locations. Ideally, the units of replication for all four hypotheses, and for fish variables in general, would be stations within areas. These stations would be the same stations used for benthos community and toxicity test responses and exposure indicators. In practice, collection of adequate numbers of fish from the same stations used for other purposes will generally be impossible (i.e., fish are sampled on larger spatial scales). As a result, fish variables cannot be matched (i.e., cannot be synoptic) with other variables within areas in a Triad approach. This has some important implications for tests of integrative hypotheses and for the overall 1997 study design (Section 3.2).

Tests of H7 and H8 should follow the approach used in the pulp and paper EEM adult fish survey (AFS), in which mature fish of one or more sentinel species are analyzed, and the sexes are analyzed separately (EC/DFO, 1995). Where only juvenile fish are present (e.g., Heath Steele), analyses would be limited to assessing effects on growth and size, and the sexes cannot be separated. However, where adult fish are present, and gonads easily removed, the hypotheses tested should be expanded to include hypotheses related to effects on reproduction as well as growth. Fish catch and community studies (H5, H6) should follow the general approach used for assessment of "Biological Integrity" in U.S. stream fish communities (e.g., Karr, 1981; Karr et al., 1986) to Canadian communities stream and lake communities. However, the specific indices used in these studies should be avoided; more objective procedures (e.g., Principal Components Analysis) for combining variables are preferable (Green, 1979).

A1.2 Statistical Power and Sample Sizes

Power analyses for CI designs are described, and formulae provided, in the summary of the 1996 studies (EVS, ESP and JWEL, 1996; see also Alldredge, 1987; Cohen, 1988; Green, 1989). Estimates of variances of life history parameters for AFS studies and catch/community metrics for CPUE/community studies were rarely available from the 1996 studies. However, variance estimates for these life history variables can be found in any First Cycle interpretative report from the pulp and paper EEM program; EVS (1996) provides variance estimates for two sucker species in a northern Alberta river, based on a multi-year study (1989-94). Estimates of variance in CPUE and fish community metrics are rarer, especially for northern Canada. Peterman (1990) provides a discussion and review of statistical power in fisheries studies; many of the examples cited were based on CPUE and

other catch/abundance data. Peterson and Rabeni (1995) discuss statistical power for fish community studies, although for a warmwater U.S. stream. Green (1989, 1994) describes methods for estimating variances of abundance data using Taylor's Power Law.

Based on the abundances of potential sentinel fish species in the 1996 studies, target sample sizes for the pulp and paper EEM AFS (20 fish per sex per area) may be the maximum achievable at most sites. Increasing sample sizes to >30 fish per sex per area provides limited gains in power (EVS, ESP and JWEL, 1996; next paragraph). Sample sizes of 5-10 geographically separated catch locations per area are probably the maximum achievable. Statistical power for these studies will be low, and arguably inadequate, because of the practical limitations on sample sizes, and because fish community and CPUE data are variable. Analyzing biomass rather than numbers may reduce variances and increase statistical power. Variances of community-level measures (total richness, abundance and biomass) are lower than variances of individual species' abundances or biomass (i.e., CPUE) (Peterson and Rabeni, 1995).

Although no formal power calculations for CI designs are presented in this appendix, Table A1 summarizes the sample sizes necessary to detect various differences (d), expressed as standard deviation (SD) units. SD refers to SD among replicates within areas. If differences are expressed in SD units, then the sample sizes necessary to detect those differences are constant for all variables. Table A1 and SD or CV (coefficients of variation) provided in either EVS, ESP and JWEL (1996) or the literature can be used to calculate the absolute (i.e., unstandardized) differences which can be detected (i.e., multiply d by SD or CV). However, standardized differences may be superior conceptually as well as practically to absolute differences (Cohen, 1988).

First, Table A1 and any power curve show that differences of <1 SD are difficult to detect with reasonable sample sizes for field projects (i.e., 5-30) or, conversely, that increased sample sizes beyond ~20-30 provide diminishing returns in terms of the differences which can be detected. This is a general limitation for any variable. The most effective strategy to increase power is to reduce SD rather than increase sample sizes. Second, d are signal:noise ratios (i.e., how large is the difference or signal relative to background variation or noise?) (Green, 1994). Third, d can be converted to % overlap of the frequency distributions of values of Y for the two populations compared (Table A1). This is an intuitively appealing concept (Cohen, 1988). For example, if d=3, then only 7% of values from the two populations (areas) overlap. Differences of this magnitude are usually obvious without statistical tests, and may be greater than many differences among species! At d=1, overlap approaches 50%, arguably the point at which populations are as similar as they are different. Again, $d \approx 1$ seems to be a reasonable difference to target, and sample sizes of 15-30 adequate to detect that difference.

Green (1994) cautions that if *d* are used as effect sizes for power analyses, there may be no incentive to reduce SD. In fact, a focus on *d* could be used to conceal inflated SD due to sloppy field and laboratory work. Recognizing this, every effort has been made to recommend procedures which will reduce SD, and the focus on *d* is only valid if those recommendations are followed in the 1997 studies.

A1.3 Sample Allocation

The optimal sample allocation for CI designs is equal replication (*n*) within each area (reference and exposed). If *k* reference areas are used, the optimal sample allocation is to allocate *n* replicates to the exposed area and *n/k* replicates to each reference area, assuming that the reference versus exposed contrast is the most important. Similarly, if *j* exposed areas are used, the optimal allocation is to use *n* replicates in the reference area, and *n/j* in each exposed area. Analyses of optimal allocations for contrasts, and other power analyses, can be conducted using the formulae for standard errors of contrasts given in Snedecor and Cochran (1980) in standard power formulae (e.g., Allard et al., 1995).

Replication within stations, fishing locations or fish is never preferable to adding stations, fishing locations or fish, unless there are significant added costs for the latter relative to the former (Cuff and Coleman, 1979; Green et al., 1993; this conclusion can also be reached using power-cost formulae for nested designs). Within the same area, the added travel and time costs of sampling additional stations or locations, as opposed to collecting additional samples from within stations or locations, are trivial (i.e., it is usually a question of walking 10's or 100's of m versus a few m). This is particularly true if the major costs are associated with processing or analyzing the samples, or with the base (i.e., fixed) costs of conducting any field study (travel to the site, accommodation, boat rental, etc.). Furthermore, composites can be used within stations or locations to improve precision without increasing sample processing or analytical costs. Note that travel and time costs for adding reference areas are often significantly greater than those for adding replicates within areas, which is why the number of reference areas is usually limited to one or a few.

A2.0 COMPARISONS OF TOOLS

Tools to be compared in 1997 field studies include:

- different types of toxicity tests (i.e., different test species, although different responses for the same species can also be compared) (H1)

- different fish tissues, for measurements of metals and metallothionein (H2,H3)

A comparison of different sampler sizes (i.e., composites from smaller samplers vs single samples from larger samplers), originally proposed, will no longer be conducted for reasons

detailed in Section A2.4. A comparison of composites of several smaller samples versus a single larger sample or a composite of fewer larger samples was an issue identified in Beak (1995) and in Taylor (1996).

More generally, any tools which are measured on the same replicates in the 1997 studies can be compared using either the approach in this section or in Section 3.2.1.3.

A2.1 Statistical Model and Analyses

For these types of comparisons, a univariate split-plot or repeated measures design, or its multivariate analogs (profile analysis), is appropriate. In the univariate approach, tools to be compared are levels of a fixed factor; in the multivariate approach, tools are variables. Green (1993) reviews the application of repeated measures designs to environmental monitoring; "times" in his examples can be replaced with "tools". Tabachnik and Fidell (1989, Chapter 10) provide methods, examples and a good discussion of profile analysis and its relationship with repeated measures.

In the split-plot design, each tool is "applied" to (i.e., used in or measured on) each of n replicate stations or fish within each area. Table A2 provides the ANOVA model, with the appropriate error terms for F-tests. The interaction between area and tool is the test of interest because it tests whether differences between areas (i.e., exposure differences or effects) differ among tools. In profile analysis, the relevant test is the test of parallelism of profiles among areas. If data are standardized for each tool by dividing by the pooled within-area standard deviation (SD), the tool providing the greatest difference between areas is the most powerful (see below). If multiple reference or exposure areas are used, contrasts among areas can be used to subdivide the area term and the interaction between area and tools. Contrasts can also be used on the tools, and area and tool contrasts can be combined for more extensive analysis of the interaction term. In SYSTAT, the area contrasts are A matrix contrasts and the tool contrasts are C matrix contrasts (Wilkinson and Hill, 1994, pp. 305-324); Tabachnik and Fidell (1989) provide examples of the use of contrasts with other statistical programs.

In most situations, the univariate split-plot is more powerful than profile analysis (Green, 1993). When only two variables (i.e., tools) are compared, both tests are equally powerful, and profile analysis is preferred because there are fewer statistical assumptions to be met. However, in these cases, it is usually simpler to conduct the analyses as a one-way ANOVA comparing the differences between tools for each replicate among areas. In the two-variable case, the difference between the two tools is the only contrast on tools possible.

Variables (i.e., values for tools) should be standardized in either univariate or multivariate analyses by dividing by the pooled within-area SD obtained form ANOVA or t-tests comparing each tool among areas (Tabachnik and Fidell, 1989). This step is necessary if the

tools are measured on different scales; it also equalizes variances within areas and tends to remove other statistical problems. Furthermore, standardization is necessary if the tests of hypotheses are considered comparisons of the power of the tools, and is the most meaningful way of expressing differences between tools. If variables are standardized, then the differences (D) between areas for each tool are equivalent to d in Table A1 (D is used to avoid confusion when calculating d as differences in D). The tool providing the largest D between areas is the most powerful for future programs. The practical significance of differences in D can readily be appreciated by converting D to sample sizes (n) required to detect D at some specified power. The differences in n could then be converted to differences in costs.

A2.2 Statistical Power and Sample Sizes

Power calculations for the split-plot design are the same as those for the CI design (i.e., t-test or one-way ANOVA), except that the variance required is the variance of the difference between tools among stations (e.g., Green, 1993). The variances of those differences were not measured in the 1996 studies, because most of the tools to be compared in 1997 were not used. The variances of the differences could theoretically be calculated if the variances for the two tools (A and B) within areas were available from other studies, using (Snedecor and Cochran, 1980, p. 99):

$$Var\ (A-B) = Var\ (A) + Var(B) - 2Cov\ (A,B) \qquad (1)$$

where Var=variance (i.e., SD squared) and Cov=covariance.

However, if A and B are standardized, Var(A)=Var(B)=1 and Cov(A,B)=r^2(A,B). Then:

$$Var\ (A-B) = 2 - 2\ r^2 \qquad (2)$$

The variances of differences can then be estimated from Equation (2) without any estimates of the variances of A and B (the probable correlation between A and B must be specified or assumed). Equation (2) also demonstrates the advantage of pairing (i.e., synoptic sampling or measuring A and B on the same replicates) versus re-randomization (i.e., measuring A and B on different samples within areas): on a standardized scale, $2r^2$ is the reduction in variance achieved.

Table A1 applies to split-plot analyses, if differences to be detected (d) are expressed as:

$$\frac{D_A - D_B}{\sqrt{2 - 2r^2}}$$

Power analyses could be conducted, assuming different r, but that is largely unnecessary. Equation (2) indicates that unless $r^2 \geq 0.5$ (i.e., $r > 0.7$), the variance and SD of differences between standardized variables will be >1 (i.e., the variances of each variable). Therefore, tests for differences of differences (i.e., comparisons of tools) are more powerful than simple tests of differences between areas (i.e., environmental effects) for the same variables when $r^2 > 0.5$ ($r > 0.7$), but less powerful when $r^2 < 0.5$ ($r \leq 0.7$). The sample sizes in Table A1 apply exactly to $r^2 = 0.5$; required sample sizes will be smaller when $r > 0.7$ and larger when $r \leq 0.7$.

Obviously, small differences in D between tools will be easy to detect if those variables are strongly correlated ($r > 0.7$); there is no need to increase sample sizes beyond those required for tests of environmental effects. Unfortunately, differences between tools which are weakly correlated ($r < 0.7$) will be more difficult to detect but may be the most important for environmental monitoring, because the weak correlations suggest that the tools may be measuring different aspects of exposure, availability or response. Therefore, consultants analyzing the 1997 data must be careful about concluding that two or more weakly correlated tools are redundant simply because the area * tool interaction is not significant. In these cases, estimates of the differences in D and their confidence limits should be provided, or *a posteriori* power analyses conducted. Alternatively, tools could be considered redundant only if $r > 0.7$ (or some other criterion) *and* there was no significant area * tool interaction. The third alternative of increasing sample sizes beyond those required for the tests of environmental effects was rejected because of increased costs; because increasing the number of replicates (e.g., individual fish) may not be achievable; and because correlations among the tools to be compared are expected to be reasonably strong.

A2.3 Sample Allocation

Optimal sample allocation for split-plot designs is the same as for CI designs - equal replication in reference and exposed areas.

A2.4 Benthos Sampling

The need to test the hypothesis "The choice of sampler size does not influence the ability to detect effects in benthic community characteristics" in the 1997 field studies has been reconsidered and will no longer be done. The issue of many, smaller samples versus fewer or one larger sample has already been addressed in two previous AETE reports (Beak, 1995; Taylor, 1996) and does not need to be repeated at additional cost. These two reports reached the same conclusions. These conclusions will now be adopted, and samples in 1997 should consist of composites of several smaller samples rather than one or a few larger samples. Compositing is desirable in depositional habitats, because variances (as CV or on a log scale) of most measures tend to decrease with increasing total numbers per sample (EVS {1996} provides empirical evidence, based on six years of sampling a northern Alberta river; Green {1979, 1989} provides theoretical support). Richness and abundance also tend to be highly

correlated when abundances are low. Even in erosional habitats, several Surber or Hess samples could be composited and processed for the same cost as single samples, if the composites were subsampled in the laboratory. The best strategy is to pool samples and/or subsample composites so that some desired total number of organisms is achieved (Green, 1989). Empirical data from both erosional and depositional habitats suggests that ~500 organisms per sample is a reasonable target (EVS, 1996). Bob Wissemann of Aquatic Biology Associates (ABA) of Corvallis, Oregon suggested a similar target, based on his extensive experience in western North America and his specific experience with Contwoyto Lake, the site of the Lupin mine.

Subsampling composites in the laboratory will always be superior to analyzing single samples, and no more expensive, if:

- composite samples can be subsampled prior to sorting (i.e., removing organisms from debris, which is the most labour-intensive part of processing)

and that:

- variance among lab subsamples of a composite is less than variance among the individual field samples used to make up the composite

The first proviso is easily met by using the Oregon DEQ (Department of Environmental Quality) or similar devices widely used in the U.S. (Mr. Wissemann, pers. comm.). The second proviso is best addressed in a targeted study comparing variances among lab subsamples versus field replicates building on work by Beak (1996).

A3.0 COMPARISONS OF CORRELATIONS (INTEGRATIVE HYPOTHESES)

A3.1 Statistical Model and Analyses

Hypotheses H9 - H12 refer specifically to comparisons of correlations of two or more *Y* variables with one or more *X* variables, or vice versa. Designation of variables as *Y* and *X* is somewhat arbitrary, although *C* variables presumably cause the responses measured by the *T* and *B* variables. The same general approaches used for those specific comparisons can be used for almost any other comparison of Triad *C*, *T* and *B* variables. Hypotheses H9 - H12 are partly or mostly redundancy questions (i.e., do we need to measure more than one of two or more *C*, *T* or *B* variables?). However, the ultimate integrative hypotheses of interest are presumably related to the strength of the overall relationship among the Triad components (i.e., H13; or: is a Triad approach justifiable for mine monitoring?).

One important restriction on tests of integrative hypotheses, and specifically H12 and H13, should be noted. Since tests of integrative hypotheses, and calculations of correlations, require synoptic sampling of *C*, *T* and *B* variables, fish variables cannot be used as *B* variables. Therefore, fish variables have been excluded from tests of integrative hypotheses in 1997, and more generally, from sites using gradient designs; H12 is untestable. H13 is also untestable because sublethal toxicity tests will be conducted on effluent samples and not on water samples from each station. H12 and H13 can only be examined qualitatively, by determining if differences in fish variables between reference and exposed sites in CI designs are associated with differences in exposure (H12) or are consistent with effects observed in toxicity tests at effluent concentrations similar to those in receiving waters (H13). Correlations between fish life history variables and metal or metallothionein levels in the same fish can be used to examine *C-B* relationships.

For convenience, H1 has been rephrased as a comparison of correlations (Table 3-1):

> *The strength of relationships between sediment toxicity responses [i.e., T variables] and any exposure indicator is not influenced by the use of different sediment toxicity tests or combinations of toxicity tests.*

Environmental effects are simply redefined as correlations between *T* variables and exposure variables, rather than as differences in *T* variables between reference and exposed areas. The *T* variables are measured for comparisons of other correlations; if H1 is rephrased, then it becomes another hypothesis to test in those designs.

The optimal design for comparisons of correlations, or tests of $r=0$, is to sample many stations representing a gradient of exposure. The problem is essentially one of regressing many *Y* on *X*, where *X* is a single or composite measure of exposure. Obviously, a relationship between *Y* and *X* is easier to detect if the range of *X* values is broad. Ideally, the *X* values should be uniformly distributed (i.e., evenly spaced), but that is rarely achievable in Triad studies.

In some cases, exposure may follow a discrete rather than continuous distribution, with large differences in exposure between several relatively homogeneous areas. Analyses then become analyses of contrasts among areas in ANOVA, and the design an extension of a CI design; Chapman et al. (1996) provide an example. Because this type of analysis is effectively a regression analysis on a few area means, a continuous exposure gradient is likely to provide more power and is preferable. Many of the tests described below would be difficult to conduct in a CI design with several exposed areas.

Formal methods for testing H9 - H11 (i.e., comparing correlations) are difficult to construct and/or interpret. As an initial screen, redundant variables can be identified from bivariate correlations. If two variables are strongly correlated (e.g., $r \geq 0.80$?), the correlations between

each and a third variable will usually be similar. However, when the two variables are weakly correlated, their correlations with a third variable could be either different or similar. Furthermore, even if their correlations with the third variable were similar, the variables might not be redundant (i.e., they may measure different things. For example, Green et al. (1993) provides the following simple correlation matrix from a Triad study; *C*, *T* and *B* refer to Principal Components (PC) based on the original variables:

	C	*T*	*B*
C	1	0.65	0.49
T		1	0.05
B			1

Are *B* and *T* redundant as response variables, since their correlations with *C* are similar? No - they are uncorrelated and represent relatively independent responses to the chemical gradient (C; which may have been confounded with a habitat gradient affecting benthos) (see below).

Partial correlation analysis could be used to address H9 - H11 more directly (Sokal and Rohlf, 1981; Cohen, 1988). Partial correlations are correlations between *Y* and *X*, with the effects of the correlation of each with a third variable (*Z*), removed (i.e., partialled out). Partial correlations between *Y* and *X* can be considered the correlation between those variables for any fixed value of *Z* (Cohen, 1988). For H9, we might ask if there is any additional correlation between biological parameters (*B*) and dissolved metals (C_2), once the effects of correlations between either and total metals (C_1) have been removed. Designation of C_1 and C_2 is admittedly arbitrary, but that is unlikely to affect conclusions in most cases. Because partial correlations can be calculated from the bivariate correlations between any three variables, they can be calculated for the Triad example above from Green et al. (1993). The partial correlations between either *B* or *T* and *C*, with the effects of the other partialled out, are actually larger than the bivariate *r* of each with *C*, a clear indication that *B* and *T* are measuring different responses and are not redundant (hence the Triad instead of a Duad).

There are two problems with using partial correlation analysis to address H9 - H11 and related hypotheses. First, the null hypothesis is that the partial $r=0$, which may not be appropriate. For example, if total metals account for 90% of the variance in *B* (i.e., $r^2=0.9$), is it important if dissolved metals or any other variable accounts for a significant portion of the remaining variance in *B*? Probably not, given the costs of measuring those variables. Conversely, if total metals account for only 10% of the variance in *B*, any other *C* variable which accounts for >11% of the remaining variance in *B* (i.e., 11% of the remaining 90% is

~10% of the total variance) would arguably not be redundant with total metals even if the partial r were not significant.

The second and most important problem with partial correlation analyses with few variables is that the overall problem is essentially multivariate. For example, before H9 is tested, single composite measures of total and dissolved concentrations of the ~20 metals in ICP scans, and some composite measure for B, are required. The first PC (i.e., PC1) from any analyses of metal concentrations in water (total or dissolved) is likely to be adequate, since concentrations of all metals tend to be positively correlated and PC1 usually accounts for most of the variance (NDM, 1996). However, both PC1 and PC2 from analyses of benthic community data may contain important information. Consequently, answers to H9 and H10 might depend on whether one or both B PC were analyzed. For C-B relationships for fish, competition for binding sites among metals may make it difficult to generate a single composite C variable (e.g., metals in mussels in Chapman et al., 1992). More generally, once a decision is made about which composite measures to use, tests of subsequent hypotheses become contingent on those decisions.

For the above reasons, multivariate methods are recommended for 1997 studies. Green et al. (1993) describe methods appropriate for Triad analyses. The methods to be used should be specified before data analyses by the consultants conducting the 1997 studies; there is no "correct" method or set of methods (Chapman, 1996). The number of sites relative to the number of variables will usually limit the analyses possible, unless the number of variables is reduced before analyses (in which case, why measure them in the field?) (Green et al., 1993). H9-11 should be addressed as a subset of the overall analyses. For example, partial correlation analyses on PC or other vectors derived from original variables could be used to address H9 and H10. H11 could be addressed in a number of different ways. For example, B-T relationships could be examined using partial correlation or regression analyses, with responses from each of t toxicity tests representing a separate T variable (i.e., $T_{1..t}$). In all cases, careful interpretation of test results will be as important as the statistical significance of those results.

A3.2 Statistical Power and Sample Sizes

Formal power analyses are impossible for comparisons of correlations because of the myriad of methods which could be justifiably used for analyses. Cohen (1988) provides power formulae for multiple regression and correlation analyses, which might be applicable to specific tests. However, the basic problem in any Triad study is to simply detect correlations considered biologically significant. If $r \geq 0.50$ (i.e., either variable "explains" ≥25% of the other) are considered biologically significant, then the sample sizes necessary to detect correlations of that magnitude are easily calculated (Table A3). The selection of $r \geq 0.50$ is partly arbitrary, but also matches the correlation defined as a "large" effect by Cohen (1988) and the size of most correlations between C, T and B variables measured in marine Triad

studies (e.g., Chapman et al., 1992, 1996; Green et al., 1993; SeaConsult and EVS, 1996). Table A2 also applies to partial correlations among three variables. Sample sizes should be increased by one to account for the additional variable, and most tests of partial correlations will be two-tail.

Table A3 indicates that $n \approx 10$ exposed stations will be adequate to detect high correlations in the 1997 studies. If correlations of this magnitude are considered indicative of redundancy, then the sample sizes proposed for 1997 (see below) will have a high probability of identifying redundant variables. Correlations of 0.50-0.80 cannot reliably be detected unless sample sizes are ≥20. However, Table A2 underestimates the power of the gradient designs proposed for 1997, because it applies to samples drawn randomly from a population. In contrast, the 1997 studies will use stations deliberately selected to represent a broad range of exposure (there is some statistical "cheating" associated with this approach). Second, using multivariate approaches, and using composite variables such as principal components instead of individual *C*, *T* or *B* variables, generally increase power relative to bivariate correlation studies.

Note the paradox in Table A2. Stronger correlations are easy to detect with smaller sample sizes; are often the most interesting environmentally; and usually identify the strongest predictive relationships. However, weaker and arguably less important correlations require larger sample sizes and greater costs to detect. For that reason, sample sizes for Triad studies should probably focus mostly on using detecting r in the mid-range of Table A2 (i.e., 0.60-0.80), rather than exclusively on detecting r=0.50. Large sample sizes are required to detect r<0.50; $r \approx 0.50$, like $d \approx 1$, marks the point at which increasing sample sizes produces diminishing returns in power.

Although sample sizes of ≥20 stations are recommended in this report, sample sizes >50 and even >100 are desirable for many multivariate analyses of observational data in the biological and social sciences (Green, 1979; Tabachnik and Fidell, 1989). Chemometricians use much smaller sample sizes, often with more variables than replicates (NDM, 1996). However, many of the methods used by chemometricians are intended for analyses of controlled laboratory experiments, not field studies. Chemical and toxicological data will generally be less variable than benthic community data, and analyses more robust. However, even with samples sizes >50, and in one case >200, secondary trends (i.e., PC2) from analyses of metal concentrations in water are suspect and probably meaningless (NDM, 1996); in some cases, PC2 simply identify outliers (e.g., Paine, 1996). Since sample sizes >50 are not feasible for the 1997 studies, investigators analyzing data from those studies must be careful not to over-interpret the data. The specifics of many results (e.g., loadings of metals or species on multivariate vectors) are unlikely to be robust and repeatable in future studies. The analyses should be treated as exploratory analyses rather than strict experimental hypothesis-testing (Tabachnik and Fidell, 1989; Everitt, 1994).

A3.3 Sampling Allocation

The optimal sample allocation for a gradient design is to sample many stations along the gradient in the exposed area and only one or a few reference stations. The reference stations represent exposure=0, and there is no need to replicate that particular X value as opposed to any other. However, some replication within the reference area is useful if, for example, exposure-response relationships observed for exposed stations are compared to reference responses (i.e., to determine the exposure level at which responses decline to reference values). Therefore, the field plans in this report assume that 5 stations will be sampled in the reference area at any sites where gradient analyses are conducted.

A4.0 Multiple Mine Sites

Hypotheses will be tested at more than one mine site in 1997, which is an additional form of replication. Theoretically, mine sites could be combined in CI designs, with site as a random factor in ANOVA. However, fish and benthos communities would differ radically among mine sites, which might render results meaningless. Furthermore, adding another factor to any ANOVA model usually leads to more complex interactions and increases the probability of violating statistical assumptions. Using mine sites as a factor in gradient designs would be much more difficult because gradients would differ among sites.

Sokal and Rohlf (1981, pp. 779-782) provide a simple test which combines the probabilities from two or more tests of the same or similar hypotheses. The test would be useful for pooling results from different mine sites. Note that the simple probability test can be used even when different statistical tests are used for different mine sites (e.g., parametric versus non-parametric tests; correlation analyses of exposure gradients among stations versus ANOVA analyses of exposure gradients among areas). The probability test and some simple parametric (Everitt, 1994, pp. 46-51) and non-parametric tests (Sokal and Rohlf, 1981; Neave and Worthington, 1988) should be used to combine tests of hypotheses from different mine sites.

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Table A1. Sample sizes per area required to detect standardized differences (*d*; in SD units) between two areas, with α=0.05 (one- or two-tail) and power (1-β)=0.80 and 0.95 (from Cohen {1988} and Alldredge {1987}; % overlap refers to the overlap between frequency distributions from the two populations compared).

D	% OVERLAP	A_1=0.05 (A_2=0.10)		A_2=0.05 (A_1=0.025)	
		POWER=0.80	POWER=0.95	POWER=0.80	POWER=0.95
0.5	67	51	88	64	105
1.0	45	14	23	17	27
2.0	19	4	7	5	8
3.0	7	3	4	3	4

Table A2. Split-plot ANOVA for comparing tools.

SOURCE	ABBREVIATION	ERROR TERM FOR TESTS
Area	A	R{A}
Replicates within areas	R{A}	T*R{A}
Tool	T	T*R{A}
Area*Tool	A*T	T*R{A}
Residual error = Replicates*Tool	T*R{A}	T*R{A}

Table A3. Sample sizes required to detect correlations (r) ≥0.50 between two variables, with α=0.05 (one- or two-tail) and power (1-β)=0.80 and 0.95 (from Cohen, 1988).

R	PV[1] (%)	A_1=0.05 (A_2=0.10)		A_2=0.05 (A_1=0.025)	
		POWER=0.80	POWER=0.95	POWER=0.80	POWER=0.95
0.3	9	68	116	85	139
0.5	25	22	39	28	46
0.6	36	15	25	18	30
0.7	49	10	16	12	19
0.8	64	7	11	9	13
0.9	81	5	7	6	8

[1] - Percentage of variance in either variable "explained" by the other variable (i.e., $100*r^2$)

APPENDIX B

Quality Management Plan (QMP)

B1.0 INTRODUCTION

Appropriate quality assurance and quality control (QA/QC) protocols are essential to ensure that environmental data achieve a high level of quality commensurate with the intended use of the data. The purpose of this quality management plan (QMP) is to serve as a general set of protocols, for both field and laboratory, which is to be used by all members of the 1997 AETE field program. Use of this QMP will ensure both high quality of data as well as uniformity and comparability in the data generated at each study site.

B2.0 DATA QUALITY OBJECTIVES

For all field and laboratory measurements, data quality objectives (DQOs) have been set where applicable. Data quality objectives are defined by the US EPA as "qualitative and quantitative statements of the level of uncertainty that a decision maker is willing to accept in decisions made with environmental data" (QAMS, 1990). DQOs define the degree to which the total error in the results derived from the data must be controlled to achieve an acceptable confidence in a decision that will be made with the data.

B3.0 QUALITY CONTROL OFFICER

A quality control officer (QCO) must be designated for the project. This quality control officer will have the following responsibilities:

- To ensure that all data quality objectives are known to both field personnel and the chosen analytical laboratories;
- To ensure that standard operating procedures (SOPs) are followed for each field component at each study site;
- To ensure that both the toxicity and analytical laboratories follow established SOPs for each analysis; and
- To ensure the all analyses were under statistical control during each analytical run. This requires that the quality control data for each analysis be reviewed and compared with historic control limits to be requested from the analytical and toxicity laboratories. The QC data should include percent recoveries of spiked samples, and results for blanks, replicates and certified reference materials. Logical checks of the data should also be conducted, especially for toxicity.

The quality control officer will have authority to require corrective actions (e.g., repetition of the analysis) if the SOPs are not followed or the analytical systems were not under control.

B4.0 Field Protocols for 1997 Study Components

B4.1 Responsibilities and Training

For each field team, a team leader must be chosen with authority to make decisions in the field related to implementation of the study plan. The team leader must be familiar with all aspects of the study plan and is responsible for communicating any changes in the study plan to the project manager. The team leader will also be responsible for ensuring that:

- All field personnel are trained and competent in use of each field instrument;
- All SOPs for each program component are followed; and
- Adequate heath and safety measures are followed.

B4.2 Standard Operating Procedures (SOPs)

B4.2.1 Habitat Characterization and Classification

Habitat characterization must be conducted in each reference and exposure area and include each sample station. To ensure habitat is characterized in a consistent manner, the Department of Fisheries and Ocean and the New Brunswick Department of Natural Resources and Energy (DFO/DNRE) *Stream Survey and Habitat Assessment Table* will be used as a guide at all mine sites (DFO and NBDNRE 1994; Appendix C). In addition to completing the habitat assessment table, the following information will be recorded on the habitat assessment table as well as on field data sheets for the other study components:

- Location;
- Date and time;
- Wind and climatic conditions;
- Field crew members;
- Sampling methods; and
- Deviations in field sampling protocols.

For stream and river habitats the habitat surveyed should be divided into discrete habitat units based on stream type (e.g., fall, run, riffle, pool). For each unit the length, average width, average depth, current velocity, substrate composition, embeddedness, percent

undercut bank, percent over-hanging bank vegetation, percent shade, percent stream bank vegetation and percent bank erosion should be determined. Water depth should be measured using a gauging rod. Current velocity should be measured in the middle of the stream and at 1/4 and 3/4 distances in the stream channel using a current velocity meter positioned at 0.6 m water depth.

For lakes, habitat should also be visually surveyed using the DFO and NBDNRE (1994) methodology as a guide. Water depth should be measured using a weighted survey tape. Substrate characterization should include sampling of sediments along a transect using, for instance, a Petit Ponar grab sampler.

Based on the substrate types identified in the habitat characterization, the study area should be classified into constituent habitats based on the habitat classification scheme of Cowardin et al. (1979) developed for the Fish and Wildlife Service.

B4.2.2 Sample Station Selection and Location Identification

Sample stations should be selected in as uniform habitats as possible based upon the following criteria:

- Uniform substrate;
- Uniform current velocity; and
- Uniform depth.

Global Positioning System (GPS) positions should be recorded for each sampling station to record the station location. The accuracies of the positioning should be known to the nearest 30 m.

B4.2.3 Water Chemistry Sampling

B4.2.3.1 Field Protocols

Field measurements of temperature, conductivity, dissolved oxygen and pH should be taken at each station using, for example, a Hydrolab H20 or YSI meters. The analytical methods for calibration and use of each field instrument should be those outlined in each respective instruction manual. A log should be kept of each field instrument indicating its usage and any problems encountered. In using an oxygen electrode, care must be taken to change the membrane on a regular basis, or if it becomes dried out, torn or damaged in any way. All values including calibration readings must be recorded on the field sheets.

In shallow stream/river environments (< 2 m) water samples should be collected at the surface from each station with clean bottles prepared by the analytical laboratory. Clean

sampling techniques must be used at all times to minimize sources of contamination. Sample bottles should be triplicate rinsed with sample media. Samples should then be collected by removing the cap below the surface (approximately 15 cm depth) to avoid any surface contamination. In deeper receiving environments (> 2 m) sub-surface grab samples should be collected at each station using a Van Dorn-type sampler. Sample bottles should also be triplicate rinsed with sample media. Latex (or nitryl) gloves should be used during this procedure to avoid all contamination.

Separate samples should be collected for total and dissolved metals. Dissolved metals are operationally defined as water filtered through a 0.45 μm filter. The dissolved sample should be field filtered according to standard methods (APHA 1995 -Section 3030B). Differences in filtering protocol can affect data quality. Therefore filtering protocols must be reviewed with field staff prior to sample collection at each mine site. In addition, all filters, filter holders, syringes and preservatives should be provided by the same analytical laboratory to ensure quality control. Gloves or other equipment which may cause metals contamination (e.g., due to powdered zinc) should not be used. Metals samples (total and dissolved) should be acidified with ultrapure HNO_3 (provided by the analytical laboratory) to pH <2. Other samples should also be preserved with the appropriate preservative after sample collection (e.g., nutrient samples preserved with H_2SO_4). All sample containers should be labelled on the side with identification numbers also recorded on the top of each container to ensure sample identification if the label is damaged during shipping. All samples should be cooled and shipped on ice to the same analytical laboratory. The same analytical laboratory should be used to ensure consistency in sample analyses and QA/QC. Chain-of-custody sheets must accompany the water chemistry samples to the laboratory. Copies should be retained by the field team leader for sample tracking. Sample collection information should be recorded onto field data sheets.

B4.2.3.2 Quality Control Protocols for Water Chemistry

Prior to commencement of the field programs, the QCO should discuss the objectives of the water chemistry sampling program, sampling protocols (SOPs), analyses protocols and DQO with the analytical laboratory. To meet study objectives, a detection limit of 1/10 of the CCME guidelines for protection of the aquatic environment is required for analytical measurements. The quality control officer should ensure that the required detection limits are made known to the analytical laboratory well in advance. In this way, the correct methodology, volume of samples and methods of preservation can be established before the field work commences.

At each mine site, on each sampling event, quality control samples for water chemistry should include collection and analysis of trip blanks, filter blanks and field replicates. If subsurface samples are collected using a Van Dorn-type sampler, then sampler blanks should also be collected. Transport blanks consist of sample bottles filled with distilled, deionized

water in the laboratory. These transport blanks are brought to the field, opened, and then shut immediately. The water for the transport blanks should be provided by the analytical laboratory. A filter blank consists of a field-filtered sample of distilled, deionized water. Distilled water for the filter blank should also be provided by the laboratory. When a Van Dorn-type sampler is used to collect samples, sampler blanks should also be collected. To collect sampler blanks, the sampler is triplicate rinsed with distilled water, deionized water is poured into the sampler, and then normal samples are taken. Field replicates should be collected from a station located in the exposure area.

The field QC samples are required to assess the quality of field sample collection. These field QC samples are exclusive of those analysed routinely in the laboratory as part of normal laboratory QC. Field QC samples should be cooled and shipped on ice to one of up to three analytical laboratories. Comparison of QC results between laboratories could be used to ensure quality control in sample analysis. Results of the QC samples should be reviewed by the QCO to ensure DQO are met.

B4.2.4 Sediment Sampling

B4.2.4.1 Field Protocols

Sediment samples should be collected only if a station has an area > 1 m^2 of depositional habitat. If not, detailed notes on the site should be made and pictures taken to provide evidence that the station is not suitable for sediment collection. Sediment samples should be collected using a stainless steel Eckman or Petite Ponar grab sampler. Grabs may be accepted as representative samples based on several criteria (Environment Canada, 1995):

- Sediment is not extruding from the upper face of the sampler;
- Overlying water is present indicating minimal leakage;
- Overlying water is not excessively turbid and the sediment surface within the grab is relatively flat indicating minimal disturbance/winnowing; and
- The penetration depth is at least 4 - 5 cm.

Samples which do not meet these criteria should be rejected. Replicate grab samples should be collected at each station to test sediment chemistry; the number of replicates should be as specified in the site specific study designs. A pre-cleaned plastic spoon should be used to take the upper 2 cm of the sediment column from each of the replicate grabs and deposit it into a glass mixing bowl. The replicate samples should be thoroughly stirred until homogenous in color and texture. Homogenized samples should be placed into sample jars provided by the laboratory (e.g., pre-cleaned glass with Teflon-lined lids). Jars should be filled to the top to minimize air space. Mixing bowls and plastic utensils should be cleaned between sampling stations by using the following protocol:

- Water rinse;
- Phosphate-free soap wash;
- Deionized water rinse;
- 5% HNO_3 rinse; and
- Final deionized water rinse.

All sample jars should be labeled on the side with identification numbers also recorded on the top of each container to ensure sample identification if the label is damaged during shipping. All samples should be cooled and shipped on ice to the same analytical laboratory. The same analytical laboratory should be used to ensure consistency in sample analyses and QA/QC. Chain-of-custody (COC) sheets must accompany the sediment chemistry samples to the laboratory. Copies should be retained by the field team leader for sample tracking. Sample collection information should be recorded onto field data sheets.

B4.2.4.2 Quality Control Protocols for Sediment Sampling

Prior to commencement of the field programs, the QCO should discuss the objectives of the sediment chemistry sampling program, sampling protocols (SOPs), analyses protocols and DQO with the analytical laboratory. The quality control officer should ensure that the required detection limits are made known to the analytical laboratory well in advance. In this way, the correct methodology and volume of samples can be established before the field work commences.

In addition to all samples being sent to the same analytical laboratory, swipe blanks should also be collected to determine the effectiveness of field decontamination procedures. The swipes consist of acid-wetted, ashless filter papers wiped along the inside of the sampler and mixing bowl/spoon surfaces that are likely to contact sample media. These samples should be placc in whirl-pack bags and sent to the analytical laboratory for extraction and metals analysis.

The field QC samples are required to assess the quality of field sample collection. These field QC samples are exclusive of those analysed routinely in the laboratory as part of normal laboratory QC. Field QC samples should be cooled and shipped on ice to one of up to three analytical laboratories. A three-way comparison of QC results could be used to ensure quality control in sample analysis. Results of the QC samples should be reviewed by the QCO to ensure DQO are met.

B4.2.4.3 Quality Control Requirements for Choice of an Analytical Laboratory

A common analytical laboratory should be selected for samples collected at all mine sites. The laboratory must be certified by CAEAL and the project QCO should ensure that the laboratory follows the following quality control practices:

- Written (or referenced) SOPs for each analytical system;
- Instrument calibration and maintenance records;
- Clearly enunciated responsibilities of Q/A officer;
- Adequate and training of personnel;
- Good Laboratory Practices (GLPs);
- Sample preservation and storage protocols ;
- Sample tracking system (e.g., LIMS system);
- Use of QC samples to ensure control of precision and accuracy (blanks, replicates, spikes, certified reference materials (minimum effort should be 15-20%) ;
- Maintenance of control charts and control limits on each QC sample;
- Data handling and reporting (blanks, replicates, spike recovery, significant figures);
- Policy for reporting low level data (e.g., ASTM L,W); and
- Participation in external audits and round robins.

The QCO should request that all QC data (including control limits) be contained in the analytical reports and ensure that all analytical runs were under statistical control at the time of analysis. The QCO should also ensure that the analytical laboratory has attained the required detection limits or have a valid technical reason when these limits were not attained. Such values should be flagged in the analytical report. The QCO should examine all outliers and can request repeat analysis if the data are questionable.

B4.2.5 Benthic Invertebrate Sampling

B4.2.5.1 Field Protocols

Benthic samples should be collected at stations of similar substrate and habitat type. Samples should be collected with a Hess sampler, Eckman or Petite Ponar grab depending upon the substrate type. A Hess or Surber sampler (250 μm mesh size) should be used for collection of benthic samples in shallow (<32 cm), flowing waters on rocky substrates where a grab sample cannot be taken. The Surber sampler consists of two square frames hinged together; one frame rests on the surface while the other remains upright and holds a nylon collecting net with a collection cylinder attached. A base extension is used when sampling areas of fine, loose sediments or rubble. The base frame fits into the base extension which is pushed into the sediments to decrease the lateral movement of invertebrates out of the area to be sampled. The sampler is positioned with its net mouth open, facing upstream. When in use, the two frames are locked at right angles, the base frame (and base extension) marking off the area of substrate to be sampled (0.093 m^2) and the other frame supporting a net to strain out organisms washed into it from the sample area. General operating procedures for the Surber sampler are as follows:

- Position the sampler securely to the bottom substrate, parallel to the water flow with the mouth of the net facing upstream;

- Bring the sampler down quickly to reduce disturbance and the escape of rapidly-moving organisms;

- Eliminate gaps under the edges of the frame, which could result in loss of invertebrates, by working the sampler into the substrate and shifting rocks and gravel along the outside edge of the sampler;

- Avoid excessive drift into the sampler from outside of the sample area by minimizing disturbance of substrate upstream from the sampler;

- Maintain the sampler in position during sampling by holding with one hand or bracing with the knees from behind so that the area delineated remains constant;

- Wear heavy gloves when handling dangerous debris (e.g., glass or other sharp objects) present in the sediment;

- Turn over and examine carefully all rocks and large stones. Rub carefully in front of the net with the hands or a soft brush to dislodge the organisms and pupal cases, etc., clinging to them before discarding;

- Wash larger components of the substrate within the enclosure with stream water; water flowing through the sampler should carry dislodged organisms into the net;

- Stir the remaining gravel and sand vigorously with the hands to a depth of 5-10 cm where applicable, depending upon the substrate, to dislodge bottom-dwelling organisms;

- Hand pick some of the heavier mussels and snails from the substrate, if necessary, that are not carried into the net by the current;

- Remove the sample by washing out the sample collection cylinder into a plastic basin;

- Homogenize replicate samples to obtain one composite sample;

- Place sample into a plastic sample bottle (wide-mouthed) and preserve with 10% buffered formalin fixative;

- Examine the net carefully for small organisms clinging to the mesh, and remove them (preferably with forceps to avoid damage) for inclusion in the sample; and

- Rinse the sampler net after each use.

An Eckman or Petite Ponar grab sampler should be used for collection of benthic samples in soft sediments. The Eckman is used primarily on soft sediments in deep water (>2 m), although a pole mounted version can be used in harder substrates and shallower waters. Ponar grabs are used for substrates consisting of hard and soft sediments such as clay, hard pan, sand, gravel and mud where penetration of the substrate by the sampler is possible. The standard Ponar is set with a spring loaded pin, lowered to the bottom and allowed to penetrate the substrate. When the Ponar penetrates the sediment, the pin is released and the jaws are allowed to close on the sediment sample when the sampler is withdrawn. The Ponar (plus sample) is then pulled through the water column and placed into a plastic basin on the bottom of the boat. After the sample has been removed and whenever the Ponar is not being used, the safety pin must be inserted into the lever bar to prevent the bar from closing on the operator. The Petite Ponar sampler is similar to the standard Ponar but is considerably lighter, safer and easier to use.

Both the Eckman and Ponar samplers should be made of stainless steel rather than brass. The choice of using an Eckman or Ponar sampler depends on the nature of the sediment and the depth of the water column. In hard sediments, use of the Eckman sampler is limited as penetration is poor. The pole mounted Eckman is able to penetrate some hard substrate, but its use is limited to shallow depths. If sediments are very soft, the Eckman may be preferable to the Ponar because the latter tends to fill entirely with sediments, thereby obliterating the sediment-water interface. At depths greater than 20 m the Ponar may be more successful because of its greater weight and stability in the water column.

Benthic samples collected with the Eckman or Petite Ponar should be collected synoptically with sediment chemistry samples (if applicable). Benthic samples are considered acceptable if there is full penetration of the grab and it remains closed at the surface. Benthic samples should be sieved in the field using a 250 μm stainless steel mesh sieve. Each sample from each station should consist of five replicates pooled to form one composite sample. Pooled, replicate samples are taken to account for and to include the natural variability in the distribution of discretely distributed, individual organisms, and aid in the detection of area differences (Beak, 1996). Samples should be placed into plastic collection bottles (wide-mouthed) and preserved with 10% buffered formalin.

All sample containers should be labeled on the side with identification numbers also recorded on the top of each container to ensure sample identification if the label is damaged during shipping. All samples should be cooled and shipped on ice to the same analytical laboratory. Chain-of-custody (COC) sheets should accompany the benthic invertebrate samples to the laboratory. Copies should be retained by the field team leader for sample tracking. Sample collection information, including the water depth and velocity at the sample station, should be recorded onto field data sheets.

All benthic samples should be sorted with the use of a stereomicroscope (10X) and keyed to the generic level. To expedite sorting, all samples may be stained with a protein dye that is absorbed by aquatic organisms but not by organic material such as detritus and algae. Samples should be washed through a 250 μm sieve and sorted entirely except in the following instances: those samples with large amounts of loose organic matter, and samples with high densities (>100) of major taxa. In these cases, samples should first be washed through a large mesh size sieve (e.g., 3.36 mm) to remove all coarse detritus, leaves, and rocks. Large organisms such as leeches, crayfish, and molluscs, retained in the sieve should be removed from the associated debris. The remaining sample fraction should be subsampled before sorting as follows. Sample material should be distributed evenly on a 250 μm sieve and divided in two. One half of the material should be removed and represerved while the remaining half is distributed evenly on another 250 μm sieve. Organisms and debris washed from the algae should be added back to the loose fraction. The algae should be sorted separately from the loose material. Where subsampling is warranted, algae should be distributed evenly on a 250 μm sieve and again divided in two. This procedure should be repeated until an appropriate subsample fraction remains. A minimum of 200 organisms should be sorted from each sample, up to a maximum of 500.

In samples containing large quantities of filamentous algae, the algae should be separated from the loose material and washed separately in a 250 μm sieve. Organisms and debris washed from the algae should be added back to the loose fraction. The algae should be sorted separately from the loose material. Where subsampling is warranted, algae should be distributed evenly on a 250 μm sieve and cut into appropriate subsample fractions. The loose material from the same sample may also be subsampled, depending on the densities of organisms. A minimum of 200 organisms should be sorted from both algae and loose material, up to a maximum of 500.

For those samples that are subsampled, sorted and unsorted fractions should be represerved separately. Sorted organisms should be placed in 1 oz. glass bottles and represerved in 80% ethanol. Each bottle should be labelled externally and internally with survey name, date, station and replicate number, and sorter's name.

Chironomids and oligochaetes should be mounted on glass slides in a clearing medium prior to identification. In samples with large numbers of oligochaetes and chironomids, a random sample of no less than 20% of the picked individuals from each group should be removed from the sample for identification, up to a maximum of 100 individuals. Following identification and enumeration, a detailed species list should be prepared for each station and replicate, summarizing the total organism density and total number of taxa. The species list should be in a standard spreadsheet format and of a high quality, ready for submission in final reports.

B4.2.5.2 Quality Control Protocols for Benthic Invertebrate Collection and Enumeration

Prior to commencement of the field programs, the QCO should discuss the objectives of the benthic invertebrate sampling program, field sampling and sieving protocols, analyses protocols and DQO with the analytical laboratory. The quality control officer should ensure that the required level of taxonomic resolution is made known to the analytical laboratory well in advance.

Quality control protocols should include:

- Collection of benthic samples by the same individual at one mine site to ensure collection consistency;
- Adherence to sampling protocols outlined above to ensure sampler consistency between mine sites;
- Use of the same taxonomist for processing and enumeration of samples from all mine sites;
- When samples arrive at the laboratory, they should be checked for adequate preservation and proper labelling before being logged and kept secure;
- Staining samples (if required) to facilitate accurate sorting;
- Use of the most updated and widely used taxonomic keys for all taxonomy;
- Confirmation of sorting efficiency. Ten percent of processed samples should be resorted by a second person to confirm 95% recovery of all organisms;
- Estimation of subsampling error in a minimum of 20% of samples subjected to subsampling. This can be accomplished by entirely sorting 20% of the samples that were subjected to subsampling;
- Verification of taxonomy by an independent expert;
- Retention of all unsorted and sorted fractions of samples until taxonomy and sorting efficiency are confirmed;
- Preparation of a voucher collection of identified organisms for both reference and exposure stations; and

- Review of all tabulated benthic data to ensure there has been no data entry errors or incorrect spelling of scientific nomenclature.

B4.2.6 Fish Sampling

B4.2.6.1 Fish Population Sampling

Gill netting, trap netting and backpack (or boat) electrofishing are expected to be the primary capture methods employed in the field program. The standard operating protocols for these capture methods are outlined below.

Protocol for Gill Netting

The protocol employed during gill netting should be as follows:

- Individual panels of various mesh sizes should be assembled to comprise a gang of nets of required sizes. The order of assembly of sizes should be the same for each gang. A bridle should be attached to each end, and anchor/float lines should be attached to the bridle that are appropriate for the water depth in which the nets should be deployed. The section of rope between the anchor and the bridle should be of sufficient length that the anchor can be placed on the bottom before any netting is deployed.

- Netting locations should be selected that are free of major bottom irregularities or obstructions (steep drop-offs, tree stumps, etc). Upon selection of the preferred site, the net should be deployed in a continuous fashion along the selected route. Care should be taken to avoid tangles or twists of the net, and to ensure that marker buoys at each end are visible (i.e., above water) after setting. Water temperatures should be taken on the bottom and at 2 m above the bottom at each end of the net if other than isothermal conditions are present. The location and orientation of the net relative to shoreline features should be marked on an appropriate map and/or obtained by electronic positioning equipment (GPS). The above noted information, the water depth at each end of the net, the date, time of day and other relevant information (wind direction and weather conditions, wave height, etc.) should be recorded in the field book for each netting location.

- Upon retrieval, the same information as noted above (as applicable) should be recorded. All fish collected should be identified and enumerated as described below. Those fish not required for further testing/analysis should be live released provided they are in good condition.

Protocol for Trap Netting

The protocol for trap netting is as follows:

- Prior to use in the water, the net should be spread out on land and examined for holes and signs of excessive wear (broken and/or frayed lines or attachment points) if the condition of the net cannot be determined from previous users. The lead, wings, house and all attachment lines should be examined, as well as the house access point opening. All damage should be repaired, the house opening should be secured and the net should be repacked to facilitate ease of deployment.

- Netting sites should be selected that are relatively smooth bottomed, of a suitable substrate for anchoring (i.e., mud, sand, and/or gravel; smooth bedrock not suitable) and free of major irregularities (large boulders, tree stumps or snags, etc.). If water visibility permits, the selected location should be examined from above to confirm its suitability.

- The net should be set perpendicular to shore such that the lead is in shallow water near shore and the house is in deeper water offshore. The net is continuously deployed from the bow of the boat, while backing offshore, until all parts of the net and all anchors are in the water. Upon setting the house anchor, the net is then tensioned. The wing anchors are then lifted and repositioned such that the wings are aligned at a 45° angle to the lead, and lightly tensioned. The date, time of day, water temperature and other appropriate information is recorded in the field book.

- When servicing the net, the house float is lifted and the boat (assuming a small aluminum boat is used) is pulled under the anchor line between the house and the house anchor. The boat is then manually pulled sideways to the house of the net, which is then passed over the boat until all fish are concentrated at the near shore end of the house. The house access point is then opened and the fish are removed, identified and enumerated. The fish required for analysis are retained, while the remainder are released live. The catch and the ancillary environmental data (as described below) are recorded in the field book. The house opening is then closed and the boat is backed out from beneath the net. Anchors are lifted and reset to re-tension the net as required.

Protocols for Back-Pack or Boat Electrofishing

The operators of the electrofishing gear should follow procedures outlined in standard fisheries text books. The description below represents only a brief summary of the operations to be followed for back-pack electrofishing.

- Before electrofishing operations begin, the amount of effort, either by distance, time or desired sample size should be agreed upon in order to calculate catch per unit effort. Duration of electrofishing is generally a good measure of effort. In a small river, a known area of the river (~100 m^2) should be enclosed with block nets downstream and on both banks (if necessary). The area thus delineated should be swept by the operator in an upstream- downstream direction along transects running perpendicular to the shoreline, starting downstream and going upstream. Two other persons should stand about 1 m downstream of the operator to capture the fish with dipnets. Between each sweep, the block nets should be checked for immobilized fish. The number of fish obtained in each sweep should be noted and placed in an appropriate holding tank.

- Health and safely procedures must be followed strictly. These are also outlined in standard text books.

Fish Data Collection

Fish captured at a site should be identified and enumerated as described below. The biological variables to be measured include:

- fork length;
- fresh weight;
- external conditions;
- sex (if possible); and
- age.

Information on each fish species should be recorded on standard data logging sheets. Fork length should be measured to the nearest ± 0.1 mm. Fish should be weighed to the nearest 1.0 g or 5% of total body weight. Calibration of the balance should be checked with certified standards each time the instrument is moved. Age should be determined by the appropriate structure (scales, otoliths, pectoral spines) following established protocols. A single person should perform the age determinations on all the fish for consistency and quality control. Aging structures should be archived for future reference and fish age should be confirmed by a second expert (minimum 10%).

B4.2.6.2 Fish Tissue Sampling

As recommended by Beak (1996) all histopathology, metallothionein and metals analyses should be conducted on the same fish. The number of fish to be collected at each site are presented in the site specific study designs (Section 4.0). Tissue samples should be obtained from fish that are alive after collection and immediately before tissue removal. For large fish (>15 cm) the organs for metallothionein and metals analysis (kidney, gill, liver) should be

divided in half; one half for metals and the other for metallothionein analysis. Small fish (<10 cm) should be frozen whole and submitted for analyses. Fish between 10 to 15 cm should not be collected.

The biological variables to be measured on each fish collected for tissue dissection include:

- fork length;
- fresh weight;
- external/internal conditions;
- sex;
- age;
- gonad weight;
- kidney weight;
- egg size and mass (if appropriate); and
- liver weight.

External examinations should be conducted on fish collected for tissue analyses for lumps and bumps, secondary sexual characteristics, missing fins or eyes, opercular, fin or gill damage, external lesions, presence of parasites, and other anomalous features. All external lesions should be recorded as to position, shape, size, color, depth, appearance on cut surface and any other features of note. Photographs should be taken of lesions to aid in their interpretation. The external conditions should be assessed according to the health assessment index of Adams et al. (1993) or Goede and Barton (1990) on data logging sheets. If the fish are large enough (> 15 cm), the body cavity should be opened to expose the internal organs; this is most easily done by using a blunt pair of scissors. Latex gloves are recommended for the dissection. The body cavity may be opened either from the pectoral fin back to the anus or by inserting one blade of the scissors into the vent then proceeding anteriorly to the isthmus of the gills . Avoid cutting internal organs in this process (other than the extreme end of the anus). Puncturing the swim bladder may be necessary to allow access to the internal organs. Internal examination of each fish should include the recording and/or photographing of evident tumors, neoplasms and lesions in major organs including the liver and skin. Internal conditions should be assessed according to the health assessment index of Adams et al. (1993) or Goede and Barton (1990) on data logging sheets.

All internal organs should be examined for lumps, bumps or abnormal features. The lower intestine and oesophagus should be cut to allow total removal of the gastrointestinal tract. The liver should be removed and weighed to 0.1 g on pre-weighed aluminum pans. The liver samples must be weighed immediately to avoid loss of water. Care must be taken to avoid rupturing the gall bladder and to remove the spleen before weighing. If the liver tissue is diffuse, it should be teased from the intestines starting from the posterior and proceeding anteriorly. The liver should be weighed, divided in half and frozen in separate plastic bags on dry ice for metals and metallothionein analysis.

The gonads should be removed from the dorsal wall of the body cavity from the anterior to the posterior and weighed on a pre-weighed pan to the nearest 0.1 g or ±1% of the total organ weight. Care should be taken to remove external mesenteries and visceral lipid deposits before weighing the gonads; gonadal membranes, however, should remain intact. Egg volume and mass should be measured on fresh eggs. One hundred eggs should be counted in a stereoscopic microscope and added to a small graduated cylinder containing a known volume of water. The cylinder should be placed on a balance so that the mass of the 100 eggs can be measured. The volume of the eggs should then be determined from the displacement of the water in the cylinder.

The kidneys should be removed by making lengthwise incisions along each edge of the tissue and then detached using the spoon end of a stainless steel weighing spatula by applying firm but gentle pressure against the upper abdominal cavity wall (dorsal aorta). In this procedure the kidney is scraped away from the dorsal aorta and associated connective tissue. The kidney should be divided in half, placed in separate whirlpack bags and frozen on dry ice for both metals and metallothionein analysis.

The gills arches and attached filaments should be removed by severing the dorsal and ventral cartilaginous attachment of the arches to the surrounding oral cavity. The gill arches should be placed in whirlpack bags and frozen on dry ice for metals and metallothionein analysis.

If whole fish are collected due to small fish size (< 10 cm), each specimen should be placed in a numbered plastic bag and kept on dry ice in a cold box during shipment to the analytical laboratory. The samples must arrive at the laboratory frozen. Chain-of-custody sheets should accompany the fish tissue samples to the laboratory. Copies should be retained by the field team leader for sample tracking. Laboratory analyses of fish tissues should follow procedures outlined by the AETE Committee.

B4.2.6.3 Quality Control Protocols for Fish Sampling

Prior to commencement of the field programs, the QCO should discuss the objectives of the fish population and fish tissue sampling programs, field sampling protocols, analyses protocols and DQO with the analytical laboratories. The following quality control protocols should be followed for the fisheries assessment:

- All aging structures should be sent to the same laboratory for analyses to ensure consistency and quality control;

- 10% of the structures from each mine site should be verified for age by an independent expert;

- The same individual should conduct the external and internal fisheries health assessment at one mine site;

- Photographs should be taken of the range of values assigned to various health assessment parameters to ensure consistency in assessment between mine sites; and

- A sample numbering system should be designed to facilitate tracking of all tissue sub-samples taken from the same fish.

B4.2.7 Toxicity Samples

The following general QA/QC protocols apply to all toxicity tests:

Negative Controls — All tests must be conducted using well-established negative (clean) controls. For every toxicity test, one series of test chambers must contain clean diluent water (or clean diluent water and clean sediment) only. The complete test series is repeated if the mean control response does not meet the acceptability criteria for a particular test.

Positive Controls (Reference Toxicants) — All toxicity tests include positive (toxic) controls, conducted with well-established standard reference toxicants. Reference toxicants are used to provide insight into mortalities or changes in sensitivity that may occur as a result of acclimation, disease, loading density or handling stress. For organisms obtained from outside sources, a positive control is tested for each new batch obtained. For organisms obtained from in-house laboratory cultures, positive controls are performed on a monthly basis. Control charts are constructed for each species and reference toxicant used. The cumulative mean value and upper and lower control limits (± 2SD) are plotted on each chart. These charts are kept in the laboratory and updated with the results of each reference toxicant test. The QA/QC Officer is responsible for monitoring the data for trends in increasing or decreasing sensitivity. If the results of a reference toxicant test fall outside the control chart limits, the test procedures and health/source of the test organisms are reviewed; subject to those findings, the test may be repeated.

Reference Samples — Reference samples are usually required for sediment toxicity tests and are used to separate toxicant effects from unrelated effects such as sediment grain size. Reference sediments are collected from an area documented to be free of chemical contamination and should represent the range of important physical variables found in the test sediments.

Test Organisms — Only healthy organisms of similar size and life history stage should be used for toxicity tests. All test organisms used for a batch of tests must be from the same source. Records of collection, shipping and holding should be maintained for all species obtained outside of the laboratory.

Blind Testing — All treatment containers should be randomized during test set-up. Blind testing is done periodically where laboratory personnel are not given the identity of the sample prior to testing.

Replication — The number of replicates required varies from one test protocol to another, but should always be sufficient to account for variability in test organism response. Unless otherwise specified in the experimental design, each treatment in a test series must begin with the same number of replicates.

Water Quality Measurement/Maintenance — Toxicity tests involving exposure of organisms in aqueous media require that the media be uncontaminated and that proper water quality conditions be maintained to ensure the survival of the organisms, and to ensure that undue stress is not exerted on the organisms, unrelated to the test materials. Appropriate water quality parameters must be measured at the start and end of a test as a minimum, and preferably every 24 h. If acceptable limits are exceeded at any time, the data should be reviewed by the Project Manager and QA/QC Officer and the later should recommend appropriate action.

Standard Laboratory Procedures — Standard laboratory procedures should be followed in all testing. These include use of established methods, proper documentation, proper cleaning, avoidance of contamination and maintenance of appropriate test conditions. All unusual observations or deviations from established procedures must be recorded and reported to the Laboratory Manager.

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APPENDIX C

Habitat Assessment Methodology

GENERAL INFORMATION	
This methodology is to be used for a consistent approach to habitat characterization when completing the DNRE/DFO Table- New Brunswick Stream Survey and Habitat Assessment Table.	
SIDE 1/PAGE 1	
TABLE HEADING	
River:	- the name of the river or stream being surveyed
Start Point:	- start of survey (GPS reading)
End Point:	- end of survey (GPS reading)
Drainage Code:	
Stream/River No.:	
Personnel:	- fill in each surveyors initials
Date:	- fill in date on which survey is performed
GIS Map No.:	- if known, fill in the Forest Inventory Map number pertaining to area on river/stream being surveyed
Stream Order No.:	
TABLE	
Rules for filling out the table: • for something assessed, but not observed put **(0)** • for something not assessed put **(--)** • specify orientations as: R = right L = left M = middle	
Column 1 "Reach No."	• reach number being surveyed
Column 2 **"Unit No."**	Each distinctive stream type encountered within a reach during the stream survey is denoted as a discrete unit and numbered consecutively, starting with one, from the start point to the end point of each reach surveyed.

Fish Habitat Assessment Methodology (continued)

<table>
<tr><td>Column 3
"Stream Type"</td><td>Identify and record stream type by number from the "Stream Type" table below.</td></tr>
<tr><td colspan="2">
<table>
<tr><th colspan="4">STREAM TYPE</th></tr>
<tr><th colspan="2">FASTWATER</th><th colspan="2">POOLS</th></tr>
<tr><td>1. Fall
2. Cascade
3. Riffle (Gr/Rb)
4. Riffle (R/B)
5. Riffle (Sand)</td><td>6. Sheet (ledge)
7. Chute
8. Run
9. Rapid</td><td>10. Midchannel
11. Convergence
12. Lateral
13.Beaver
14.Trench
15. Plunge
16.
17. Bogan</td><td>18. Eddy
19. Gabion
20. Log Structure
21. Road Crossing
22. Wood Debris
23. Man-Made Dam
24. Natural Deadwater</td></tr>
</table>
</td></tr>
<tr><td>Column 4
"Channel Type"</td><td>Two or more stream types may occupy the width of a river/stream. In such cases the location of the stream type must be denoted as R, L or M.

Right and left are with respect to the right and left sides of the surveyor, as the surveyor is moving from upstream to downstream.

Main Channel: used when the stream identified encompasses the entire width of the river.

Side Channel: used when an island divides the river into two or more channels. One channel would be identified as the Main (1) and the other as a Side channel (2).
- specify if the side channel is to the left (L) or the right (R) of the Main Channel.

Split: used when there are two or more stream types encompassing the entire width of the river/stream use R, L to divide right and left sides.

Bogan: used when there is a backdrop of water due to an incoming tributary. Substrate normally consists of sands and fines
- specify if the bogan is on the left (L) or on the right (R).

(e.g., The survey for reach one has just begun. The river or stream has three stream types encompassing the entire width of the river or stream. To the left is a riffle (stream type 3, 4 or 5, depending on substrate composition); In the middle is a pool (stream type 14 to 24, depending on pool characteristics); To the right is a run (stream type 8). The riffle would be unit 1, the pool would be unit 2 and the run would be unit 3. The channel type of unit 1 would be written as 3L. The number designates the riffle as a split, with the unit being on the left side of the stream (L). The channel type for unit 2 would be written as 3M, and that for unit 3 would be 3R.)</td></tr>
</table>

Fish Habitat Assessment Methodology (continued)

Column 5 **"Length (m)"**	Length of the stream type being measured (*i.e.* the length of the unit)
Column 6 **"Average Width (m)"**	**Wet Width:** -The width of the river/stream system, in metres, from the edge of the existing water line of one bank to the edge of the existing water line of the opposite bank. Measurement is based on low water. The wet width is measured throughout the unit and the average is calculated. **Bank Channel Width:** -The channel width of river/stream system in, metres, based on the high water mark from one bank to the opposite bank. The channel width is measured throughout the unit and an average is calculated.
Column 7 **"Substrate (%)"**	Based on the chart below, use the criteria to identify the percent (%) of each substrate within the stream type. The total of all substrate types must equal 100%

SUBSTRATE AND CRITERIA

1. Bedrock, Ledge		
2. Boulder	=	> 461 mm
3. Rock	=	180 - 460 mm
4. Rubble	=	54 - 179 mm
5. Gravel	=	2.6 - 53 mm
6. Sand	=	0.06 - 2.5 mm
7. Fines	=	0.0005 - 0.05 mm

Column 8 **"Average Depth - Wet Width (m)"**	The wet depth is measured in metres from the stream bed to the water surface. Measure wet depth throughout each stream type, within the boundaries of the left and right bank waterlines (as determined during the measurement of the average wet width). An average is calculated from the measured wet depths.
Column 9 **"0-50% Undercut Bank"**	The bank overhang above the water edge for each stream type, based on low water. The left and right sides each represent 50% of the total stream type. Identify the percent of the length of each side (left and right) that is undercut. *(i.e., if a stream type is 10 m long and 5 m of the left side has an undercut and 4 m of the right side has an undercut bank then 25% (5m / 10m x 50%) of the left hand bank is undercut and 20% (4m / 10m x 50%) of the right hand bank is undercut.)*

Fish Habitat Assessment Methodology (continued)

Column 10 **"0-50% Overhanging Bank Vegetation"**	Vegetation at or near the water surface. The left and right sides each represent 50% of the total stream type. Identify the percent of the area of both the left side and the right side of the stream type influenced by overhanging vegetation. *(i.e., if a stream type is 10 m long and 5 m of the left side is influenced by overhanging vegetation and 2 m of the right side is influenced by overhanging vegetation then 25 % (5m / 10m x 50%) of the left hand bank has overhanging vegetation and 10% (2m / 10m x 50%) of the right hand bank has overhanging vegetation.)*
Column 11 **"Large Woody Debris in Stream (m)"**	The additive length of in-stream woody debris for each stream type. Only consider woody debris that is 10 cm in diameter or greater.

Fish Habitat Assessment Methodology (continued)

Column 12 **"Flows"**	**Type:** - determined from the "Flow Type " table presented below: **Flow Type:** 1. Survey Stream 2. Spring 3. Tributary 4. Spring Seep **Flow (cms):** to determine flow, first fill out the Water Flow Measurement Table on side 2 of the form: Unit no.- is the unit number for which the flow is being determined (from Side 1). Stream type - is the stream type for which the flow is being determined (from Side 1). Wet width (m) (W) - record corresponding data from Side 1 Depth (m) (D) - the wet depth is taken at ¼, ½ and ¾ of the distance across the wet width, and measured from the stream bed to the water surface - the average of the depth is calculated (depth sum divided by 4) Coefficient (A) - 0.9 (smooth) is used when stream bed is mud, sand, bedrock - 0.8 (rough) is used for all other stream bed types Length (m) (L) - the distance over which an object is floated (not less than 3m), and should be done over an homogenous area Float Time (seconds) (T) - time it takes for a floatable object (*i.e.*, a dry stick, a whiffle ball) to travel the designated length - taken at ¼, ½ and ¾ of the distance across the wet width - the average is calculated (float time sum divided by three) Comments - using the " Checklist of Land use and Attributes" on Side 1, record the number(s) which will best describe the location and/or problems affecting it. If no codes apply then write any observations that can accurately describe the area or location where the flow was measured. Flow is calculated using the equation at the bottom of side 2: W x D x A x L/T. **Time:** the time at which the flow is measured **Temperature:** the ambient and water temperatures, measured in degrees Celsius, at the time the flow is measured

Fish Habitat Assessment Methodology (continued)

Column 13 **"% Substrate Embeddedness"**	The percent of sands or fine material surrounding larger substrate (gravel through boulder). Record the number, from the chart below, which best represents the embeddedness of the large substrate in the streambed **Embeddedness Criteria** 1. ≤ 20% 2. 20% - 35% 3. 35% - 50% 4. ≥ 50%
Column 14 **"Comments"**	Using the "Checklist of Land Use Attributes", record the number(s) which will best describe the stream type location and/or problems affecting it.
SIDE 2/PAGE 2	
Column 1 **"Reach No."**	As in Side 1
Column 2 **"Site (50 m interval)"**	
Column 3 **"% Site"**	**Riffle/Run** - determine what percentage of each reach is riffle (gravel/rubble or rock/boulder or sand), and what percent of each reach is run. **Pools** - determine what percentage of each reach surveyed was pool habitat
Column 4 **"Shade (%)"**	Determine the percent of the stream type (from Side 1) which is shaded. This value will be based on the amount of the stream type which would be shaded by the sun between 10 am and 2 pm.

Fish Habitat Assessment Methodology (continued)

Column 5 **"Stream Banks"**	**Vegetation (%):** - percent of bare ground, grasses, shrubs and trees of both the left and right side from the channel bank and 15 m back (the shrubs category includes alders and willows). The total amount of stream bank vegetation should equal 100%. **Erosion (%):** - the left and right sides each represent 50% of the total stream type. - identify the percent of the length of each side that is stable, bare stable, eroding (bare stable refers to a bank that is stable but that has no vegetation on it). *(e.g., if a stream type is 10 m long and 5 m of the left bank is eroded and the remaining 5 m is stable with vegetation, and 10 m of the right bank is stable with no vegetation then the left bank is 25% (5m / 10m x 50%) stable, 0% bare stable and 25% (5m / 10m x 50%) eroding, and the right bank is 50% (10m / 10m x 50%) bare stable.)*
Column 6 **"O_2 (mg/l)"**	- the level of dissolved oxygen (mg/L) for each reach, measured in the field with a calibrated, YSI Dissolved Oxygen Meter (or equivalent)
Column 7 **"ph"**	- the pH for each reach, measured with a calibrated, field pH meter - measured in a laboratory from a grab sample taken at the time of the survey
Column 8 **"Depth"**	**Wet:** the wet depth is taken, for each stream type, at ¼, ½ and ¾ of the distance across the wet width, and measured from the stream bed to the water surface, in metres. **Channel:** the channel depth is taken, for each stream type, at ¼, ½ and ¾ of the distance across the channel width. The depth is measured in metres from the stream bed to the upper limit of the channel width.
Column 9 **"Pool Rating"**	**Number:** assign an appropriate number from the criteria column of the "Pool Rating " table from the bottom of Side 1 to each pool encountered.

Column 10 **"Pool Tail"**	The lower or downstream end of the pool. **Embeddedness:** the percent of sands or fine material surrounding larger substrate (gravel through boulder). - record the number from the column chart, presented below, which best represents the embeddedness of the large substrate in the pool tail **Embeddedness Criteria** 1 ≤ 20% 2 20% - 35% 3 35% - 50% 4 ≥ 50% **Mean Substrate Size:** - the mean size of the substrate within the pool tail column **% Fine:** - how much of the substrate is fine material (diameter 0.0005 - 0.05 mm, from "Substrate" table, Side 1)
Column 11 **"% Turbulence "**	

APPENDIX D

Estimated Costs

D-0 Estimated Costs

The unit costs for individual expenses that were used for estimating overall study costs for 1997 are provided for specific sites in the following appendix sections. These are based on 1996 costs, with approximately a 5% buffer for inflation or other adjustments. Actual costs may vary between laboratories, regions or other factors. Actual unit costs must be determined prior to undertaking the 1997 surveys.

For example, there are numerous parameters that can be measured in water and numerous ways to measure them that will all affect cost. Obtaining the lowest possible detection limits for metals for comparison with freshwater guidelines is necessary and will increase the cost. There will be some site-specific requirements (e.g., cyanide, thiosalts) that will vary from site to site.

Allowance should be made for QA/QC issues including the need to analyze filters used to filter water samples for total and dissolved metal levels. Inter-laboratory comparisons would result in additional samples and costs.

Note that costs are based on submitting numerous samples to one laboratory to obtain a volume discount. If more than one laboratory is used analyzing smaller numbers of samples, actual costs could increase by + 10 - 20%. Allowance should be made for costs associated with sending split and spiked samples to an independent laboratory.

Finally, note that costs will vary depending on the contractor(s) chosen. For instance, we have assumed that toxicity testing will be done by B.A.R. Environmental, and have used their prices for this work with the exception of the *Tubifex* 28-d toxicity tests. B.A.R.'s quotation for this test, which they do not routinely do, was much lower than quotations by U.S. laboratories; to be conservative the higher figure was used. Further, we have assumed that metallothionein analyses will be done by Dr. Klaverkamp and have not costed this work component.

D-1 Estimated Costs - Myra Falls

Estimated costs associated with testing Hypotheses 2 to 8 and 9, 12 and 13 at Myra Falls are provided in Table D-1. Labour rates used are blended rates based on those used for 1996 plus a 5% increase to account for average salary/overhead increases. It must be recognize that by deleting one hypothesis, costs may not decrease substantially as preparation time, some field time, travel costs, etc. will remain the same regardless of whether there are x or x-1 hypotheses being tested. The estimated cost for testing these hypotheses at Myra Falls is approximately $166,000. Note that these costs are for 20 samples/stations; should these increase, costs would increase proportionately except for some fixed costs (e.g., travel).

D-2 Estimated Costs - Lupin

Estimated costs associated with testing Hypotheses 1 to 14 at Lupin are provided in Table D-2. Labour rates used are blended rates based on those used for 1996 plus a 5% increase to account for average salary/overhead increases. It must be recognize that by deleting one hypothesis, costs may not decrease substantially as preparation time, some field time, travel costs, etc. will remain the same regardless of whether there are x or x-1 hypotheses being tested.

There are some important cost savings and potential overuns associated with any work conducted at the Lupin mine. During the 1996 survey the Lupin mine provided accommodation and meals for no charge, although their regular charge out rate to contractors/consultants is $150/day per person. If in 1997 the same arrangement cannot be made these costs will have to be incurred by the project. In addition the mine provided transportation around the mine sites. We have also assumed that the same arrangement regarding boats can be made in 1997 as was made in 1996. The mine provided the use of two aluminum skiffs (about 17') with 25 to 30 horsepower engines. Although we had brought zodiacs with us the work was made much easier and faster due to the size of the boats provided. Should mine boats not be available extra costs will be incurred not only for boat rental/shipping, but also time. Should the weather not be conducive to travel on the water a helicopter will be required to shuttle equipment (two to four days of either drop off or pick up) and personnel/samples (daily) to and from the study sites. Assuming that logistical difficulties will be limited (i.e., as costed in Table D-2) the estimated costs for testing these hypotheses at Lupin are approximately $268,000. Note that these costs are for 20 samples/stations; should these increase, costs would increase proportionately except for some fixed costs (e.g., travel).

D-3 Estimated Costs - Dome

Estimated costs are provided per hypothesis (Table D-3). There will be obvious efficiencies of testing several hypotheses at the same site. Some costs (e.g., travel) assume that more than one hypothesis is being tested at this site. Fees for field collection also include some time required for organization of equipment, ordering supplies, etc. not just time spent in the field.

A separate cost is provided for pulling all the information together into a comprehensive integrated report. Similarly, a separate cost estimate is provided for overall project management, client liaison including meetings, etc. Expenses (e.g., lab costs) are based on best estimates at this time. The actual costs should be finalized prior to implementing the survey for all lab costs and once the study design has been finalized.

D-4 Estimated Costs - Heath Steele

Estimated costs associated with testing Hypotheses 1 to 13 at Heath Steele are provided in Table D-4). Labour rates used are blended rates based on those used for 1996 plus a 5% increase to account for average salary/overhead increases. There is some overlap and obvious efficiencies of testing several hypotheses at the same site. Fees for field collection include field preparation time and the actual field work. Separate costs are provided for compilation of an integrated report. Costs are also provided for overall project management and client liaison (e.g., meetings). Sample analyses costs are based upon estimates available at this time. The actual costs should be finalized prior to implementing the survey. Costs for metallothionein analyses are not included.

Table D.1. Estimated costs for testing the hypotheses at Myra Falls Mine in 1997.

Expense	Unit Rate	H1		H2-H8		H9		H10		H11		H12		H13		Project Management[1]		Parameter Total	
		Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost
LABOUR	per diem																		
Sample Collection: P1	$930		$0		$0		$0		$0		$0		$0		$0		$0	0	$0
Sample Collection: P2	$555		$0		$0		$0		$0		$0		$0		$0		$0	0	$0
Sample Collection: P3	$520		$0	20	$10,400	7.5	$3,900		$0		$0	2	$1,040	1.5	$780		$0	31	$16,120
Sample Collection: P4	$488		$0		$0	7.5	$3,660		$0		$0	2	$976	1.5	$732		$0	11	$5,368
Sample Collection: P5	$335		$0	20	$6,700		$0		$0		$0		$0		$0		$0	20	$6,700
Reporting: P1	$930		$0	2	$1,860	3	$2,790		$0		$0		$0		$0	8	$7,440	13	$12,090
Reporting: P2	$555		$0	7	$3,885		$0		$0		$0	2	$1,110	0.5	$278	10	$5,550	19.5	$10,823
Reporting: P3	$520		$0	3	$1,560	10	$5,200		$0		$0		$0		$0	10	$5,200	23	$11,960
Reporting: P4	$488		$0	10	$4,880	2	$976		$0		$0	2	$976	1	$488	10	$4,880	25	$12,200
Reporting: P5	$335		$0	25	$8,375	15	$5,025		$0		$0		$0		$0	5	$1,675	45	$15,075
Labour Total			**$0**		**$37,660**		**$21,551**		**$0**		**$0**		**$4,102**		**$2,278**		**$24,745**		**$90,336**
FIELD DISBURSEMENTS	per unit																		
Field Equipment Rental	$1,500		$0	4	$6,000	1	$1,500		$0		$0		$0		$0		$0	5	$7,500
Truck Rental	$100		$0	12	$1,200		$0		$0		$0		$0		$0		$0	12	$1,200
Truck Mileage (per km)	$0.37		$0	1800	$666												$0	1800	$666
Accomodation/Meals	$150		$0	12	$1,800	12	$0		$0		$0		$0		$0		$0	24	$1,800
Miscellaneous Supplies	$500		$0	1	$500	1	$500		$0		$0	1	$500	1	$500		$0	4	$2,000
Delivery/Cartage	$500		$0	4	$2,000	1	$500		$0		$0	1	$500	1	$500		$0	7	$3,500
Field Total			**$0**		**$12,166**		**$2,500**		**$0**		**$0**		**$1,000**		**$1,000**		**$0**		**$16,666**
ANALYTICAL COSTS	per sample																		
Water Chemistry																			
Analytical Scan (RCAP-MS)	$140	0	$0	0	$0	20	$2,800	0	$0	0	$0	10	$1,400	4	$560		$0	34	$4,760
Cyanide	$30	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
Thiosalts[2]		0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
Total P, TSS	$25	0	$0	0	$0	20	$500	0	$0	0	$0	10	$250	4	$100		$0	34	$850
QA/QC costs	15%	0	$0	0	$0		$495	0	$0	0	$0		$248		$99				$842
Sediment Chemistry																			
Particle Size Analysis	$40	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
Total Organic Carbon	$30	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
Standard Metals Scan	$80	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
Nutrients (TKN,P)	$20	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
Mercury (Cold Vapour FAA)	$20	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
AVS/SEM	$150	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
Fe-Mg Hydroxide Analysis[2]		0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
QA/QC Costs	15%	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0			0	$0
Zooplankton I.D./Enumeration	$250	0	$0	0	$0	20	$0	0	$0	0	$0		$0		$0		$0	20	$0
Sublethal Toxicity Tests																			
S. capricornatum growth inhibition	$285	0	$0	0	$0		$0	0	$0	0	$0		$0	4	$1,140		$0	4	$1,140
C.dubia Survival and Reproduction	$1,020	0	$0	0	$0		$0	0	$0	0	$0		$0	4	$4,080		$0	4	$4,080
Fathead Minnow Growth and Survival	$1,045	0	$0	0	$0		$0	0	$0	0	$0		$0	4	$4,180		$0	4	$4,180
Lemna minor Growth Inhibition	$440	0	$0	0	$0		$0	0	$0	0	$0		$0	4	$1,760		$0	4	$1,760

Table D-1 (continued)

Expense	Unit Rate	H1		H2-H8		H9		H10		H11		H12		H13		Project Management[1]		Parameter Total	
		Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost
Screening Tests																			
C.dubia	$420	0	$0	0	$0		$0	0	$0	0	$0	0	$0		$0		$0	0	$0
Fathead Minnows	$450	0	$0	0	$0		$0	0	$0	0	$0	0	$0		$0		$0	0	$0
Acclimation Procedures																			
C.dubia	$1,180	0	$0	0	$0		$0	0	$0	0	$0		$0	1	$1,180		$0	1	$1,180
Fathead Minnows	$1,100	0	$0	0	$0		$0	0	$0	0	$0		$0	1	$1,100		$0	1	$1,100
Sediment Toxicity Tests																			
Hyalella azteca Growth and Survival	$650	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
Chironomus Spp. Growth and Survival	$550	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
28-day Oligochaete[2]	$2,500	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0			0	$0
Fish Age Structure Analysis																			
Scales, Otoliths, or Fin Rays	$6	0	$0	160	$960	0	$0	0	$0	0	$0		$0		$0		$0	160	$960
Fish Fecundity																			
Gonad Egg Count	$75	0	$0	80	$6,000	0	$0	0	$0	0	$0		$0		$0		$0	80	$6,000
Metals in Fish Tissues																			
Mercury (Flameless AA)	$25	0	$0	120	$3,000	0	$0	0	$0	0	$0		$0		$0		$0	120	$3,000
Arsenic, Selenium (AA)	$35	0	$0	120	$4,200	0	$0	0	$0	0	$0		$0		$0		$0	120	$4,200
Other metals (RCAP-MS)	$60	0	$0	120	$7,200	0	$0	0	$0	0	$0		$0		$0		$0	120	$7,200
Metalothionein in Fish Tissue[2]		0	$0	120	$0	0	$0	0	$0	0	$0		$0		$0		$0	120	$0
Analytical Total			**$0**		**$21,360**		**$3,795**		**$0**		**$0**		**$1,898**		**$14,199**		**$0**		**$41,252**
OFFICE DISBURSEMENTS	per diem																		
GIS/Mapping	estimate		$0		$3,000		$0		$0		$0		$0		$0		$3,000		$6,000
Computer Rental	$60		$0	8	$480	0	$0	0	$0	0	$0	1	$60	0	$0	16	$960	25	$1,500
GIS Rental	$120		$0	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0	2	$240	2	$240
Courier/Fax/Telephone/Photocopying	estimate		$0		$3,000		$0		$0		$0		$250		$0		$3,000		$6,250
Office Total			**$0**		**$6,480**		**$0**		**$0**		**$0**		**$310**		**$0**		**$7,200**		**$13,990**
HYPOTHESES TOTALS			**$0**		**$77,666**		**$27,846**		**$0**		**$0**		**$7,310**		**$17,477**		**$31,945**		
MYRA FALLS TOTAL																			**$162,243**

NOTES:

H9 not recommended for testing

H1, H11, H12 not being tested due to lack of consistent substrate between exposure and reference areas

1 includes meetings, client liason and integrated report preparation

2 costs still to be determined; if cost given it is very preliminary estimate

Table D-2. Estimated costs for testing the hypotheses at Lupin Mine in 1997.

Expense	Unit Rate	H1		H9		H10		H11		H12		H13		Project Management[1]		Parameter Total	
		Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost
LABOUR	per diem																
Sample Collection: P1	$930		$0		$0		$0		$0		$0		$0		$0	0	$0
Sample Collection: P2	$555		$0		$0		$0		$0		$0		$0		$0	0	$0
Sample Collection: P3	$520	4	$2,080	6	$3,120	8	$4,160		$0		$0		$0		$0	18	$9,360
Sample Collection: P4	$488	4	$1,952	6	$2,928	8	$3,904		$0		$0		$0		$0	18	$8,784
Sample Collection: P5	$335	3	$1,005		$0		$0		$0		$0		$0		$0	3	$1,005
Reporting: P1	$930	1	$930	3	$2,790	3	$2,790	2	$1,860		$0		$0	8	$7,440	17	$15,810
Reporting: P2	$555		$0		$0		$0		$0		$0		$0		$0	0	$0
Reporting: P3	$520	3	$1,560	7	$3,640	6	$3,120	3	$1,560		$0		$0	10	$5,200	29	$15,080
Reporting: P4	$488		$0	2	$976		$0		$0		$0		$0	10	$4,880	12	$5,856
Reporting: P5	$335	5	$1,675	3	$1,005	13	$4,355	5	$1,675		$0		$0	10	$3,350	36	$12,060
Labour Total			**$9,202**		**$14,459**		**$18,329**		**$5,095**		**$0**		**$0**		**$20,870**		**$67,955**
FIELD DISBURSEMENTS	per unit																
Field Equipment Rental	$1,500	1	$1,500		$0		$0		$0		$0		$0		$0	1	$1,500
Airfare	$1,000	2	$2,000		$0		$0		$0		$0		$0	2	$2,000	4	$4,000
Truck Rental[2]		0	$0		$0		$0		$0		$0		$0		$0	0	$0
Accomodation/Meals[3]	$150	0	$0		$0		$0		$0		$0		$0		$0	0	$0
Miscellaneous Supplies	$300	1	$300		$0		$0		$0		$0		$0		$0	1	$300
Delivery/Cartage	$500	4	$2,000		$0	2	$1,000		$0		$0		$0		$0	6	$3,000
Field Total			**$5,800**		**$0**		**$1,000**		**$0**		**$0**		**$0**		**$2,000**		**$8,800**
ANALYTICAL COSTS	per sample																
Water Chemistry																	
Analytical Scan (RCAP-MS)	$140	0	$0	3	$420	0	$0	0	$0		$0		$0		$0	3	$420
Cyanide	$30	0	$0	3	$90	0	$0	0	$0		$0		$0		$0	3	$90
Thiosalts[4]		0	$0	0	$0	0	$0	0	$0		$0		$0		$0	0	$0
Total P, TSS	$25	0	$0	3	$75	0	$0	0	$0		$0		$0		$0	3	$75
QA/QC costs	15%	0	$0		$88	0	$0	0	$0	0	$0	0	$0			0	$88
Sediment Chemistry																	
Particle Size Analysis	$40	0	$0	0	$0	20	$800	0	$0		$0		$0		$0	20	$800
Total Organic Carbon	$30	0	$0	0	$0	20	$600	0	$0		$0		$0		$0	20	$600
Standard Metals Scan	$80	0	$0	0	$0	20	$1,600	0	$0		$0		$0		$0	20	$1,600
Nutrients (TKN,P)	$20	0	$0	0	$0	20	$400	0	$0		$0		$0		$0	20	$400
Mercury (Cold Vapour FAA)	$20	0	$0	0	$0	20	$400	0	$0		$0		$0		$0	20	$400
AVS/SEM	$150	0	$0	0	$0	20	$3,000	0	$0		$0		$0		$0	20	$3,000
Fe-Mg Hydroxide Analysis[4]		0	$0	0	$0	20	$0	0	$0		$0		$0		$0	20	$0
QA/QC Costs	15%	0	$0	0	$0		$570	0	$0	0	$0	0	$0			0	$570
Benthos I.D./Enumeration	$250	0	$0	0	$0	20	$5,000	0	$0		$0		$0		$0	20	$5,000
Sublethal Toxicity Tests																	
S. capricornatum growth inhibition	$285	0	$0	3	$855	0	$0	0	$0		$0		$0		$0	3	$855
C.dubia Survival and Reproduction	$1,020	0	$0	3	$3,060	0	$0	0	$0		$0		$0		$0	3	$3,060
Fathead Minnow Growth and Survival	$1,045	0	$0	3	$3,135	0	$0	0	$0		$0		$0		$0	3	$3,135
Lemna minor Growth Inhibition	$440	0	$0	3	$1,320	0	$0	0	$0		$0		$0		$0	3	$1,320

Table D-2 (continued)

Expense	Unit Rate	H1		H9		H10		H11		H12		H13		Project Management[1]		Parameter Total	
		Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost
Screening Tests																	
C.dubia	$420	0	$0	1	$420	0	$0	0	$0		$0		$0		$0	1	$420
Fathead Minnows	$450	0	$0	1	$450	0	$0	0	$0		$0		$0		$0	1	$450
Acclimation Procedures																	
C.dubia	$1,180	0	$0	1	$1,180	0	$0	0	$0		$0		$0		$0	1	$1,180
Fathead Minnows	$1,100	0	$0	1	$1,100	0	$0	0	$0		$0		$0		$0	1	$1,100
Sediment Toxicity Tests																	
Hyalella azteca Growth and Survival	$650	20	$13,000	0	$0	0	$0	0	$0		$0		$0		$0	20	$13,000
Chironomus Spp. Growth and Survival	$550	20	$11,000	0	$0	0	$0	0	$0		$0		$0		$0	20	$11,000
28-day Oligochaete[4]	$2,500	20	$50,000	0	$0	0	$0	0	$0	0	$0	0	$0			20	$50,000
Analytical Total			**$74,000**		**$12,193**		**$12,370**		**$0**		**$0**		**$0**		**$0**		**$98,563**
OFFICE DISBURSEMENTS	per diem																
GIS/Mapping	estimate		$3,000		$0		$0		$0		$0		$0		$3,000		$6,000
Computer Rental	$60	8	$480	0	$0	0	$0	0	$0	0	$0	0	$0	16	$960	24	$1,440
GIS Rental	$120	2	$240	0	$0	0	$0	0	$0	0	$0	0	$0	2	$240	4	$480
Courier/Fax/Telephone/Photocopying	estimate		$3,000		$0		$3,000		$3,000		$0		$0		$3,000		$12,000
Office Total			$6,720		$0		$3,000		$3,000		$0		$0		$7,200		$19,920
HYPOTHESES TOTALS			**$95,722**		**$26,652**		**$34,699**		**$8,095**		**$0**		**$0**		**$30,070**		
LUPIN TOTAL																	**$195,238**

NOTES:

1 includes meetings, client liason and integrated report preparation
2 on-site vehicle/transportation was provided by the Lupin mine at no charge
3 on-site accomodation/meals were provided by the Lupin mine at no charge; regular charge of $150 per day per person
- potentially it could cost $600*18 days= $10,800 for 4 people
4 costs still to be determined; if cost given it is very preliminary estimate

Table D.3. Estimated costs for testing the hypotheses at Dome Mine in 1997.

Expense	Unit Rate	H1		H2-H8		H9		H10		H11		H12		H13		Project Management[1]		Parameter Total	
		Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost
LABOUR	per diem																		
Sample Collection: P1	$930		$0		$0		$0		$0		$0		$0		$0		$0	0	$0
Sample Collection: P2	$555		$0		$0		$0		$0		$0		$0		$0		$0	0	$0
Sample Collection: P3	$520		$0	0	$0	0	$0	0	$0		$0		$0		$0		$0	0	$0
Sample Collection: P4	$488	4	$1,952	14	$6,832	6	$2,928	5	$2,440		$0		$0	3	$1,464		$0	32	$15,616
Sample Collection: P5	$335	4	$1,340	20	$6,700	6	$2,010	8	$2,680		$0		$0	3	$1,005		$0	41	$13,735
Reporting: P1	$930	2	$1,860	3	$2,790	3	$2,790	3	$2,790	3	$2,790		$0		$0	6	$5,580	20	$18,600
Reporting: P2	$555		$0	0	$0		$0		$0		$0		$0		$0		$0	0	$0
Reporting: P3	$520	3	$1,560	6	$3,120	5	$2,600	6	$3,120	3	$1,560		$0		$0	15	$7,800	38	$19,760
Reporting: P4	$488		$0	10	$4,880	5	$2,440		$0		$0		$0		$0	0	$0	15	$7,320
Reporting: P5	$335	5	$1,675	25	$8,375	5	$1,675	12	$4,020	5	$1,675		$0		$0	10	$3,350	62	$20,770
Labour Total			**$8,387**		**$32,697**		**$14,443**		**$15,050**		**$6,025**		**$0**		**$2,469**		**$16,730**		**$95,801**
FIELD DISBURSEMENTS	per unit																		
Field Equipment Rental	$550	2	$1,100	2	$1,100	1	$550	2	$1,100		$0		$0		$0		$0	7	$3,850
Airfare	$650	1	$650	1	$650	1	$650	1	$650		$0		$0		$0	0	$0	4	$2,600
Truck Rental	$150	2	$300	10	$1,500	3	$0	2	$0		$0		$0	5	$750		$0	22	$2,550
Accomodation/Meals	$150	2	$300	10	$1,500	2	$0	2	$0		$0		$0	3	$450		$0	19	$2,250
Miscellaneous Supplies	$250	1	$250	1	$250	1	$250	1	$250		$0		$0	3	$750		$0	7	$1,750
Delivery/Cartage	$250	1	$250	2	$500	1	$250	1	$250		$0		$0		$0		$0	5	$1,250
Field Total			**$2,850**		**$5,500**		**$1,700**		**$2,250**		**$0**		**$0**		**$1,950**		**$0**		**$14,250**
ANALYTICAL COSTS	per sample																		
Water Chemistry																			
Analytical Scan (RCAP-MS)[2]	$140	0	$0	0	$0	40	$5,600	0	$0	0	$0		$0	6	$840		$0	46	$6,440
Cyanide	$30	0	$0	0	$0	20	$600	0	$0	0	$0		$0	3	$90		$0	23	$690
Total P, TSS	$25	0	$0	0	$0	20	$500	0	$0	0	$0		$0	3	$75		$0	23	$575
QA/QC costs	15%	0	$0	0	$0		$1,005	0	$0	0	$0	0			$151				
Sediment Chemistry																			
Particle Size Analysis	$40	0	$0	0	$0	0	$0	20	$800	0	$0		$0		$0		$0	20	$800
Total Organic Carbon	$30	0	$0	0	$0	0	$0	20	$600	0	$0		$0		$0		$0	20	$600
Standard Metals Scan	$80	0	$0	0	$0	0	$0	20	$1,600	0	$0		$0		$0		$0	20	$1,600
Nutrients (TKN,P)	$20	0	$0	0	$0	0	$0	20	$400	0	$0		$0		$0		$0	20	$400
Mercury (Cold Vapour FAA)	$20	0	$0	0	$0	0	$0	20	$400	0	$0		$0		$0		$0	20	$400
AVS/SEM	$150	0	$0	0	$0	0	$0	20	$3,000	0	$0		$0		$0		$0	20	$3,000
Fe-Mg Hydroxide Analysis[4]		0	$0	0	$0	0	$0	20	$0	0	$0		$0		$0		$0	20	$0
QA/QC Costs	15%	0	$0	0	$0	0	$0		$570	0	$0	0	$0	0	$0				
Benthos I.D./Enumeration	$250	0	$0	0	$0	0	$0	20	$5,000	0	$0		$0		$0		$0	20	$5,000
Sublethal Toxicity Tests																			
S. capricornatum growth inhibition	$285	0	$0	0	$0	0	$0	0	$0	0	$0		$0	3	$855		$0	3	$855
C.dubia Survival and Reproduction	$1,020	0	$0	0	$0	0	$0	0	$0	0	$0		$0	3	$3,060		$0	3	$3,060
Fathead Minnow Growth and Survival	$1,045	0	$0	0	$0	0	$0	0	$0	0	$0		$0	3	$3,135		$0	3	$3,135
Lemna minor Growth Inhibition	$440	0	$0	0	$0	0	$0	0	$0	0	$0		$0	3	$1,320		$0	3	$1,320

Table D-3 (continued)

Expense	Unit Rate	H1		H2-H8		H9		H10		H11		H12		H13		Project Management[1]		Parameter Total	
		Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost
Screening Tests																			
C.dubia	$420	0	$0	0	$0	0	$0	0	$0	0	$0		$0	1	$420		$0	1	$420
Fathead Minnows	$450	0	$0	0	$0	0	$0	0	$0	0	$0		$0	1	$450		$0	1	$450
Acclimation Procedures																			
C.dubia	$1,180	0	$0	0	$0	0	$0	0	$0	0	$0		$0	1	$1,180		$0	1	$1,180
Fathead Minnows	$1,100	0	$0	0	$0	0	$0	0	$0	0	$0		$0	1	$1,100		$0	1	$1,100
Sediment Toxicity Tests																			
Hyalella azteca Growth and Survival	$650	20	$13,000	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	20	$13,000
Chironomus Spp. Growth and Survival	$550	20	$11,000	0	$0	0	$0	0	$0	0	$0		$0		$0		$0	20	$11,000
28-day Oligochaete[4]	$2,500	20	$50,000	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0			20	$50,000
Fish Age Structure Analysis																			
Scales, Otoliths, or Fin Rays	$6	0	$0	160	$960	0	$0	0	$0	0	$0		$0		$0		$0	160	$960
Fish Fecundity																			
Gonad Egg Count	$75	0	$0	80	$6,000	0	$0	0	$0	0	$0		$0		$0		$0	80	$6,000
Metals in Fish Tissues																			
Mercury (Flameless AA)	$25	0	$0	120	$3,000	0	$0	0	$0	0	$0		$0		$0		$0	120	$3,000
Arsenic, Selenium (AA)	$35	0	$0	120	$4,200	0	$0	0	$0	0	$0		$0		$0		$0	120	$4,200
Other metals (RCAP-MS)	$60	0	$0	120	$7,200	0	$0	0	$0	0	$0		$0		$0		$0	120	$7,200
Metallothionein in Fish Tissue[4]		0	$0	120	$0	0	$0	0	$0	0	$0		$0		$0		$0	120	$0
Analytical Total			**$74,000**		**$21,360**		**$7,705**		**$12,370**		**$0**		**$0**		**$12,676**		**$0**		**$126,385**
OFFICE DISBURSEMENTS	per diem																		
Drafting/Clerical	estimate		$1,500		$3,000		$0		$0		$0		$0		$0		$2,000		$6,500
Computer Rental	$60	8	$480	8	$480	0	$0	0	$0	0	$0	0	$0	0	$0	18	$1,080	34	$2,040
GIS Rental	$120	2	$240	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0	5	$600	7	$840
Courier/Fax/Telephone/Photocopying	estimate		$2,500		$2,500		$0						$0		$0		$2,000		$7,000
Office Total			**$4,720**		**$5,980**		**$0**		**$0**		**$0**		**$0**		**$0**		**$5,680**		**$16,380**
HYPOTHESES TOTALS			**$89,957**		**$65,537**		**$23,848**		**$29,670**		**$6,025**		**$0**		**$17,095**		**$22,410**		
DOME TOTAL																			**$252,816**

NOTES:

1 includes meetings, client liason and integrated report preparation

2 number of samples doubled to account for analysis of total and dissolved metals

3

4 costs still to be determined; if cost given it is very preliminary estimate

Table D.4 Estimated costs for testing the hypotheses at Heath Steele Mine in 1997

Expense	Unit Rate	H1		H2-H8		H9		H10		H11		H12		H13		Project Management[1]		Parameter Total	
		Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost
LABOUR	per diem																		
Sample Collection: P1	$930	0	$0		$0		$0	0	$0	0	$0	0	$0		$0		$0	0	$0
Sample Collection: P2	$555	0	$0		$0		$0	0	$0	0	$0	0	$0		$0		$0	0	$0
Sample Collection: P3	$520	0	$0	20	$10,400	8	$4,160	0	$0	0	$0	0	$0	3	$1,560		$0	31	$16,120
Sample Collection: P4	$488	0	$0		$0		$0	0	$0	0	$0	0	$0		$0		$0	0	$0
Sample Collection: P5	$335	0	$0	30	$10,050	8	$2,680	0	$0	0	$0	0	$0	3	$1,005		$0	41	$13,735
Reporting: P1	$930	0	$0	2	$1,860	2	$1,860	0	$0	0	$0	0	$0	2	$1,860	6	$5,580	12	$11,160
Reporting: P2	$555	0	$0	7	$3,885		$0	0	$0	0	$0	0	$0	7	$3,885		$0	14	$7,770
Reporting: P3	$520	0	$0	5	$2,600	8	$4,160	0	$0	0	$0	0	$0	8	$4,160	8	$4,160	29	$15,080
Reporting: P4	$488	0	$0	7	$3,416		$0	0	$0	0	$0	0	$0		$0	8	$3,904	15	$7,320
Reporting: P5	$335	0	$0	30	$10,050	8	$2,680	0	$0	0	$0	0	$0	8	$2,680	8	$2,680	54	$18,090
Labour Total			**$0**		**$42,261**		**$15,540**		**$0**		**$0**		**$0**		**$15,150**		**$16,324**		**$89,275**
FIELD DISBURSEMENTS	per unit																		
Field Equipment Rental	$1,500	0	$0	1	$1,500		$0	0	$0	0	$0	0	$0		$0		$0	1	$1,500
Airfare	$1,000	0	$0	0	$0		$0	0	$0	0	$0	0	$0		$0	2	$2,000	2	$2,000
Truck Rental	$150	0	$0	10	$1,500	4	$600	0	$0	0	$0	0	$0	0	$0		$0	14	$2,100
Accomodation/Meals	$150	0	$0	50	$7,500	16	$2,400	0	$0	0	$0	0	$0	6	$900		$0	72	$10,800
Miscellaneous Supplies	$250	0	$0	1	$250	1	$250	0	$0	0	$0	0	$0		$0		$0	2	$500
Delivery/Cartage	$250	0	$0	3	$750	3	$750	0	$0	0	$0	0	$0	4	$1,000		$0	10	$2,500
Field Total			**$0**		**$11,500**		**$4,000**		**$0**		**$0**		**$0**		**$1,900**		**$2,000**		**$19,400**
ANALYTICAL COSTS	per sample																		
Water Chemistry																			
Analytical Scan (RCAP-MS)2	$280	0	$0	15	$4,200	20	$5,600	0	$0	0	$0	0	$0	6	$1,680		$0	41	$11,480
Thiosalts3		0	$0	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0
Total P, TSS	$25	0	$0	15	$375	20	$500	0	$0	0	$0	0	$0	6	$150		$0	41	$1,025
QA/QC costs	15%		$0	0	$0		$915		$0		$0		$0		$275				
Benthos I.D./Enumeration	$250	0	$0	0	$0	20	$5,000	0	$0	0	$0	0	$0		$0		$0	20	$5,000
Sublethal Toxicity Tests																			
S. capricornatum growth inhibition	$285	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0	6	$1,710		$0	6	$1,710
C.dubia Survival and Reproduction	$1,020	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0	6	$6,120		$0	6	$6,120
Fathead Minnow Growth and Survival	$1,045	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0	6	$6,270		$0	6	$6,270
Lemna minor Growth Inhibition	$440	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0	6	$2,640		$0	6	$2,640

Table D-4. (continued)

Expense	Unit Rate	H1		H2-H8		H9		H10		H11		H12		H13		Project Management[1]		Parameter Total	
		Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost	Units	Cost
Screening Tests																			
C.dubia	$420	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0	1	$420		$0	1	$420
Fathead Minnows	$450	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0	1	$450		$0	1	$450
Acclimation Procedures																			
C.dubia	$1,180	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0	1	$1,180		$0	1	$1,180
Fathead Minnows	$1,100	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0	1	$1,100		$0	1	$1,100
Fish Age Structure Analysis																			
Scales, Otoliths, or Fin Rays	$6	0	$0	100	$600	0	$0	0	$0	0	$0	0	$0		$0		$0	100	$600
Metals in Fish Tissues4																			
Mercury (Flameless AA)	$25	0	$0	0	$0	0	$0	0	$0	0	$0	0	$0		$0		$0		$0
Arsenic, Selenium (AA)	$35	0	$0	60	$2,100	0	$0	0	$0	0	$0	0	$0		$0		$0	60	$2,100
Other metals (RCAP-MS)	$60	0	$0	60	$3,600	0	$0	0	$0	0	$0	0	$0		$0		$0	60	$3,600
Metallothionein in Fish Tissue5		0	$0	60	$0	0	$0	0	$0	0	$0	0	$0		$0		$0	60	$0
Analytical Total			**$0**		**$10,875**		**$12,015**		**$0**		**$0**		**$0**		**$21,995**		**$0**		**$43,695**
OFFICE DISBURSEMENTS	per diem																		
Drafting/Clerical	estimate		$0		$3,000		$2,000		$0		$0		$0		$500		$2,000		$7,500
Computer Rental	$60	0	$0	3	$180	4	$240	0	$0	0	$0	0	$0	3	$180	8	$480	18	$1,080
GIS Rental	$120	0	$0	2	$240	2	$240	0	$0	0	$0	0	$0	0	$0	2	$240	6	$720
Courier/Fax/Telephone/Photocopying	estimate		$0		$2,500		$1,500		$0		$0		$0		$1,500		$2,000		$7,500
Office Total			**$0**		**$5,920**		**$3,980**		**$0**		**$0**		**$0**		**$2,180**		**$4,720**		**$16,800**
HYPOTHESES TOTALS			**$0**		**$70,556**		**$35,535**		**$0**		**$0**		**$0**		**$41,225**		**$23,044**		
HEATH STEELE TOTAL																			**$169,170**

NOTES:

H1, H10 and H11 not possible to test as sediments not available
H6 not recommended as outlined in hypotheses
H12 not possible to test

1 includes meetings, client liason and integrated report preparation
2 costs doubled to account for analyses of both total and dissolved metal samples
3 thiosalt analyses conducted on-site by mine personnel
4 costs estimated for analyses of 50 fish plus an additional 10 to ensure adequate sample volumes
5 costs still to be determined