presented above, only three fish species have been caught in sufficient numbers and are common to both the exposure and reference area. These include two small-bodied species (ninespine stickleback and slimy sculpin) and one large-bodied species (Arctic grayling).

4.1.4.1 Fish Capture Methods

Beach seine nets, baited minnow traps, backpack electrofishing, fyke nets, hoop nets, gill nets, drift nets, and angling will be among the methods that will be employed to capture fish. Sampling will be conducted at various times of the day and in selected habitats to maximize catch. Field personnel will collect fish following the detailed methods set out in the Golder Associates Technical Procedures for Fish Inventories (unpublished file information). Appropriate fish capture and research permits will be obtained from DFO and the Nunavut Research Institute prior to conducting the field program.

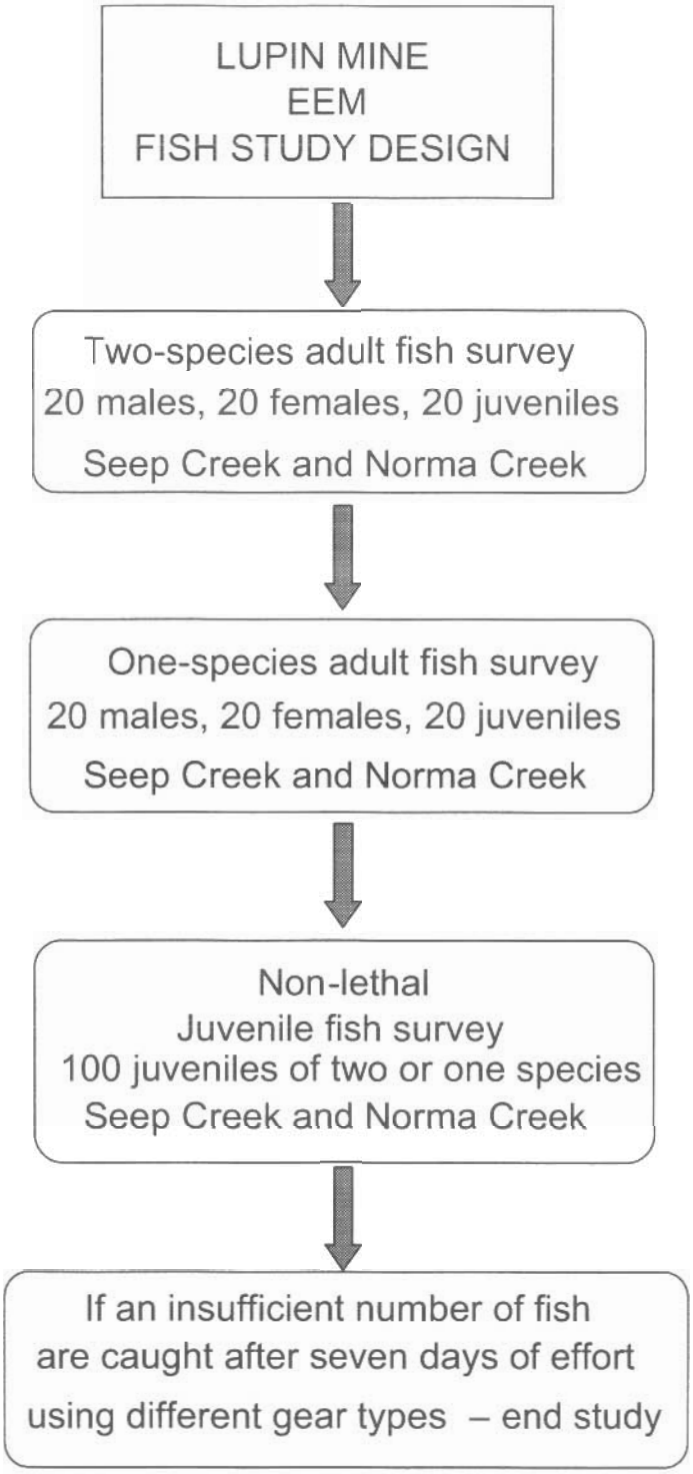
To keep track of the amount of effort put forth using the various nets, the following information will be recorded: number of net hauls, time, distance, or area sampled for each haul, Global Positioning System location, dimensions of net used, and number of target and non-target species captured. For minnow trap sets, the following information will be recorded: number of hours set, Global Positioning System location and number of target and non-target species captured. The settings on the backpack electrofishing unit and the number of seconds of active electrofishing will be recorded. Catch-per-unit-effort (CPUE) will be calculated for all gear types and expressed as number of fish captured per unit of effort. All captured individuals of the study species will be enumerated, and fish health measurements described above will be recorded within the level of precision outlined in Environment Canada's EEM aquatic technical guidance document (Environment Canada 2002).

4.1.4.2 Sampling Effort

For an adult lethal survey, 20 adult males, 20 adult females and 20 juveniles of a study species would be collected from the exposure and reference areas. For a non-lethal survey, 100 juvenile fish of a study species would be collected in each the exposure area and the reference area.

The fish survey will be conducted over a maximum of seven consecutive days in the exposure area. In the event that an insufficient number of fish are captured, the study will end, and no sampling effort will be put forth in the reference area.

Figure 4-2 Flow Chart of Potential Study Designs (options are in order of priority)



4.1.4.3 Supporting Environmental Information

Supporting environmental data will be recorded at each sampling site (i.e., air temperature, percent cloud cover, wind direction, and velocity, and water dissolved oxygen, conductivity, water temperature, pH, depth, and Secchi depth). The general aquatic habitat will also be described. Water samples will be collected during the fish study to document water quality. Two water samples will be collected in the exposure area, and two samples will be collected in the reference area. In addition, one field blank, one trip blank and one duplicate sample will be collected as quality control samples during the water quality monitoring for the fish and benthic invertebrate studies. Water samples will be collected according to protocols outlined in Golder Associates Technical Procedures for Water Quality and Limnology Inventories (unpublished file information) and Environment Canada's EEM aquatic technical guidance document (Environment Canada 2002). Field water quality measurements will also be recorded at the same location and time of sample collection.

All water samples will be analyzed by an accredited analytical laboratory for the following parameters: deleterious substances as required under the MMER (2002), pH, nutrients (total phosphorus, ammonia, nitrate, total organic carbon, dissolved organic carbon), physical characteristics (conductivity, alkalinity, total hardness), other total and dissolved metals (including aluminium, cadmium, iron, manganese, mercury, molybdenum, selenium, uranium), and major ions (chloride, calcium, magnesium, potassium, sodium, sulphate).

4.1.4.4 Fish Measurements for the Adult Survey (Preferred Option)

Measurements on fish will be completed following the EEM aquatic technical guidance document (Environment Canada 2002) and Golder Associates Fish Inventory Methods and Fish Health Assessment – Metals Technical Procedures Documents (unpublished file information). A unique biomarker number will be given to all target fish, and any non-target species will be identified, enumerated and live-released.

The following are parameters (where practicable) that will be measured on each target fish in an adult survey The level of precision required for each measure is provided (in brackets), where applicable:

- length (±1 mm);
- total body weight (±1%);
- age (by laboratory analysis, ±1 year with 10% independently confirmed);
- life stage and maturity;
- gonad weight (±1.0%);

- liver weight (±1%);
- sex; and,
- internal condition.

Additional biological variable will also be measured as supportive information should an effect in the above parameters be determined. The following supportive biological variables will be determined where possible:

- egg size (±1.0%);
- external condition; and,
- total number of eggs per female (fecundity) (±1.0%).

It is important to note that determination of fecundity in small forage fish has proven difficult and therefore the ability to accurately measure this variable will be assessed

Fork or total length (depending on species) and total body weight will be recorded for all captured target fish. Detailed observations will be made during the external examination on any features of the fish that do not appear normal (i.e., wounds, tumours, parasites, fin fraying, gill, parasites or lesions). When possible, information on developmental maturity, sex, and stage of mature fish will be recorded. This information will be verified later on mature fish during the internal examination.

For internal examinations of fish, the organ system will be examined for general appearance and the presence of any abnormalities. If abnormalities such as tumours, necrosis, or heavy parasite loads are observed, their appearance will be noted and photographs taken (where possible). After removal of the fish liver, the gastrointestinal tract will be dissected. Stomach fullness will be documented along with a general description of gut contents and parasite load. Photographs will be taken of abnormal tissue and representative normal tissues.

The reproductive tissues from the target species will be carefully excised and weighed (±0.001 g). An accurate measurement of gonad weight may not be possible due to their small size in small-bodied fish, and the fact that associated fatty tissue may be difficult to remove from the gonads. Sex and maturity of the fish will be confirmed at this time. When necessary, certain whole gonads will be preserved in 10% neutral-buffered formalin when abnormal conditions are observed or when gonad staging determinations are in question.

Liver tissue will also be weighed (± 0.001 g) and collected for examination in the laboratory. Liver tissue will be preserved in 10% neutral buffered formalin for histology evaluation (only if deemed necessary). General biological parameters and tissue

collection information for each fish examined will be recorded on autopsy forms and in the field logbooks.

Ageing structures (e.g., otoliths and pectoral fin ray sections) will be collected from all sexually mature fish and larger juvenile fish that are not young-of-the-year (YOY) that are sacrificed for internal health assessments. If non-lethal sampling is required, only pectoral rays and/or scales will be collected from fish. The collected ageing structures will be placed in envelopes and labelled with the fish biomarker number.

As part of routine quality assurance and quality control (QA/QC) for field operations, samples will be collected by experienced personnel and will be labelled, preserved and shipped according to the foregoing technical procedures. Detailed field notes will be recorded in waterproof field books and on pre-printed waterproof field data sheets. Specific work instructions outlining each field task in detail will be provided to the field personnel by the task manager.

Laboratory Methods

Ages of the fish collected during the fish study will be determined following the methods outlined in Mackay et al. (1990). Independent confirmation of at least 10% of the ageing structures will be provided by a second fish ageing expert. Length-frequency analysis will be used to correlate ages determined from structures, if necessary (i.e., the analysis is not complicated by large differences in sexes and if an adequate number of juveniles are not captured) (Mackay et al. 1990).

Gonad samples will be sent to Golder's laboratory for detailed weighing and egg counts. If histopathology is deemed necessary, gonad and liver samples will be delivered to the Department of Veterinary Medicine at the University of Saskatchewan, Saskatoon for sectioning and mounting as per their standard methods. These samples will then be examined by Dr. Richard Schryer of Golder (Saskatoon).

Statistical Design and Data Analysis

The fish population study is based on a control/impact design using statistical analysis to detect differences between discrete exposure (Seep Creek) and reference (Norma Creek) areas. *A priori* determination of the test hypothesis, effect size, probability of a Type I Error (α), probability of a Type II error (β), and Power ($1-\beta$) is discussed below.

The null hypothesis for the study design is stated as "There is no significant negative effect on the fish population exposed to mine effluent relative to a reference fish population not exposed to effluent."

Effect size refers to the difference between the predicted hypothesis (null hypothesis) and the specified alternate hypothesis. Effect sizes are used to assess the degree to which the research hypothesis under study is actually observed via the sample data. EEM guidance document (Environment Canada 2002) states that a difference between 20% and 30% would be acceptable.

Probability of a Type I error, denoted "α", occurs if the null hypothesis "that there is no effect" is rejected, when in fact it is true (i.e., the exposure area is declared as being different from the reference when it is not). This type of error can be costly to industry. The α has been set at 0.05 (or 95% confidence) to reduce the likelihood of a Type I error. The probability of a Type II error, denoted "β", occurs if the null hypothesis is accepted when in it is false (i.e., there is no effect detected in the exposure area when in fact there is an effect relative to the reference area). This type of error is a risk to the environment β will be set at 0.05 to reduce the risk of a Type II error, which will result in a Power of the statistical tests of 0.95. This Power is the probability of correctly rejecting the null hypothesis when it is false (i.e., there is a high probability associated with correctly identifying an affected fish population in the exposure area).

All data will be entered into the EEM Metal Mining database for environmental data, which is scheduled to be completed in 2004. Once entered, the data will be extracted from the database and independently reviewed for transcription errors using several screening and reviewing methods (graphical and statistical). Data will be screened for potential outliers by visual examination of linear regression scatter plots and box plots. Data may require transformation, depending on the data distribution. Also, linear regression analysis will be completed on parameters used in EEM analysis. As part of the linear regression analysis, an evaluation of studentized residuals (SR) will be used as an additional screening tool. Observations that are more than three standard deviations (i.e., SR>3) from the cell mean will be checked and validity confirmed. The data will be removed from the database only if warranted. Adopting SR>3 as a cut-off is considered conservative, as greater than 95% of SR are expected to have lower values. If data are removed for obvious reasons, then screening will be re-run (i.e., scatter plots, box plots, linear regression) and outliers checked and validity confirmed. Outliers that are removed will be reported and reasons (labelling errors, analytical errors, etc.) for removal described. Decision on removal of outliers will be determined prior to parametric analysis. Any data that have a SR>3 and can not be reasonably explained will result in that particular analysis being performed with and without that data to determine the overall effect on the conclusion of the results. This will be reported and aid in determining if the outlier is an effect or a result of natural variability or some other error.

Descriptive statistics on biological variables including mean, median, minimum, maximum, standard deviation, standard error, and sample size will be summarized

according to area, species, sex (when possible) and sexual maturity. Biological indices, including condition factor (k), gonadosomatic indices (GSI), and liversomatic index (LSI) will also be calculated and included in the summary tables.

CPUE will be calculated to document the effort expended in collecting the required number of fish. This calculation will also provide a measure of relative abundance between sampling areas by standardizing the catch data for the exposure and reference areas.

Statistical comparisons between areas and estimates of variability for power analysis will be derived for the parameters as outlined in the EEM guidance document (Environment Canada 2002) (Table 4-2). Only a number of the endpoints can constitute an effect on the fish population in the exposure area when significantly different from the reference area; these endpoints are designated as "Effect" in the last column of Table 4-2. The remaining endpoints are designated as supportive analyses and will be analyzed only if data for these endpoints can be collected accurately (i.e., fecundity information) and provide additional relevant information supporting the effect analyses.

Survival is measured by comparing age distribution within a population. A healthy population should exhibit variability in age. A portion of the population should be made up of young, maturing fish, and older fish past their peak reproductive years.

Energy use is a measure of the ability of a fish population to utilize resources in their environment to grow and reproduce. It is also a measure of the hindrances that can deter fish from growing and reproducing normally and successfully.

Energy storage is a measure of the present condition of the fish population. A healthy fish will exhibit good body weight for its length and have a healthy liver weight that is not disproportionate relative to body size. Stressors from the environment, whether natural or anthropogenic, can affect fish condition within a population.

Mean age will be calculated as an indication of the age distribution for each species. All fish for which age has been determined (i.e., juveniles and adults) will be used in statistical comparison between the exposure and reference areas. For each species the variability of fish ages will be assessed using analysis of variance (ANOVA).

The following protocol will be used to determine significant effects on the dependent response variables. A general linear model will be used to test for homogeneity of slopes between the dependent variable and covariate for each area (i.e., test for significant area – covariate interaction). In cases where a significant interaction between area and covariate is found, analysis of covariance (ANCOVA) will not be conducted (Environment Canada

2002). Instead, a least-squares regression of the response variable and the covariate will be performed, independently, for each area. This analysis enables a determination of how the response variable is interacting with the covariate. Parameter estimates will be based on the y-intercept from each regression equation. It should be noted that the presence of a significant interaction between areas and covariate will be used as an indication of a significant difference between areas for a particular parameter (Environment Canada 2002).

Table 4-2 Health Response Endpoint Variables and Statistical Procedures Used for Identifying Differences Between Exposure and Reference Areas

Type of Response	Endpoint	Dependent Variable (Y)	Covariate Variable (X)	Statistical Procedure	Endpoint - Effect or Support Analysis?[a]
Survival	Age	n/a	n/a	ANOVA	Effect
Energy Use	Size at age	Total Body weight	Age	ANCOVA	Effect
		Length	Age	ANCOVA	Support
	Relative Gonad Weight	Gonad weight	Body weight[b]	ANCOVA	Effect
		Gonad weight	Length	ANCOVA	Support
	Total Body Weight	n/a	n/a	ANOVA	Support
	Length	n/a	n/a	ANOVA	Support
	Relative Gonad Weight	Gonad weight	Body weight[b]	ANCOVA	Effect
		Gonad weight	Length	ANCOVA	Support
	Relative Fecundity	# of eggs/ female[c]	Body weight[b]	ANCOVA	Support
		# of eggs/ female[c]	Length	ANCOVA	Support
Energy Storage	Relative Liver Weight	Liver weight	Body weight[b]	ANCOVA	Effect
		Liver weight	Length	ANCOVA	Support
	Condition	Total body weight	Length	ANCOVA	Effect

[a] indicates how statistical results will be interpreted; [b] gonad and liver weight removed from body weight; [c] measured only if egg counts can be accurately quantified

In cases where a general linear model produces no significant interaction between area and the covariate (i.e., homogeneity of slopes will not be violated), ANCOVA will then be performed and the effect of area on the response variable will be determined using Type III sum of squares. ANCOVA will then be used to test the effect of area on the endpoints. Parameter estimates are based on the adjusted mean (least-squared mean) after removing the effect of the covariate.

In cases where no significant difference is noted in the parametric analysis, power analyses will be performed to determine if there was sufficient power in the particular analysis to detect a change of 20, 25, and 30% between reference and exposure areas. Statistical results will be summarized in tabular format including p-values for each step of data analysis. Results of power analyses will be provided in the summary tables where no significant difference was noted, to determine if the sample size was sufficient to detect an effect size of 20, 25, and 30% with 95% power.

All data and analyses will be reviewed as part of Golder's quality assurance/quality control process to confirm results. A subset of each analysis will be checked including data screening, data analysis, and interpretations.

4.1.4.5 Fish Measurements for the Non-lethal Juvenile Fish Study (back-up study design)

As discussed above, if there are insufficient numbers of adult fish available for the study, a non-lethal survey of 100 juveniles per site will be conducted as an alternative monitoring method. This approach would entail that some biological endpoints could not be examined. Much of the study design for the adult survey (Section 4.1.4.4) is applicable to a non-lethal survey of juveniles; however, some modifications would be required and they are outlined below.

A non-lethal survey of juvenile fish will evaluate effects of mine effluent on energy use (growth, reproductive effort) and energy storage (condition). Fish will be examined for length, weight, abundance (catch per unit effort), age (subset of samples only), external condition, and life stage. Endpoints requiring invasive measurements such as fecundity, liver weight, and gonad weight, will not be examined in a non-lethal survey.

Field Methods

Field collection and measurement methods for a non-lethal survey will be the same as for an adult survey; however, the number of parameters that will be measured on fish for a non-lethal survey is reduced compared with an adult survey because invasive measurements and observations (e.g., liver and gonad weight) are not applicable for a non-lethal survey. All captured individuals of the study species will be enumerated, and the measurements and observations listed below will be recorded within the level of precision required of MMER/EEM biological studies (Environment Canada 2002):

- fork or total length, where appropriate (±1 mm);
- total body weight (±1%);

- age, by use of non-lethal structures such as scales (by laboratory analysis, ±1 year with 10% independently confirmed) (a subset of 20 juveniles [aged 1+] per study area);
- life stage; and,
- external condition.

Data Analysis

The data management, statistical procedures, and QA/QC outlined for the above adult survey (Section 4.1.4.4) are for the most part applicable to the analysis of data collected for a non-lethal survey; however, only a portion of the endpoints listed in Table 4-2 will be examined because some measurements will not be recorded for juvenile fish (i.e., liver and gonad weight). Further, some statistical procedures will be different.

The endpoints that will be examined for a non-lethal sampling survey are presented in Table 4-3. A length-frequency distribution will be used as surrogate of an age frequency distribution because ageing will only be conducted on a subset of older fish (aged 1+ or greater) (Environment Canada 2002). Length-frequency distributions will be compared between the exposure and reference areas with a non-parametric two-sample Kolmolgorov-Smirnov test.

Table 4-3 Endpoints and Variables to Identify Differences Between Exposure and Reference Areas for a Non-Lethal Survey

Endpoint	Variable(s)	Statistical Procedure	Endpoint - Effect or Support Analysis?[a]
Age	Length-frequency distribution	Two sample Kolmolgorov-Smirnov test	Effect
Condition	Total body weight, length	ANCOVA	Effect
Size at age (YOY)[b]	Length of YOY fish	ANOVA	Effect
	Total body weight of YOY fish	ANOVA	Effect
Size at age (Fish aged 1+)	Length of fish aged 1+	ANOVA	Support
	Total body weight of fish aged 1+	ANOVA	Support
Reproductive performance	Percent abundance of YOY fish	Not applicable	Support

[a] Indicates how statistical results will be interpreted; [b] young-of-the-year

Growth (size at age) will be examined by comparing the size of fish between the reference and exposure areas with a parametric *t*-test (Environment Canada 2002).

ANOVA will indicate if there is a statistically significant difference in mean length or mean body weight of fish between the study areas (Table 4-3). The growth of fish will be examined separately for YOY and fish aged 1+ (or greater). Reproductive performance will be examined by qualitatively comparing the relative abundance of YOY fish. The condition of fish in the study areas will be compared with an ANCOVA test. Performance will also be evaluated by the relative condition (k) of the fish examined.

4.1.5 Communication with Environment Canada during Field Sampling Sessions

During the field survey, Environment Canada will be given updates on fish capture in the exposure area and contacted to obtain advice and approval for decisions made on the study design. We therefore request that an Environment Canada contact be provided for communications regarding the fish study during the field survey period.

4.2 Fish Tissue Analysis

According to the metal mining EEM aquatic technical guidance document (Environment Canada 2002), fish tissue analysis is not required if effluent mercury concentrations are less than 0.10 µg/L for 12 consecutive sampling periods. The Lupin Mine has not monitored mercury concentrations in their effluent, thus a mercury fish tissue sampling program is required as part of the MMER/EEM biological studies.

Arctic grayling are common to both the exposure (Seep Creek, Unnamed Lake and Inner Sun Bay) and reference (Norma Lake and Norma Creek) areas of the proposed Lupin Mine EEM biological monitoring studies. This fish species is commonly fished and consumed (muscle tissue) by local residents and mine personnel. Lake trout would be another candidate species for a tissue monitoring program; a considerable historical tissue metal database is available (RL&L and DFO 1991; RL&L 1995) and they are a target species for human consumption; however, lake trout are highly mobile. Lake trout specimens tagged in Inner Sun Bay (exposure area) have later been tracked at locations throughout Contwoyto Lake (RL&L 1996c).

Eight 50-g muscle tissues samples will be collected from each study area (total number of samples will be 16). Adult male Arctic grayling of age 5+ to 6+ will be targeted, corresponding to size classes of 250 to 300 mm fork length (RCPL and RL&L 1985; RL&L and DFO 1991; AQUAMIN 1996b). Arctic grayling specimens, morphometric measurements, and aging structures will be collected using standard non-lethal sampling and data collection techniques described above for the fish population studies (Section 4.1).

Special precautions to prevent contamination of samples collected for mercury analysis will be used. For example, fresh filleting knives and dissecting equipment (e.g., titanium blades) for each fish is required. All processing and storage (appropriate plastic) containers must be washed with a detergent solution, rinsed with distilled water, soaked in 5% nitric acid bath for 12 to 24 h. Samples must be frozen (-20 °C) as soon as possible following collection and kept frozen until processed in the analytical laboratory. Technical procedures for the handling of fish tissues are kept on file at Golder offices (Edmonton and Calgary) and routinely updated.

4.3 Benthic Invertebrate Community Survey

4.3.1 Objectives

The objective of the EEM benthic invertebrate community (BIC) survey is to provide sufficient data to prove whether or not mine effluent has had a significant negative effect on the BIC within the aquatic receiving environment relative to a reference area. A control/impact study design, as recommended by Environment Canada (2002), will be implemented.

4.3.2 Habitat Selection and Study Areas

Many factors are to be examined before final determination of sampling areas. Care must be taken when selecting replicate stations to ensure habitats sampled in the reference area are similar to those found in the exposure area. Seasonal flow and discharge levels should be known before sampling commences. Low flow conditions, effluent discharge conditions, freezing, water depth, sediment characteristics and other factors can influence the effectiveness of sampling.

Habitat within the Seep Creek drainage downstream of the Lupin Mine treated effluent release point consists of both erosional and depositional habitats. The erosional areas are either large cobble areas with shallow fast flowing water, or more gentle sloped regions with embedded cobble in silt/sand. The nature of the erosional habitats in the downstream receiving environment would likely result in highly variable habitat characteristics and make interpretation of resulting data difficult. Similar habitat variability was noted for the proposed fisheries reference stream (Norma Creek; Section 3.8). A more practical sampling region in the exposure area would be depositional habitats (e.g., Inner Sun Bay), while better controlling for habitat features that seems to have confounded previous benthic macroinvertebrate assessments (e.g., Mudroch and Sutherland 1988; Porter et al. 1992; EVS 1996).

Proposed benthic invertebrate sampling areas for the MMER EEM study include Inner Sun Bay as an exposure area and similar sized bay of Contwoyto Lake as the reference area. For example, a bay is located approximately 9 km to the northeast of the Lupin Mine (Figure 2-1). Attempts will be made to collect all samples from locations that feature water depths between 2.5 and 3.5 m and similar substrates.

4.3.3 Collection Methods and Sample Size

Samples for the BIC survey will be collected using an Ekman grab with a surface area of 0.0225 m^2, following procedures described in EEM guidance document (Environment Canada 2002). Each sampling area will contain five replicate stations that will be spaced 20 m apart, if practical and allowing for maintaining consistency in habitat characteristics. At each replicate station, three field sub-samples will be collected from within a 100 m^2 area.

Each field sub-sample will be sieved using a 243 µm mesh. The sub-samples will be combined in the field to form one composite sample. This collection method is used to minimize variability between replicate stations. The sample from each replicate station will be preserved in a 10% formalin solution and sent to a certified taxonomist for enumeration and identification to the family taxonomic level. Organisms that cannot be identified to the family level of taxonomic precision (e.g., immature or damaged) will be reported as a separate category (at the lowest level of taxonomic resolution possible). A reference collection will be available for all taxa identified. These collections will be maintained for taxonomic verification, ensuring consistent taxonomy, and for quality control checks.

4.3.4 Statistical Design and Analysis

The BIC survey is based on a control/impact design used to detect differences between discrete exposure and reference areas. The null hypothesis for the study design is stated as, "there is no significant negative effect on the benthic invertebrate community exposed to mine effluent relative to a reference benthic invertebrate community not exposed to effluent."

To determine if there was a correlation between the biological endpoints (density and richness) and environmental variables and whether ANOVA or ANCOVA analysis of the data is required, a Pearson correlation matrix will be generated. Correlation analysis will be completed for the following:

- water depth;
- sediment particle size (sand, silt, and clay); and,

- sediment total organic carbon content.

Data received from the taxonomist will be collected in electronic format and hard copy. The electronic copies will be imported directly into Golder's database to minimize transcription errors. Data will be queried from the database and random checks performed to confirm validity and accuracy. Data will be screened for potential outliers by visual examination of scatter plots and box plots. Outliers will be checked and validity confirmed similarly as in the Fish Population Survey (Section 4.1.5).

Summary statistics and biological indices will be calculated for each replicate station and include total benthic invertebrate density, taxa richness, Simpson's diversity index, and Bray-Curtis index. Density is calculated as the number of individual organisms per m^2. Richness is identified as the total number of taxonomic groups in a specific area. In this study, richness data will be identified to the taxonomic level of family.

Simpson's diversity index measures the proportional distribution of organisms in the community given that not all organisms have the same success in the environment, (i.e., certain conditions favour or affect one organism over another). Simpson's diversity index will be calculated for each area taking into account the abundance patterns and taxonomic richness of the community. Simpson's diversity index values are between zero and one, whereby higher values indicate a community consisting of more taxa that are more evenly distributed among the taxonomic groups. Low numbers indicate a community dominated by few taxonomic groups; these are often referred to as stressed communities. The stress may be caused by natural and/or anthropogenic conditions.

The above indices are measures of total abundance and taxonomic richness, but do not take into account any quantitative information on the type of organisms present. A similarity index such as the Bray-Curtis index, summarizes the overall difference in community structure between the reference and exposure areas. The more similar the reference community is to the exposure community, the lower the index value, which can range from zero (similar) to one (dissimilar).

Descriptive statistics on BIC descriptors will include mean, median, minimum, maximum, standard deviation, standard error, and sample size for each area, including values for each replicate station. In addition, the following descriptors will be reported to support the conclusions made on the above descriptors that can determine effects to BIC:

- evenness;
- taxonomic (i.e., family) density;
- taxonomic proportion (i.e., family); and,
- taxa presence/absence.

Statistical analyses will be conducted on the abundance, richness, and index data calculated for each area. If necessary, BIC descriptors will be transformed using an appropriate transformation to facilitate the normalization of the data. Replicate station means will be used to determine significant differences between areas. Parametric analyses will be used to determine if there is a significant difference (α and β=0.05) between the reference and exposure areas. To compare replicate station means, ANOVA or ANCOVA (statistical method chosen will depend on the results of the Pearson correlation) will be performed on the data.

Non-parametric analysis will also be performed on the Bray-Curtis index and Simpson's diversity index as an alternative to ANOVA because these are "derived" variables with unusual statistical properties, and in general their sampling distributions are unknown (Rosenberg and Resh 1993). For example, the Bray-Curtis index represents comparisons back to the same mean reference community; consequently, the assumption of independence is violated when running parametric analysis on this variable. To verify ANOVA results, the Kruskal-Wallis test, which is the non-parametric equivalent of a one-way ANOVA, will be performed on untransformed data, followed by a comparison of mean ranks. The Kruskal-Wallis test transforms the variable to ranks to test that there is no shift in the center of the groups (i.e., the centers do not differ). Significant Kruskal-Wallis tests will be followed by pair-wise *a posteriori* comparisons of sampling area means using a non-parametric test (i.e., comparisons of mean ranks procedure).

In cases where no significant difference is noted, power analyses will be performed to determine if there was sufficient Power (95% confidence) in the particular analysis to detect a two standard deviation (2SD) difference between reference and exposure areas. Statistical results will be summarized in tabular format including p-values for each step of data analysis. Results of the power analyses will be provided in summary tables when no significant differences are noted. This will confirm if the sample size was sufficient to detect an effect size of 2SD.

Data analysis and interpretations will undergo independent quality assurance to confirm results. A subset of each analysis will be checked including data screening, data analysis, and interpretations.

4.3.5 Supporting Environmental Data

Supporting environmental variables, such as dissolved oxygen, conductivity, temperature and pH measurements, are useful for data interpretation and for understanding the basis for certain biological responses and behaviours. Potentially confounding factors that may influence the BIC will be documented during the survey and will be provided in the interpretive report.

Dissolved oxygen, conductivity, temperature, and pH will be measured at each sample area. Data collection methods will follow Golder's Technical Procedures for Basic Limnology & Bathymetry (unpublished file information). Water depth and water transparency will be measured with a Secchi disk. The information will be displayed in a tabular format that provides a comparison among the areas.

Sediment samples will be collected at the same time as benthic invertebrate sampling. As per the EEM aquatic technical guidance document (Environment Canada 2002), a single standard Ekman grab will be used to collect the top 10 cm of sediment, to support benthic habitat characterization at each station. Sediments will be analyzed for total organic carbon content, as well as particle size according to the following classification:

- gravel (2-16 mm);
- course sand (2-0.2 mm);
- fine sand (0.2-0.062 mm);
- silt (0.062-0.0039 mm); and,
- clay (<0.0039 mm).

Samples will be stored in sterile sediment sampling polyethylene bags, double bagged and double labelled, then kept cool (~4°C) for shipment to the analytical laboratory. Descriptive statistics on the particle size composition and total organic carbon content will be summarized according to mean, median, minimum, maximum, standard deviation, standard error and sample size for each area, and will include values for each replicate station.

In addition, sediment samples (stainless steel Ekman grab) will be archived (frozen) and stored for possible future chemical analyses if warranted, (i.e., if an effect in the benthic community is noted from this study). One sediment sample will be collected from each station within the exposure (Inner Sun Bay) and reference (embayment of Contwoyto Lake) area.

4.4 Schedule

Fish Population Surveys

The field survey will be conducted in mid-July 2005 rather than later in the season (i.e., August or September) because this period of the year is considered as early to mid-summer; fall-like weather conditions typically begin sometime in late August (Canadian Climate Normals 1961-2000; Section 3.3.1). Little is known about the biology of ninespine stickleback and slimy sculpin in the vicinity of the Lupin Mine, but according to Scott and Crossman (1973), they spawn in. The timing of the survey will

also depend on when treated effluent is released. To protect grayling that spawn in Seep Creek, the mine is not allowed to commence effluent discharge prior to 15 July of any year unless otherwise approved by the NWB (NWB 2000). Historically, effluent discharge periods have ranged between two and eight weeks (Table 3-1).

Fish Tissues

The timing of fish tissue sample collections will correspond with the fish population survey of mid to late July 2005. As Arctic grayling are spring spawners, collection of tissues from specimens of this species will not interfere with spawning behaviour.

Benthic Invertebrates

Due to variability of the benthic invertebrate population during the summer months, it is recommended that the community survey occur in the fall (early September 2005) prior to freeze-up. This minimizes the collection of emerging larvae or adults. In addition, this season corresponds to collection periods of several studies (e.g., RCPL and RL&L 1985b; Porter et al. 1992; RL&L 1996a).

Reporting

The interpretive report, based on results of the completed field surveys and data analysis, will be submitted to Environment Canada no later than 6 June 2006.

5.0 CLOSURE

This site characterization and study design was completed by Golder on behalf of Kinross. We trust that the information provided meets the requirements of the MMER submission for the Lupin Mine. Golder wishes to thank Kinross for the opportunity to complete this work. Should there be any further requirements or clarification, please do not hesitate to contact the undersigned individuals.

GOLDER ASSOCIATES LTD.

Report Prepared by:

Mark Dunnigan, M.Sc., P.Biol.
Aquatic Biologist
Project Manager

Report Reviewed by:

Gary Ash, M.Sc., P.Biol.
Senior Fisheries Biologist
Principal

Bernard Trevor, M.Eng., E.I.T.
Water Resources Engineer

Vanessa Bourhis, M.Sc.
Aquatic Biologist

MD/BT/GA/VB/aw

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