Some of the limitations of the marine fish study are outlined below.

- The only fish species that is known to be present in the area of the mouth of Twin Lakes Creek in numbers sufficient for the study, and is expected to be relatively sedentary, is shorthorn sculpin (*Myoxocepalus scorpius*);
- Due to the timing of reproduction for shorthorn sculpin (eggs appear to be deposited during the winter months), it is likely that fish that are caught during the summer sampling period will not be sexually mature.
- Due to the behaviour of fresh water entering the marine environment, it is predicted that the freshwater plume will form a thin layer on the surface, subject to winds and tidal (east-west) movement, and will be highly dispersed at the surface before mixing downwards. Therefore, the exposure of the fish to the plume may be limited to very high dilutions.
- The creek water is modified by a number of potentially confounding factors, in addition to receiving the mine effluent. These confounding factors include heavy metals from areas of waste rock and natural mineral exposures, and sewage inputs from the townsite.

However, the advantages of the marine fish study, listed below, are felt to outweigh these disadvantages.

- The marine environment is the nearest location where fish and fish habitat are exposed to the mine effluent, even if there are confounding factors present.
- The primary question asked by the first *MMER* EEM program is whether there is a statistically significant difference between areas exposed to the effluent, and a reference area. While the exact cause of the difference may not be identified by the first biological survey, a statistically significant difference may be observed.

3.2 Past Monitoring Results and Conclusions

There are two recent reports that provide some information on environmental effects of Twin Lakes Creek water entering the marine environment. These are: *Toxicity Testing, Mouth of Twin Lakes Creek in Strathcona Sound* (Lorax, 2000); and *Marine Sampling in Strathcona Sound, July, 2003* (JWEL, 2003c).

3.2.1 Toxicity Testing at the Mouth of Twin Lakes Creek

In compliance with a condition of the Nanisivik Mine water license issued by the Nunavut Water Board, water samples were collected from the estuarine mixing zones of Twin Lakes Creek and Kuhulu Creek (reference site) in late August, 2000. The purpose of these samples was to examine water quality, and to carry out toxicity testing with sand dollar (*Dendraster excentricus*) and Microtox tests, in the mixing zone. At the time of collection, there was no evidence of stratification in the upper 5 m of the water column. However, the samples were collected at a time of low flow in the creek, and the nearest sampling station to the creek was 60 m offshore, at a sample depth of 0.1 m.

Surface waters at 60 m offshore from the mouth of Twin Lakes Creek and 0.1 m depth were characterized by higher concentrations of cadmium (0.16 µg/L), copper (0.9 µg/L), lead (1.11 µg/L) and zinc (217 µg/L) when compared with another station 75 m offshore at a depth of 5 m, or with the reference station near the mouth of Kuhulu Creek.

The metal concentrations at a depth of 0.1 m, 25 m off the mouth of Kuhulu Creek were cadmium (0.06 µg/L), copper (0.35 µg/L), lead (0.22 µg/L) and zinc (23 µg/L).

Toxicity testing with the sand dollar demonstrated that fertilization was inhibited 50% at the Twin Lakes Creek station, when compared with the reference stations. Microtox testing did not indicate differences at any station. It was concluded that the fertilization tests demonstrated that loadings from Twin Lakes Creek have the potential to exert fertilization inhibition on biota in the local receiving environment at Strathcona Sound, however, the spatial extent of potentially toxic waters was not established.

3.2.2 Marine Sampling in Strathcona Sound, 2003

JWEL collected samples of fish tissues and sediments from Strathcona Sound for analysis during 2003 (JWEL, 2003c). The full report is included with this document as Appendix C. The fish tissue sampling was for trace metal analysis, although a number of parameters typically required during the fish study were also measured (including length, weight, gonad and liver weight). Sediment samples were also collected and analysed for trace metals. In addition, incidental observations on the stratification of Strathcona Sound in mid July were also documented.

The fish were collected at the Nanisivik wharf using rod and line, with baited jigs. Shorthorn sculpin were readily caught at this location. During the period when 12 shorthorn sculpin were collected, a single Arctic cod was also taken, and released. The fish were frozen shortly after being collected, and were subsequently thawed and dissected at the JWEL office in Fredericton, New Brunswick. Dorsal muscle samples (fillet with skin on) were submitted for chemical analysis of trace metal concentrations.

The Technical Guidance Document for Metal Mining EEM cites an Ontario guideline of 0.45 mg/kg for mercury as being the level above which an impairment of the usability of the fish resource may have occurred. At the Nanisivik wharf, the mean mercury concentration for 12 fish analysed was 0.15 mg/kg, and the range of observed concentrations was 0.07 to 0.26 mg/kg. Therefore, there is no reason to believe that either natural processes or human activities have led to a situation where there is an impairment of the usability of the fish resource at Nanisivik. Moreover a comparison of fish tissue metal data collected in 2003 with data reported by BC Research (1975) showed that there did not appear to have been a significant change in tissue metal concentrations over that period.

The sediment cores that were collected were sectioned into surface and deep layers. The sediments were highly calcareous, reflecting the local geological environment. Trace metal concentrations observed in the sediments are summarized in Table 3.1 below:

Table 3.1 Observed Metal Concentration Ranges in Marine Sediments Near the Mouth of Twin Lakes Creek, July, 2003.

Element	Observed Concentrations
Arsenic	9 to 14 mg/kg, above the CCME (1999) ISQG value of 7.24 mg/kg, but below the PEL value of 41.6 mg/kg in all cases.
Cadmium	1.6 to 10.2 mg/kg, spanning the range of the CCME (1999) PEL guideline of 4.2 mg/kg.
Chromium	23 to 27 mg/kg, below the CCME (1999) ISQG value (52.3 mg/kg) in all cases.
Copper	26 to 40 mg/kg, between the CCME (1999) ISQG value (18.7 mg/kg) and the PEL value (108 mg/kg) in all cases.
Lead	88.4 to 568 mg/kg, spanning the range of the CCME (1999) PEL guideline (112 mg/kg).
Mercury	0.03 to 0.1 mg/kg, below the CCME (1999) ISQG value (0.13 mg/kg) in all cases.
Zinc	481 to 2,740 mg/kg, above the CCME (1999) PEL guideline (271 mg/kg) in all cases.

Although concentrations of some of the metals are elevated in comparison with the CCME (1999) marine sediment quality guidelines, the values reported by BC Research (1975b) occupy similar ranges. Moreover, while one core had higher concentrations of cadmium, lead and zinc in the surface section, the other core had higher concentrations of these elements in the deeper section.

Therefore, there is not a clear pattern that would suggest that the mining activities have caused a substantive change in metal concentrations in the biota or sediments near the mouth of Twin Lakes Creek.

3.3 Fish Study Design for EEM

This section provides the proposed Fish Study design for the Nanisivik Mine EEM program.

3.3.1 Rationale

A fish study in the marine environment of Strathcona Sound is the most appropriate study to meet the requirements of the *MMER* because this is the nearest location to the point of effluent discharge where fish populations, or fish habitat, might be affected by the mine effluent released from the West Twin Lake tailings area.

3.3.2 Hypotheses

The null hypothesis to be evaluated is:

- Measurements of length-at-age, weight-at-age, condition, liversomatic index, and gonadsomatic index for fish collected near the mouth of Twin Lakes Creek (the exposure area) are not significantly different from those for fish collected at a reference area.

If this null hypothesis is rejected ($p<0.05$) for any of the biological measurements, it will be concluded that there is a statistically significant difference present.

The proposed study, and the null hypothesis to be tested, applies only to the comparison of Twin Lakes Creek with a reference location. Due to confounding factors present in Twin Lakes Creek (see below), the study design can not uniquely attribute an effect observed at the mouth of Twin Lakes Creek to the release of mine effluent, or other mine-related activities or effects. This statement of the null hypothesis, and the scientific limitations of a first study design, is consistent with and meets the requirements of the *MMER*.

3.3.3 Confounding Factors

As was described in Section 2 of this document, there are two main confounding factors present in Twin Lakes Creek that limit the ability for this investigation to identify a cause-effect relationship, if it is concluded that there is a significant difference between fish from the exposure and reference areas. The confounding factors include:

- sources of metal loading to Twin Lakes Creek other than the WTDA; and
- deposit of untreated sewage from a sewage station owned by the Government of Nunavut into Twin Lakes Creek.

Twin Lakes Creek cuts through a naturally occurring zone of metal sulfide mineralization about half way down its length, as well as passing by several waste rock areas and the former mill. Seasonal thaws, and episodes of rain periodically wash high loadings of zinc, lead and other metals from various sources into the creek. As a result of these high metal loadings, which are substantially larger than the metal loadings from the mine effluent, the concentration of zinc at the creek mouth is usually elevated. In addition, raw sewage from a sewage station owned by the Government of Nunavut, is deposited into the creek at a location close to the former Mill. These two factors (other metal loadings and nutrient addition due to raw sewage discharge) are considered to be confounding factors that will make it impractical to isolate the effects of mine effluent discharge on fish in the marine environment.

Adding to the confounding factors, the behaviour of the creek waters upon entering the marine environment also merits consideration. When visited in July 2003, the combination of melting sea ice and fresh water inflows resulted in the formation of a layer of fresh water on top of the marine water. Although the freshwater lens was probably at its thickest and greatest extent at this time (due to melting sea ice, as well as relatively high stream flows), the freshwater plume from the creek can be expected to "float" in a thin layer on top of the marine waters at all times, until wind and wave action physically mix the fresh water into the underlying sea water. In addition, the freshwater plume will be advected east-west with the rising and falling tide. This is typical behaviour of freshwater streams entering the marine environment.

The predominant species of fish in this area appears to be shorthorn sculpin (*Myxocephalus scorpius*), a bottom-dwelling species. The water column near the mouth of Twin Lakes Creek drops off rapidly from approximately 1 m at the creek mouth, to in excess of 200 m offshore. As a result, marine fish may not be exposed to creek water containing the effluent, except at very high dilution.

3.3.4 Statistical Design

The proposed fish study will focus on adult shorthorn sculpin, which may not be in reproductive condition (*i.e.*, the gonads of fish sampled may not be fully ripe) at the time of sampling due to the necessity of sampling in the summer months, while these fish spawn during the winter. The study will be an Initial Monitoring study, as defined in the *MMER*. In order to try to optimize the study, the field program will be scheduled later in the season while the mine is still releasing effluent (likely during late July or early August 2004).

The exposure area will be located near the mouth of Twin Lakes Creek. A reference area has been selected, in part based upon the advice of members of the TAP, at the mouth of the Strathcona River, on the opposite side of Strathcona Sound (the stations are approximately 7 km apart). Shorthorn sculpin are known to be abundant at both locations. The selection of the Strathcona River as the reference area should minimize the potential for fish to move between the two areas, due to the physical separation.

The recommended minimum sampling size for an Initial Monitoring study is 20 sexually mature males and 20 sexually mature females from both exposure and reference areas (80 fish in total per species sampled). This sample size is based upon the rationale that there is little change in the 95% confidence limits with increasing sample size beyond 20 fish.

The measures to be recorded on each fish will include:

- body total length (±1 mm);
- body weight (g, ±1%);

- external and internal appearance (normal, abnormal, lesions or parasites)
- sex (male or female) and state of maturity (mature or immature);
- liver weight (g, ±1%);
- gonad weight (g, ±1%);
- egg size (±1%, based upon measurements of eggs in preserved gonad samples)
- fecundity (number of eggs per female ±1%, based upon egg counts and diameters in preserved gonad samples);
- age (years, ±1) based on otoliths to be removed from the skull; and
- exposure, based on metals concentrations to be measured in liver samples removed from each fish.

Fish usability will not be investigated in this fish study, and mercury will not be requested as an analyte for fish liver samples, because a recent investigation (JWEL, 2003c) has shown that mercury concentrations in the edible portion of shorthorn sculpin collected near the mouth of Twin Lakes Creek are not greater than 0.45 mg/kg wet weight.

3.4 Fish Study Data Analysis and Interpretation

In the data assessment and interpretation step of the EEM study, the following questions are normally answered:

- is there an effect?
- is the effect mine related?
- is the magnitude and extent of the effect known?
- is the mine-related cause of the effect known?

However, since the Nanisivik mine has applied for recognized closed mine status, and the present investigation will be both an Initial Monitoring study, and the Final Biological Monitoring study prior to closing mine, there will be inherent limitations on the questions that it will be possible to answer. Specifically, it will be possible to determine whether there is an "effect" on the fish population as defined in the *MMER*, and what the magnitude of effects may be. However, due to confounding factors present, it will not be possible to determine whether the effect is mine related. Since this is the Final Biological Monitoring study, there will be no follow-up to evaluate any possible mine-related cause of effects. Finally, the study will not provide specific information on the spatial extent of possible effects, except to the extent that the exposure area represents the mouth of Twin Lakes Creek.

It is possible that the study will conclude that there is no significant effect on fish. The Metal Mining EEM Guidance Document states that there are at least three reasons that a mine in compliance with the *MMER* may not have receiving water effects:

a) the mine may not produce compounds capable of causing those effects,
b) the dilution at the site may be sufficient that the effects are not manifested,
c) there may be insufficient critical habitat in the exposure area, such that fish do not spend sufficient time in the effluent to demonstrate impacts (if any were possible).

To this we would add a fourth possible reason, that fish in the exposure area may not be significantly exposed to the effluent, as may be the case when a freshwater plume enters the marine environment is carried on the surface while slowly being diffused and dispersed, and the fish study examines a bottom-dwelling species.

The statistical analysis of the Fish Study data will mainly rely upon Analysis of Covariance (ANCOVA), although Analysis of Variance (ANOVA), regression, and simple "t" or χ^2 tests may be applied, where appropriate. Analysis will be carried out separately for male and female fish. The following parameters will be derived and analyzed:

- growth, as size-at-age;
- reproductive investment, as gonad weight relative to body weight or length;
- fecundity, as number of eggs per female relative to body weight, length, or age;
- egg size, as mean egg weight against body weight, or age;
- survival, from age structure and length frequency analysis;
- energy storage, from condition factors (body weight relative to length), liver weight relative to body weight, and liver weight relative to body length; and
- frequency of physical abnormalities such as tumors, lesions, or parasites.

3.5 Quality Assurance and Quality Control

All work will be performed by suitably qualified and trained staff (biologists and technicians), under the direction of Dr. Malcolm Stephenson, Senior Aquatic Scientist for JWEL in Fredericton, New Brunswick. Where subcontractors are used (such as for analysis of otoliths to determine age, or for chemical analysis) they will be selected for their specialist expertise. Labs that are used for chemical analysis will be chosen on the basis of their being accredited by the Canadian Association of Environmental Analytical Laboratories (CAEAL) to perform the required analyses, where such accreditation exists.

All field work will be carried out following Standard Operating Procedures, to ensure overall consistency and that appropriate procedures are followed. All field measurements will be made using appropriately calibrated instruments (for example, field balances will be calibrated before and after use with standard calibration weights). All field data will be recorded using standard forms, to ensure that all of the required data are collected, in a reproducible and standardized format.

To the extent practical, all field sampling bottles and containers will be pre-labelled and packaged in an orderly fashion, to minimize the potential for labelling errors.

Where appropriate, replicate samples will be collected for duplicate analysis, and/or replicate analysis of subsamples will be requested, in order to verify the accuracy and reproducibility of both field measurements and laboratory analyses. These replicate analyses will be performed at a frequency of approximately 10% of the overall sample stream.

In data analysis, the first step will be the screening of the data for outliers. A rapid way to screen for outliers is to create scatterplots of pairs of variables, with 95% confidence ellipses superimposed. Potential outlier data points can then be identified as those that lie outside the confidence ellipses. Outliers can result from a number of causes, including data entry or transcription errors. Where outliers are detected, the data records will be reviewed in order to attempt to isolate and if possible correct the source of a potential error. Where no such identification is possible the analysis will be performed both with and without the outlier, in order to evaluate the influence that the outlier exerts on the results of the data analysis.

Statistical data will be examined to evaluate the degree to which the data conform to the underlying assumptions of the analysis (such as normality and homogeneity of variance, or equality of slopes in ANCOVA). Where appropriate, transformations may be applied in order to reduce the magnitude of violations of the underlying assumptions.

4.0 BENTHIC INVERTEBRATE COMMUNITY SURVEY

According to *MMER*, the objective of the EEM program is to evaluate the effects of effluent on the aquatic environment. The benthic invertebrate community survey component of the EEM program is intended to evaluate whether the mine effluent has effects on the benthic invertebrate community (considered to be a component of fish habitat) within the aquatic environment most proximate to the effluent discharge. At mines where the EEM study will be periodically repeated (*i.e.*, at mines that have not applied for recognized closed mine status), it is expected that follow-up rounds of EEM will evaluate trends over time, and determine the magnitude and geographic extent of effects that are related to mine effluent. The Nanisivik Mine has applied for recognized closed mine status, therefore the benthic invertebrate component of the Final EEM Study described in this document will not include follow up rounds of EEM.

The Nanisivik Mine discharges effluent from the West Twin Lake tailings area, via a weir, to Twin Lakes Creek. A secondary effluent discharge point, at the East Adit Treatment Facility, did not discharge effluent in 2002 or 2003, and is not expected to discharge effluent in future, due to mine closure and reclamation. Therefore, the benthic invertebrate community study design will focus on the effluent discharged from the West Twin Lake tailings area.

4.1 Goals and Objectives of the Benthic Invertebrate Community Survey

The objective of the benthic invertebrate community survey is to evaluate the effects of effluent on the aquatic environment in the immediate vicinity of the effluent discharge by sampling, identifying, and statistically analyzing the benthic community that is highly exposed to the effluent. The reach of Twin Lakes Creek that is located immediately downstream from the West Twin Lake tailings area discharge point provides suitable conditions for this study.

The Metal Mining EEM Guidance Document describes the expectations for the benthic invertebrate community survey, as summarized below. The study design for the First EEM Study should include:

- one ecologically relevant season;
- sampling in reference and high exposure areas (proximate to the effluent discharge);
- sampling in ecologically relevant habitat;
- one of five site-specific sampling design;
- collection of data for site-specific supporting variables; and
- use of standard field and laboratory methods.

The benthic invertebrate community descriptors which are required to be calculated and reported include:

- total invertebrate numerical density;
- taxonomic richness;
- Simpson's diversity index; and
- Bray-Curtis index.

The above list does not exclude the use of other (additional) benthic invertebrate community descriptors or analyses.

4.2 Past Monitoring Results and Conclusions

Twin Lakes Creek has been sampled to investigate the benthic invertebrate community on at least two occasions in the past. The first was prior to the mine development (1974), and is reported by BC Research (1975a). The most recent was during the summer of 2003, and is reported in JWEL (2003b). The latter document is included as Appendix C with this EEM Study Design document.

4.2.1 The Benthic Community Prior to Mine Development

BC Research (1975a) reported collecting a number of benthic invertebrate samples from Twin Lakes Creek, and other creeks in the area, using a Surber sampler. Although mesh size was not reported, it is reasonable to suppose that it would have been nominally 0.5 mm. It was reported that "invertebrate samplings revealed the presence of a few individuals of only three types of organisms ... indicating low stream productivity and low community stability. Stations sampled by BC Research included one station (WS-17) a short distance downstream from the outlet to West Twin Lake, and a second station (WS25) near the mouth of Twin Lakes Creek at Strathcona Sound. Five replicate samples were collected at each station. The collected invertebrate community was represented by 1 annelid and 3 Tipulidae at Station WS-17, and 1 annelid and 1 Tendipedidae (Chironomidae) at station WS-25.

4.2.2 The Benthic Community in 2003

The full report from which the following information is an extract is reproduced as Appendix C to this document.

Benthic samples were collected in July 2003 using a U-frame kick net, having a mesh size of 0.25 mm. This gear encloses a section of creek bed having an area of one square foot (0.093 m^2). In operation, the net is similar to a Surber sampler, in that the net encloses an area of substrate that is disturbed (by kicking). Invertebrates that are dislodged are collected in the net. Five replicate benthic samples were collected at stations located on the stream flowing out of East Twin Lake (ETL-1 to ETL-5), below the effluent discharge from West Twin Lake (WTL-1 to WTL-5) and in Twin Lakes Creek (TLC-1 to TLC-5) below the confluence of these two flows. The samples were sieved in the field using a 0.25 mm mesh, and were preserved using 10% formalin solution. After being sorted from the associated debris, the benthic invertebrates were identified to the Order/Family level, by an experienced benthic invertebrate taxonomist. Quality assurance checks included re-sorting two samples (TLC-2 and ETL-4) to verify sorting efficiency, which met the requirements of the *MMER*.

The benthic invertebrate community was dominated by Chironomidae, although a few Tipulidae and one other unidentified dipteran larva (Tabanidae?) were collected. Some Cladocerans (*Daphnia*?) were also collected, but these were presumably drifting down from the zooplankton population present in East Twin Lake, and do not represent true benthic fauna.

The assemblages present at the ETL site were qualitatively and quantitatively similar to the assemblages present at the TLC site (see Table 4.1). However, no benthic invertebrates were recovered from the WTL site, immediately below the effluent discharge weir. The effluent is not acutely lethal to fish, however, it appears to be toxic to *Daphnia magna*, at least at some times of the year (Nanisivik Mine monitoring data). Therefore, it is possible that the absence of benthic invertebrates immediately below the effluent discharge point (where the exposure to effluent is essentially 100%) may be an effect of the mine effluent.

Table 4.1 Benthic Invertebrates Collected in Twin Lakes Creek July 11, 2003

Taxa	Chironomidae	Tipulidae	Other Diptera	Cladocera
TLC1	3			1
TLC2	12			
TLC3	5			
TLC4	7	2		1
TLC5	7			
WTL1				
WTL2				
WTL3				
WTL4				
WTL5				
ETL1	9			
ETL2	9			
ETL3	14			
ETL4	3		1	
ETL5	23			

4.3 Benthic Invertebrate Community Survey Design for EEM

This section provides the proposed benthic invertebrate community survey design for the Nanisivik Mine EEM program.

4.3.1 Rationale

A benthic invertebrate community survey is possible in Twin Lakes Creek, as has been demonstrated by the 2003 field investigation. It is proposed to carry out the survey in a portion of the creek proximal to the point of effluent discharge.

4.3.2 Hypotheses

The null hypothesis to be evaluated is:

- benthic invertebrate community metrics (total invertebrate numerical density, taxonomic richness, Simpson's diversity index and Bray-Curtis index) are not significantly different in areas exposed to the effluent than in an upstream reference area.

If this null hypothesis is rejected ($p<0.05$) for any of the biological measurements, it will be concluded that there is a statistically significant difference present.

4.3.3 Confounding Factors

If carried out in the upper reach of Twin Lakes Creek, proximal to the point of effluent discharge, then there are no known confounding factors present.

4.3.4 Field Study and Statistical Design

The total number of invertebrates recovered from kick samples (0.25 mm mesh) having an area of 0.093 m^2, was typically less than 20 per sample. In order to obtain larger numbers of invertebrates for analysis, and to reduce the inter-replicate variability, it is proposed to collect and combine in the field three individual benthic invertebrate sub-samples at each replicate station. There will be five replicate stations for each sampling area. Four sampling areas will be defined as follows.

- A Reference Area (Twin Lakes Creek upstream of the effluent discharge point).
- A High Exposure Area in the effluent mixing zone of Twin Lakes Creek, at nominally 80% effluent concentration, at locations between 50 and 200 m downstream from the point of effluent discharge. Effluent concentration will be determined in the field using specific conductance. The five replicate stations will each be spaced approximately 30 m apart.
- A Low Exposure Area in the effluent mixing zone of Twin Lakes Creek, at nominally 10% effluent concentration, at locations between 50 and 200 m downstream from the point of discharge. The five replicate stations for this Exposure Area will correspond with the stations for the High Exposure

Area, but will be located near the opposite bank of the creek, with low exposure to the effluent. Exposure will be assessed in the field using specific conductance.

- A Terminal Exposure Area, located below a waterfall approximately 600 m downstream from the point of effluent discharge, where the effluent is fully mixed across the stream width. It is anticipated that when the study is carried out in August 2004, the terminal dilution for the effluent in Twin Lakes Creek may be approximately 30%.

Although it is not strictly required to sample more than a single exposure area in the Initial Monitoring study, the Metal Mining EEM Guidance Document notes that there may be situations where questions regarding the magnitude and extent of effects can be addressed by additional sampling during the same field trip. The present study design recognizes the benefits of doing so.

The appropriate ecologically relevant habitat type for sampling is cobble in riffles, since this is the predominant habitat type in the stream. The benthic invertebrate samples will be collected in late July or early August, 2004. This timing is driven in part by a need to coordinate sampling with the timing of the fish study and because effluent is discharged only during the months of June, July, and August. The summer season at Nanisivik is short, and the creek is fully frozen during the winter. In the early summer, flows in the creek can be very large due to spring runoff, and this would introduce a working hazard.

It is proposed to use a U-frame kick net having a mesh size of 0.25 mm for sampling. This is finer mesh size than is standard for metal mining EEM (0.5 mm). However, given the low numbers of invertebrates present in Twin Lakes Creek, and the fact that many of the chironomids collected during 2003 were extremely small, the selection of 0.25 mm mesh size will be critical to a successful survey. As recommended by the Metal Mining EEM Guidance Document, the samples will be sorted and reported in two stages. The first stage will recover those organisms that are retained on a mesh size of 0.5 mm, so that overall comparisons to other mines can be entertained by Environment Canada. The second stage will recover those organisms that are retained on a 0.25 mm mesh. The statistical analysis will be carried out only once, and will include all recovered organisms. Based upon our experience in 2003, it is highly unlikely that any of the benthic invertebrate samples will require subsampling.

The samples will be processed in accordance with the guidance provided in the Metal Mining EEM Guidance Document. The selection of five replicate stations per sampling area is consistent with generic guidance for EEM studies, and provides statistical power of $\alpha = \beta = 0.05$ and Effect Size = 2 standard deviations.

Due to the dimensions of the stream, it will not be possible to separate replicate stations in Twin Lakes Creek at a distance that corresponds to the riffle-pool sequence. Twin Lakes Creek has a width of approximately 20 m. Assuming that the riffle-pool sequence is approximately 6 times the stream bankfull width, the replicate stations would have to be separated by a distance of 120 m to follow the guidance. This distance is too large for optimal study design. It is proposed here to separate replicate stations by a distance of 30 m in order to optimally locate stations at exposure levels that will provide meaningful information about effluent effects within the stream.

4.3.5 Benthic Invertebrate Taxonomy

Benthic invertebrates will be identified by an experienced taxonomist, to the Family level, using appropriate taxonomic keys. A reference collection will be prepared, and will be held by the mine for a period of six years following submission of the Interpretive Report. Although initially fixed and preserved in 10% formalin, the invertebrate specimens will be transferred at the first opportunity to 70% ethanol for long-term storage. Dilute formalin is preferred for initial fixation because the samples will be shipped by air, and 70% ethanol is highly flammable.

4.3.6 Supporting Environmental Measurements

Due to the characteristics of substrates in both the reference and exposure areas (*i.e.*, a predominance of boulder and cobble substrate, with essentially no fines present, no aquatic vegetation, and essentially no terrestrial vegetation) it is not practical to measure the total organic carbon content or particle size distribution of sediments. Therefore, these measures will not be reported or recorded, although the nominal dimensions of cobbles and boulders at each sampling location will be recorded.

The following variables will be measured at each replicate station, at the time of benthic sampling:

- stream width, and replicate station distance from the left bank;
- water depth (m) and velocity (m/s);
- substrate types (e.g., % cobble, gravel, sand, etc.), dominant size range (cm) and embeddedness (%);
- water temperature (°C), specific conductance (μS/cm), dissolved oxygen (mg/L) and pH; and
- water chemistry (samples to be collected for subsequent analysis of general chemistry, including alkalinity, turbidity, nutrients, and zinc).

In addition, a single set of water samples will be collected within each sampling area at the time of the benthic invertebrate community survey. These samples will be submitted for analysis of a suite of chemicals as described for Water Quality Monitoring in Section 5 of this document.

4.4 Benthic Invertebrate Community Survey Data Analysis and Interpretation

The benthic invertebrate community data will be analyzed to provide the arithmetic mean, median, standard deviation, standard error, minimum and maximum values for the total benthic invertebrate density, Simpson's Diversity Index, taxa richness, and the Bray-Curtis index. Differences between reference and exposure areas will be evaluated using ANOVA. Data will evaluated to see whether transformations would improve their normality, or the homogeneity of variances, and transformations will be applied if so justified. Following ANOVA, multiple comparison tests, such as Tukey's HSD test, will be used to determine which Exposure Area or Areas differ significantly from the others.

This analysis will allow the following questions to be answered:

- is there an effect?
- is the effect mine related?
- is the magnitude and extent of the effect known?

4.5 Quality Assurance and Quality Control

All work will be performed by suitably qualified and trained staff (biologists and technicians), under the direction of Dr. Malcolm Stephenson, Senior Aquatic Scientist for JWEL in Fredericton, New Brunswick. Where subcontractors are used (such as for benthic invertebrate taxonomy) they will be selected for their specialist expertise. Labs that are used for chemical analysis will be chosen on the basis of their being accredited by the Canadian Association of Environmental Analytical Laboratories (CAEAL) to perform the required analyses, where such accreditation exists.

All field work will be carried out following Standard Operating Procedures, to ensure overall consistency and that appropriate procedures are followed. All field measurements will be made using appropriately calibrated instruments (for example, pH meters will be calibrated at pH 4.0 and 7.0 using certified buffer solutions). All field data will be recorded using standard forms, to ensure that all of the required data are collected, in a reproducible and standardized format.

To the extent practical, all field sampling bottles and containers will be pre-labelled and packaged in an orderly fashion, to minimize the potential for labelling errors.

Where appropriate, replicate samples will be collected for duplicate analysis, and/or replicate analysis of subsamples will be requested, in order to verify the accuracy and reproducibility of both field measurements and laboratory analyses. These replicate analyses will be performed at a frequency of approximately 10% of the overall sample stream. While the benthic invertebrate samples are being sorted, 10% of samples will be subject to re-sorting to verify that the sorting efficiency is at least 95%. If less than 95% recovery of benthic invertebrates is achieved, all of the samples will be re-sorted until a minimum 95% recovery is documented.

In data analysis, the first step will be the screening of the data for outliers. A rapid way to screen for outliers is to create scatterplots of pairs of variables, with 95% confidence ellipses superimposed.

Potential outlier data points can then be identified as those that lie outside the confidence ellipses. Outliers can result from a number of causes, including data entry or transcription errors. Where outliers are detected, the data records will be reviewed in order to attempt to isolate and if possible correct the source of a potential error. Where no such identification is possible the analysis will be performed both with and without the outlier, in order to evaluate the influence that the outlier exerts on the results of the data analysis.

Statistical data will be examined to evaluate the degree to which the data conform to the underlying assumptions of the analysis (such as normality and homogeneity of variance, or equality of slopes in ANCOVA). Where appropriate, transformations may be applied in order to reduce the magnitude of violations of the underlying assumptions.