RESPONSES OF JUVENILE RAINBOW TROUT AND BENTHIC INVERTEBRATE COMMUNITIES EXPOSED TO EFFLUENTS FROM A MOLYBDENUM MINE IN CENTRAL BRITISH COLUMBIA.

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ABSTRACT

As part of the Metal Mining Effluent Regulations (MMER) under the *Fisheries Act*, mines are required to conduct a tiered Environmental Effects Monitoring (EEM) program. The objective of the EEM program is to evaluate the effects of mine effluent on fish, fish habitat (i.e., benthic invertebrates), and use of the fisheries resource. Endako Mine is an open-pit molybdenum mine located on the Nechako Plateau in central British Columbia, about 160 km northwest of Prince George. For Endako's Cycle One EEM program, a non-lethal survey of juvenile rainbow trout was conducted to assess the potential impacts of mine effluents discharged into lower Sweetnam Creek (exposure area) on fish survival, condition, growth, and reproduction relative to fish collected from a reference area (Allin Creek). Benthic invertebrate communities were sampled in the exposure area (lower Sweetnam Creek) and compared to benthic invertebrate communities sampled in two reference areas; Allin Creek and North Un-named Creek (a naturally metal-rich area). Results of the non-lethal fish survey and benthic invertebrate survey will be discussed as well as challenges associated with designing and conducting environmental monitoring programs for existing and future mining operations in this area of British Columbia.

INTRODUCTION

As part of the Metal Mining Effluent Regulations (MMER) under the *Fisheries Act*, metal mines are required to conduct a tiered Environmental Effects Monitoring (EEM) program. The objective of the EEM program is to evaluate effluent toxicity and potential effects of mine effluent discharged into the receiving environment on fish, benthic invertebrates, and use of the fisheries resource (Environment Canada 2002). The standard EEM adult fish survey involves lethal sampling a minimum of 20 sexually mature males and 20 sexually mature females of two fish species for the required measurements of age, length, weight, liver weight, gonad weight, fecundity, and egg size (Environment Canada 2002). These parameters are used to provide estimates of survival (age and/or length frequency distributions), energy use (growth and reproductive effort), and energy storage (condition and liver size), which can be used to establish the health of the fish populations (Munkittrick et al. 2000; Environment Canada 2002).

Metal mines, however, often discharge wastewater effluents into small headwater streams that typically have few fish species and low abundances of fish, and repeated lethal sampling for a monitoring program could pose a serious threat to local populations. As an alternative, non-lethal sampling methods were developed for use in the EEM program (Gray et al., 2002; Environment Canada 2002). The non-lethal sampling program requires the collection of a minimum of 100 individuals of the same species from the exposure area and at least one reference area (Gray et al., 2002; Environment Canada 2002). Measurement endpoints include: survival (e.g., length-frequency distribution), growth (e.g., length and weight of young-of-the year and older [1+] fish), condition, and reproduction (e.g., relative abundance of YOY) (Gray et al., 2002; Environment Canada 2002).

Using non-lethal sampling methods, the objectives of this study were to assess the potential effects of effluents discharged from a molybdenum mine on the survival, condition, growth, and reproductive performance of juvenile rainbow trout (*Onchorhynchus mykiss*). The potential effects of effluents from the molybdenum mine on local benthic invertebrate communities were also assessed.

MATERIALS AND METHODS

Mine Characteristics

Endako Mine is an open-pit molybdenum mine located on the Nechako Plateau in central British Columbia, approximately 160 kilometers northwest of Prince George and approximately 25 km west of the town of Fraser Lake. It is Canada's largest capacity molybdenum mine, and the only one producing molybdenum, a metal of strategic importance, as its primary product. Current production is on the order of approximately 30,000 tonnes per day (Endako Mines 2003). The processes at the mine include the removal of ore and waste rock from the host deposit and milling (i.e., processing of ore to produce a metal concentrate). Briefly, electric shovels load the rock onto haul trucks, which haul the ore to an in-pit crusher. The crushed ore is then transported by conveyor belt to the mill. Six basic operations take place within the mill areas: crushing (primary and secondary); grinding; flotation; leaching; roasting; and product packaging. Final mill product consists of powdered concentrate in the molybdic oxide form. Some purified molybdenum sulphide is also produced in a small plant outside the mill. The product is known by the trade name of "UltraPure". The tailings (which primarily consist of particles similar in size to coarse sand) and the mill process water are discharged to the tailings ponds through a pipeline network.

Fish and Benthic Invertebrate Collections

Juvenile rainbow trout and benthic invertebrates were collected during the same period, September 25 to October 1, 2005. Using a backpack electrofisher (Smith-Root Model 12B), we attempted to collected a minimum of 100 juvenile rainbow trout from lower Sweetnam Creek (LSC; exposure area) and Allin Creek (AC; reference area). Fork length and fresh weights of juvenile rainbow trout were recorded for all captured target fish. Catch-per-unit-effort (CPUE) was calculated to estimate relative abundance between sampling areas.

Benthic invertebrate communities were sampled from riffle areas within three sites: lower Sweetnam Creek (exposure area); Allin Creek (reference area); and, northwest tributary to North Un-named Creek (NW Trib; a naturally metal-rich reference area). Each sampling area contained five replicate stations (i.e., riffles) and five sub-samples from each station were collected using a surber sampler, sieved (250 μm mesh), preserved in 10% buffered formalin and sent to a qualified taxonomist for enumeration and identification.

Statistical Analyses

Length frequency distributions of juvenile rainbow trout (2-mm size classes) were compared between areas using the two-sample Kolmogorov-Smirnov test. This is a non-parametric, distribution free test that assesses the similarity of two cumulative distribution functions of two datasets (Sokal and Rohlf 1995). Log_{10}-transformed lengths and weights of juvenile rainbow trout were compared using ANOVA. Analysis of covariance (ANCOVA) was used to assess site differences in the relationship between weight and length (condition factor). Site differences in benthic invertebrate community end-points were assessed using ANOVA. All data analyses were done using SYSTAT 11. Alpha and beta were set equal to 0.10 (Environment Canada 2002).

PRELIMINARY RESULTS AND DISCUSSION

Water Quality and Physical Habitat Characteristics

Overall, most of the water quality parameters in all three Creeks were below guideline requirements. Field measurements showed specific conductance was similar in LSC and the NW Trib and elevated relative to AC; water temperature in LSC was higher relative to the reference sites; turbidity was about three times higher in AC than in LSC and the NW Trib, which may be indicative of agricultural inputs (i.e., cow manure) in the middle section of the creek. Interestingly, total phosphorus levels were elevated in AC relative to the other sites and equal to the CCME freshwater guideline value of 0.05 mg/L. As expected, water quality in lower Sweetnam Creek (exposure area) was most similar to the NW Trib (metal-rich reference area) and there were pronounced differences in the physical habitat of the three creeks (see Table 2). Most notably, wetted width of AC was more than four times greater than LSC and the NW Trib and mean current velocities in LSC and the NW Trib were higher than AC, about 2 to 3 fold, respectively (Table 2).

Table 1. Water quality characteristics of Allin Creek (reference area), northwest tributary of North Unnamed Creek (NW Trib, metal-rich reference area), and lower Sweetnam Creek (exposure area) during the fall 2005.

Parameter	Units	CCME Freshwater Guidelines	MMER Requirements	Allin Creek	Lower Sweetnam Creek	NW Trib
Field Measured						
pH		**6.5-9.0**	**6.5-9.0**	n/a	n/a	n/a
Specific Conductance	µS/cm			128	1306	1703
Temperature	°C			5.1	6.0	5.4
Dissolved Oxygen	mg/L			10.7	11.9	10.3
Turbidity	NTU	**8**		1.7	0.3	0.3
Conventional Parameters						
Conductance	µS/cm			130	1376	1647
Dissolved Organic Carbon	mg/L			7	9.2	5.7
Hardness	mg/L			64	607	885
pH		**6.5-9.0**	**6.5-9.0**	6.8	6.8	7.9
Total Alkalinity	mg/L			68.3	70.0	191
Total Cyanide	mg/L	**0.005**	**2**	0.0007	< 0.0012	0.003
Total Dissolved Solids	mg/L			71	1094	1397
Total Organic Carbon	mg/L			7	10	6
Total Suspended Solids	mg/L	**25**	**30**	< 3	0.8	< 3
Major Ions						
Bicarbonate	mg/L			84	86	234
Calcium	mg/L			17	187	284
Carbonate	mg/L			< 5	< 5	< 5
Chloride	mg/L			0.6	15	21
Fluoride	mg/L			< 0.1	0.22	0.2
Magnesium	mg/L			5	34	43
Potassium	mg/L			1	12	14
Sodium	mg/L			4	96	78
Sulphate	mg/L			2	707	844
Nutrients						
Nitrate + Nitrite	mg/L			< 0.05	0.3	< 0.05
Nitrate	mg/L	**13**		< 0.05	0.3	< 0.05
Nitrite	mg/L	**0.06**		< 0.05	< 0.05	< 0.05
Nitrogen - ammonia	mg/L	**1.37-2.20**		< 0.05	< 0.03	< 0.05
Phosphorus - total	mg/L	**0.05**		0.05	< 0.02	< 0.02

Oxygen Demand						
Biochemical Oxygen Demand	mg/L			< 2	< 2	< 2
Chemical Oxygen Demand	mg/L			20	30	20
Radionucleides						
Radium 226	Bq/L		**1.11**	< 0.002	< 0.005	< 0.005
Metals (Total)						
Aluminum (Al)	mg/L	**0.005-0.10**		0.06	0.036	0.05
Antimony (Sb)	mg/L			0.0006	0.0005	0.0002
Arsenic (As)	mg/L	**0.005**	**1**	0.001	< 0.0004	< 0.0004
Barium (Ba)	mg/L			0.02	0.02	0.04
Beryllium (Be)	mg/L			< 0.001	< 0.001	< 0.001
Boron (B)	mg/L			< 0.05	< 0.05	< 0.05
Cadmium (Cd)	mg/L	**0.000017**		< 0.0002	0.01	0.002
Chromium (Cr)	mg/L	**0.001/0.0089**		< 0.005	< 0.005	< 0.005
Cobalt (Co)	mg/L			< 0.002	< 0.002	< 0.002
Copper (Cu)	mg/L	**0.002-0.004**	**0.6**	< 0.001	0.002	0.002
Iron (Fe)	mg/L	**0.3**		0.59	0.10	0.05
Lead (Pb)	mg/L	**0.001-0.007**	**0.4**	< 0.0001	0.00008	< 0.0001
Lithium (Li)	mg/L			< 0.01	0.03	0.01
Magnesium (Mg)	mg/L			5	32	41
Manganese (Mn)	mg/L			0.01	0.05	0.03
Mercury (Hg)	mg/L	**0.0001**		< 0.0002	< 0.0002	< 0.0002
Molybdenum (Mo)	mg/L	**0.073**		< 0.005	6.0	1.6
Nickel (Ni)	mg/L	**0.025-0.150**	**1**	< 0.002	< 0.002	< 0.002
Potassium (K)	mg/L			1	11	12
Selenium (Se)	mg/L	**0.001**		< 6.66667E-05	0.0006	< 0.0001
Silver (Ag)	mg/L	**0.0001**		< 0.0004	< 0.0002	< 0.0004
Sodium (Na)	mg/L			4	88	72
Thallium (Tl)	mg/L	**0.0008**		< 0.0001	< 0.0001	< 0.0001
Tin (Sn)	mg/L			< 0.05	< 0.05	< 0.05
Titanium (Ti)	mg/L			0.005	0.003	0.003
Vanadium (V)	mg/L			0.001	< 0.001	< 0.001
Zinc (Zn)	mg/L	**0.03**	**1**	< 0.001	< 0.004	< 0.004

Table 2. Physical habitat characteristics of Allin Creek (AC, reference area), northwest tributary of North Un-named Creek (NW Trib, metal-rich reference area), and lower Sweetnam Creek (LSC, exposure area), and during the fall 2005. Values are mean ± 1 SD.

Station	**Units**	**AC**	**NW Trib**	**LSC**
Physical Features				
Wetted channel width	m	8.5 ± 3.2	1.7 ± 1.0	2.0 ± 0.8
Mean depth	m	0.15 ± 0.03	0.16 ± 0.04	0.17 ± 0.02
Mean current velocity	m/s	0.26 ± 0.05	0.51 ± 0.12	0.70 ± 0.12
Substratum				
Sand/silt/clay (<2 mm)	%	6 ± 2	12 ± 7	16 ± 12
Gravel (2-64 mm)	%	30 ± 7	45 ± 9	50 ± 30
Cobble (64-256 mm)	%	59 ± 4	43 ± 13	33 ± 31
Boulder (>256 mm)	%	5 ± 6	0 ± 0	0 ± 0

Population Distributions

Length-frequency analysis was used to determine whether there was an effect on the survival of fish between lower Sweetnam Creek (exposure area) and Allin Creek (reference area). The length-frequency data were plotted for 395 fish from the exposure area compared to 177 fish from the reference area (Figure 1). Alpha-values for the K-S test were set at 0.10 to be consistent with other hypothesis-testing. Results from the K-S test indicate there was a significant difference in the population distributions between the two areas ($p<0.0005$) and is likely due to the higher proportion of YOY in the exposure area (LSC, see Figures 1, 2).

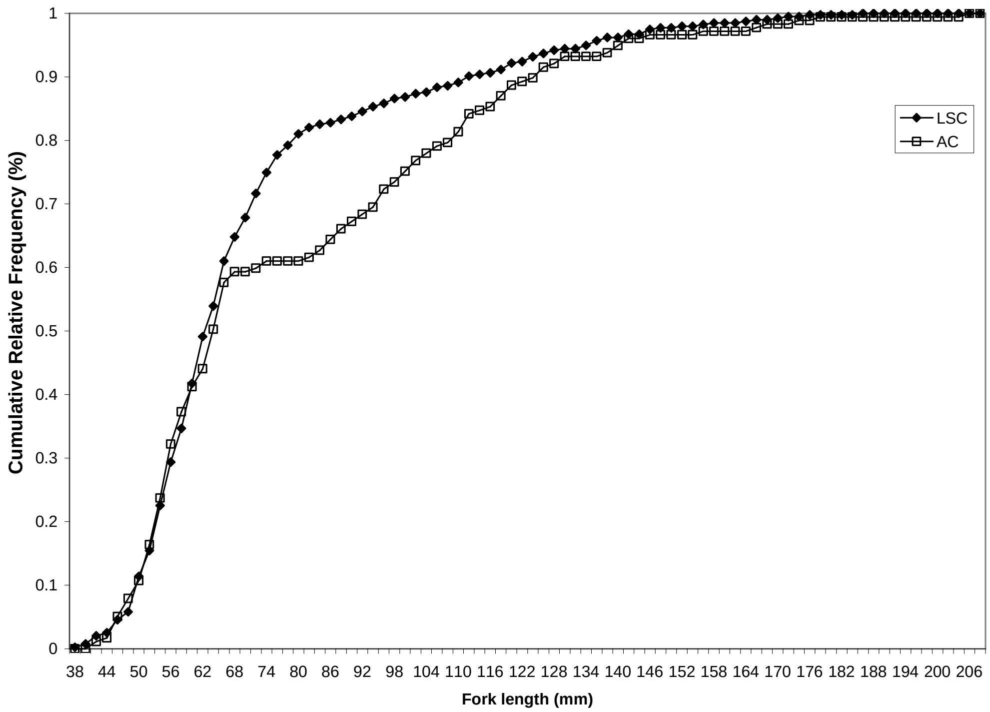

Figure 1. Cumulative length frequency distributions for juvenile rainbow trout collected from the exposure area, lower Sweetnam Creek (LSC; solid black diamonds) and the reference area, Allin Creek (AC; open circles).

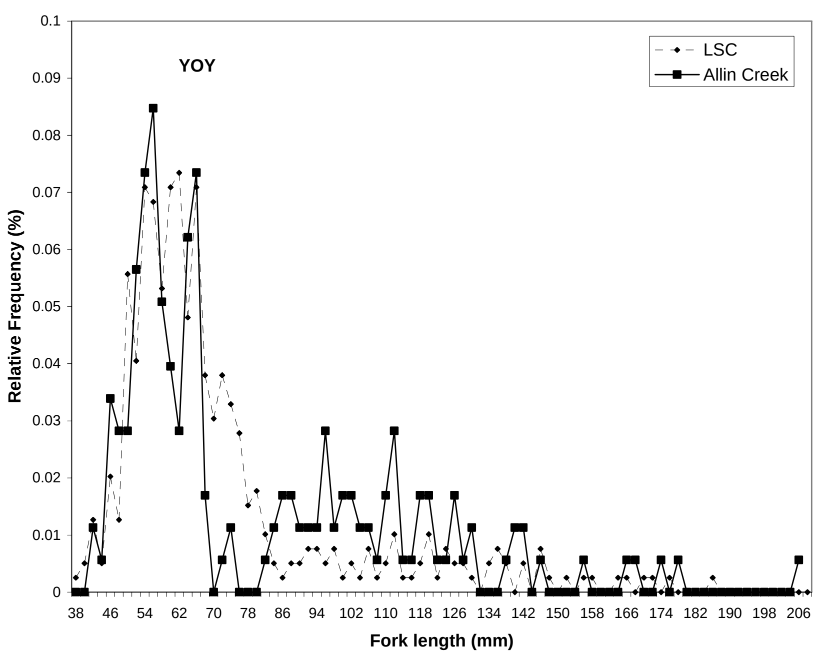

Figure 2. Length-frequency distributions for juvenile rainbow trout collected from the exposure area, lower Sweetnam Creek (LSC; dotted line) and the reference area, Allin Creek (AC; solid line).

Growth

Young-of-the-year rainbow trout from LSC were significantly longer ($p<0.0005$) and heavier ($p<0.0005$) relative to fish from AC (Table 3). Similarly, older (1^+) rainbow trout from LSC were significantly longer ($p=0.058$) and heavier ($p=0.030$) when compared to fish from Allin Creek (Table 3). Taken together, fish in LSC (the exposure area) exhibited increased growth relative to fish from the reference area (AC). Increased growth is a common response in fish collected downstream of some wastewater effluent discharges. A national assessment of data collected for the pulp and paper EEM program showed increased fish growth was a predominant response observed across the country and is likely due to a combination of increased food abundance and water temperature (Environment Canada 2003). Interestingly, benthic invertebrate density was higher in the exposure area (lower Sweetnam Creek) than in the reference areas (see Figure 5A). Water temperature recorders were placed in the exposure and reference areas, but they have not been retrieved yet. Reduced ice-cover in lower Sweetnam Creek suggests water temperatures are higher in the exposure area.

Table 3. Characteristics of juvenile rainbow trout collected from the exposure area, lower Sweetnam Creek (LSC) and the reference area, Allin Creek (AC) during the fall, 2005.

Age Class	Site	Maximum size of YOY (mm)	% of sample	Mean length (± SE, N) (mm)	Mean weight (± SE, N) (g)
YOY	LSC	85	83	61 ± 0.5 (327) A	2.9 ± 0.08 (327) A
	AC	81	62	57 ± 0.7 (109) B	2.3 ± 0.08 (109) B
Older fish	LSC	n/a		122 ± 3 (68) A	23 ± 2 (68) A
	AC	n/a		114 ± 3 (68) B	19 ± 2 (68) B

Reproduction

Reproductive performance is estimated from the proportion of the population that is represented by YOY individuals. Results indicate reproduction and survival of young trout was not adversely affected in the exposure area, since the relative proportion of YOY was greater in LSC than in AC (Table 3). Catch-per-unit-effort (CPUE) data provided additional evidence that reproduction was not adversely affected in the exposure area. In fact, about 3 times more fish were captured for every 1000 seconds of backpack electrofishing in LSC than AC (Figure 3).

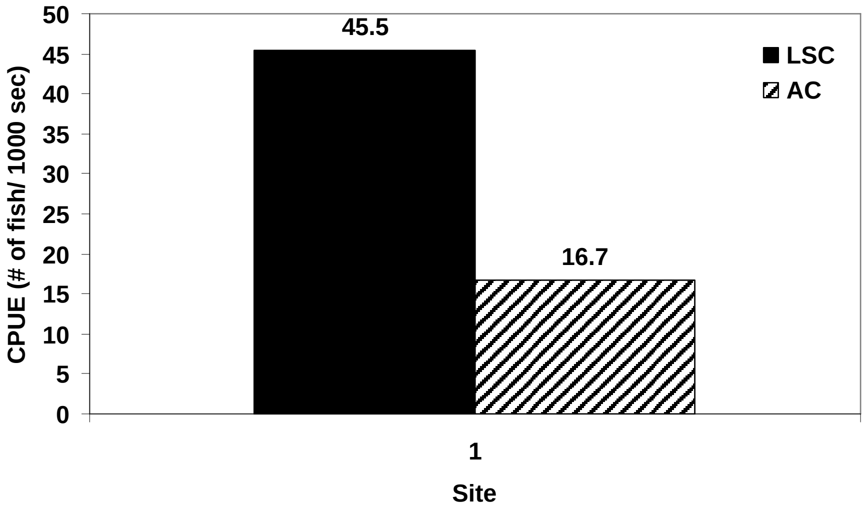

Figure 3. Catch-per-unit-effort (CPUE) for juvenile rainbow trout collected from the exposure area, lower Sweetnam Creek (LSC) and reference area, Allin Creek (AC) during the fall 2005.

Energy Storage

Condition factor is used within the EEM program, along with relative liver size, to give an indication of energy storage. Since liver size cannot be measured non-lethally, only condition can be used as an estimate of energy storage for fish that are live-released. While condition factor serves as a summary estimate, statistical comparisons must be made as an ANCOVA of fork length versus total body weight by site. There were no statistically significant site differences in the condition of juvenile rainbow trout ($p=0.24$; Figure 4).

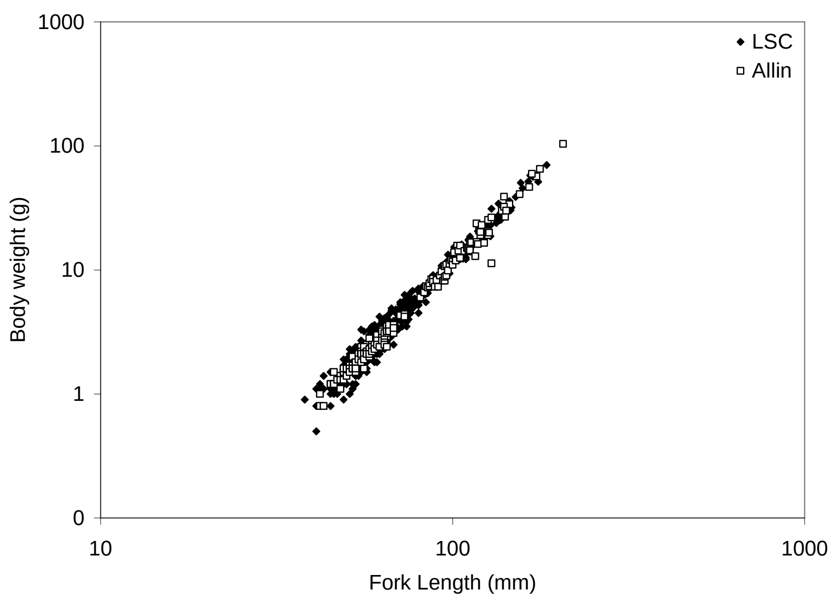

Figure 4. Condition ($\log_{10}$ fork length versus $\log_{10}$ body weight) for all of the juvenile rainbow trout collected in the fall 2005 from the exposure area, lower Sweetnam Creek (LSC) and reference area, Allin Creek (AC).

Benthic Invertebrate Community

Total benthic invertebrate density was significantly greater in lower Sweetnam Creek (LSC; exposure area) compared to Allin Creek (AC) (p=0.032) and the northwest tributary to North Un-named Creek (NW Trib; a naturally metal-rich reference area) (p=0.021) (Figure 5A). It is important to note that total density was similar (p=0.126) between LSC and the NW Trib when an outlier in the NW Trib dataset was included in the analyses. Richness and Simpson's Diversity Index at LSC were similar compared to the metal-rich reference area (p=0.81 and p=0.35, respectively), but were significantly reduced when compared to AC (p<0.001 and p=0.033, respectively) (Figures 5B, 5C). Bray-Curtis similarity indices indicated invertebrate taxonomic composition at LSC was significantly different when compared to AC (p=0.039) and NW Trib (p<0.001) (Figure 5D). There were obvious differences in community composition among areas, even at the level of major invertebrate groups (Figure 6).

The differences in community structure among areas were likely due to habitat differences, rather than exposure to mine effluent. The direction of the difference in density among areas was inconsistent with an adverse effect on the benthic community in LSC. Richness and diversity in LSC were not significantly different from one of the reference areas (NW Trib). In addition, the difference in BCI between the two reference areas was larger than the difference between LSC and NW Trib. There were habitat differences among areas that were sufficiently large to account for the at least some of the significant differences among areas. For example, Allin Creek is a larger stream than lower Sweetnam Creek and North Un-named Creek, and the variation in current velocity among areas was relatively large.

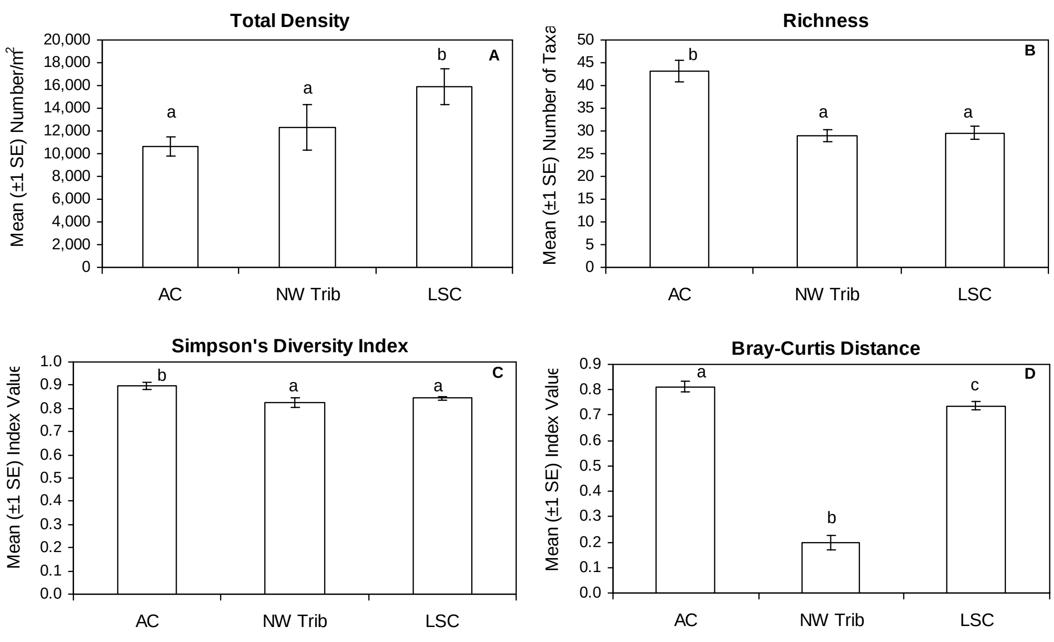

Figure 5. Comparison of invertebrate community end-points (Total density [A], Richness [B], Simpson's Diversity Index [C], Bray-Curtis Distance [D]) of benthic invertebrates collected during the fall 2005 from Allin Creek (AC), northwest tributary to North Un-named Creek (a naturally metal-rich reference area, NW Trib), and lower Sweetnam Creek (LSC). Different letters indicate a difference between the exposure area and each reference area.

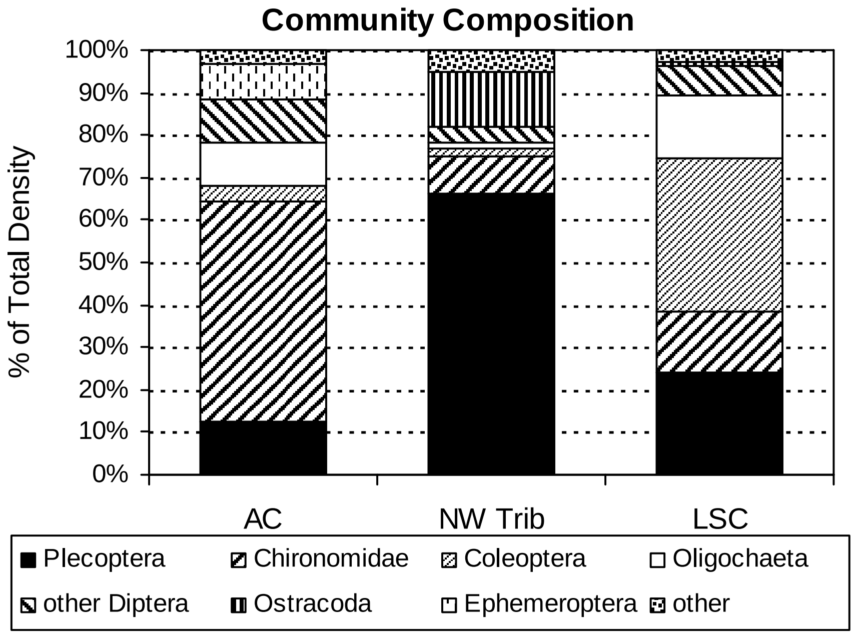

Figure 6. Benthic invertebrate community composition in Allin Creek (AC), northwest tributary to North Un-named Creek (a naturally metal-rich reference area, NW Trib), and lower Sweetnam Creek (LSC) during the fall 2005.

PRELIMARY CONCLUSIONS

- Trout collected from the exposure area (lower Sweetnam Creek) exhibited increased growth relative to fish collected from the reference site, Allin Creek. The differences were likely due to increased food abundance (i.e., benthic invertebrates) in the exposure area. Increased water temperatures probably play a role, but this will need to be confirmed.
- Reproduction and survival were not adversely affected in rainbow trout collected from the exposure area.
- The condition or “well-being” of juvenile rainbow trout is similar in the exposure and reference areas.
- Lower Sweetnam Creek had more YOY and fewer older age/size classes than Allin Creek, which is likely due to habitat differences. Mean wetted channel width of Allin Creek is 8.5 m where as mean wetted channel width of LSC is 2 m. Due to limited habitat and faster growth of fish in the exposure area, it is likely that older/larger trout from the exposure area move into the Francois Lake to continue to grow. This would need to be confirmed through additional studies.
- Benthic invertebrate community end-points (total density, richness, Simpson’s Diversity Index) in the exposure area were similar to the metal-rich reference area, but not Allin Creek, suggesting differences were likely due to habitat differences, rather than exposure to mine effluent.
- Bray-Curtis similarity indices indicated differences in community composition among areas, which were likely due to habitat differences, not exposure to mine effluent. Multivariate analyses will be done in an attempt to further characterize these differences and evaluate their potential causes.

- There are several challenges associated with designing and conducting environmental monitoring programs for existing and future mining operations in this area of British Columbia. Many creeks are ephemeral or have intermittent flows making it difficult to select suitable reference sites. Reconnaissance surveys are often required, since most small creeks have never been surveyed and baseline data required to define the system is not available, such as: geology, hydrogeology, industrial developments, physical structure, water chemistry, and resident biota. Selecting a suitable sentinel fish species is a key component of many monitoring programs. The key to selecting a suitable species is to understand the characteristics of the study site, determine what species and life-stage are available, and then decide on a scientific basis what makes most sense to examine.
- Overall, effluent from the Endako molybdenum mine discharged into lower Sweetnam Creek is not causing adverse effects on the survival, condition, growth, or reproductive performance of resident rainbow trout or benthic invertebrate communities.

REFERENCES

Endako Mines. 2003. http://www.endakomines.com/

Environment Canada. 2002. Metal mining guidance document for aquatic environmental effects monitoring. EEM/2002. June 2002.

Environment Canada. 2003. National assessment of pulp and paper environmental effects monitoring data: a report synopsis. National Water Research Institute, Burlington, Ontario. NWRI Scientific Assessment Report Series No. 2. 28p.

Gray, M.A., Curry, R.A., and Munkittrick, K.R. 2002. Non-lethal sampling methods for assessing environmental impacts using a small-bodied sentinel fish species. Water Quality Res J Can 37: 195-211.

Munkittrick, K.R., McMaster, M.E., Van Der Kraak, G., Portt, C., Gibbons, W.N., Farwell, A., and Gray, M. 2000. Development of methods for effects-driven cumulative effects assessment using fish populations: Moose River Project. SETAC Press, Pensacola, FL, USA.

Sokal, R.R., and Rohlf F.J. 1995. Biometry: the principles and practice of statistics in biological research. WH Freeman & Company, New York.