6.3.3.1 Arctic Grayling

Preliminary examinations of Arctic grayling data indicated that there were no significant differences in length or weight between sexes, therefore data were pooled across sexes for all analyses.

6.3.3.1.1 General Health

In total, 36 Arctic grayling from the reference area and 42 Arctic grayling from the exposure area were sacrificed. External and internal health examinations were conducted on each of these fish. The majority of the fish from both areas did not have any recordable abnormalities; however, one fish from the exposure area had severe fin erosion, and one fish from the exposure area had abnormal liver texture.

6.3.3.1.2 Summary Statistics

Summary statistics were calculated for fork length, weight, liver weight, and carcass weight for each area (Table 6.5). Only fish that were included in the statistical assessment of energy storage and energy use were included (i.e., age 1 fish only).

Table 6.5. Summary statistics for age 1 Arctic grayling

Area	Variable	*n*	Median	Mean	SD	SE	Minimum	Maximum
Exposure	Fork length (mm)	51	101	103	9.29	1.3	86	122
	Total weight (g)	47	10.43	11.3	3.25	0.47	7.06	19.62
	Carcass weight (g)	37	7.37	8.15	2.54	0.42	4.89	13.37
	Liver weight (g)	37	0.12	0.12	0.04	0.01	0.05	0.2
Reference	Fork length (mm)	31	111	110	3.28	0.48	92	123
	Total weight (g)	31	17.34	16.39	3.53	0.64	8.2	22.88
	Carcass weight (g)	29	13.23	13.12	3.15	0.59	6.69	18.87
	Liver weight (g)	29	0.17	0.16	0.04	0.01	0.09	0.24

Note: *n*=sample size; SD=standard deviation; SE=standard error

6.3.3.1.3 Energy Use

Total body weight and length of age 1 Arctic grayling were statistically different between the two study areas (*t*-test, $P<0.1$; Table 6.6). Age 1 Arctic grayling weighed 31.0% more and were 6.8% longer in the reference than in the exposure area (Table 6.6; Figure 6.3).

Table 6.6. Summary of statistical analyses for Arctic grayling

Type of Response	Endpoint	Endpoint Effect or Support Analysis	Primary Analysis				
			Interaction Statistic *P*	Intercept Statistic *P*	SSD	Direction of Difference	Magnitude of Difference (%)
Energy Use	Total Body Weight	Support	n/a	<0.0001	yes	ref<exp	31.0
	Length	Support	n/a	0.0004	yes	ref<exp	7.0
Energy Storage	Condition	Effect	0.30	<0.0001	yes	ref<exp	7.5
	Relative liver weight (liver weight-at-carcass weight)	Effect	0.14	0.29	no		
	Relative liver weight (liver weight-at-fork length)	Support	0.84	0.01	yes	ref<exp	9.5

Note: SSD=statistically significant difference; statistical significance assessed at $P \leq 0.1$

6.3.3.1.4 Energy Storage

Condition of age 1 Arctic grayling, as determined by comparing the length-weight relationships, was significantly different between the exposure and reference areas (Table 6.5; Figure 6.4). The interaction term was not significant, indicating that the slopes were similar between reference and exposure areas; however, the intercept was significantly higher in the reference area than in the exposure area. This indicated that, for a given length, fish from the reference area were heavier than those from the exposure area. The condition of age 1 Arctic grayling was 7.5% higher in reference fish than exposure fish (Table 6.5). The linear length-weight relationships were described by the following equations, where W is total body weight in grams and L is fork length in millimetres:

Reference area: $\text{Log}_{10}\ W = -4.99\ \text{Log}_{10}\ L + 3.03;\ r^2 = 0.88;\ n = 29$

Exposure area: $\text{Log}_{10}\ W = -4.26\ \text{Log}_{10}\ L + 2.64;\ r^2 = 0.77;\ n = 48$

The slope and intercept for the relationship of liver weight-at-carcass weight were not significantly different ($P>0.01$) between reference and exposure areas (Table 6.5, Figure 6.5). The linear liver weight-at-carcass weight relationships were described by the following equations, where Liver is liver weight in grams and Carcass is carcass weight in grams:

Reference area: $\text{Log}_{10}\ \text{Liver} = -1.51\ \text{Log}_{10}\ \text{Carcass} + 0.65;\ r^2 = 0.53;\ n = 29$

Exposure area: $\text{Log}_{10}\ \text{Liver} = -1.75\ \text{Log}_{10}\ \text{Carcass} + 0.91;\ r^2 = 0.61;\ n = 37$

Figure 6.3. Length-frequency distribution for age 1 Arctic grayling in the exposure and reference areas

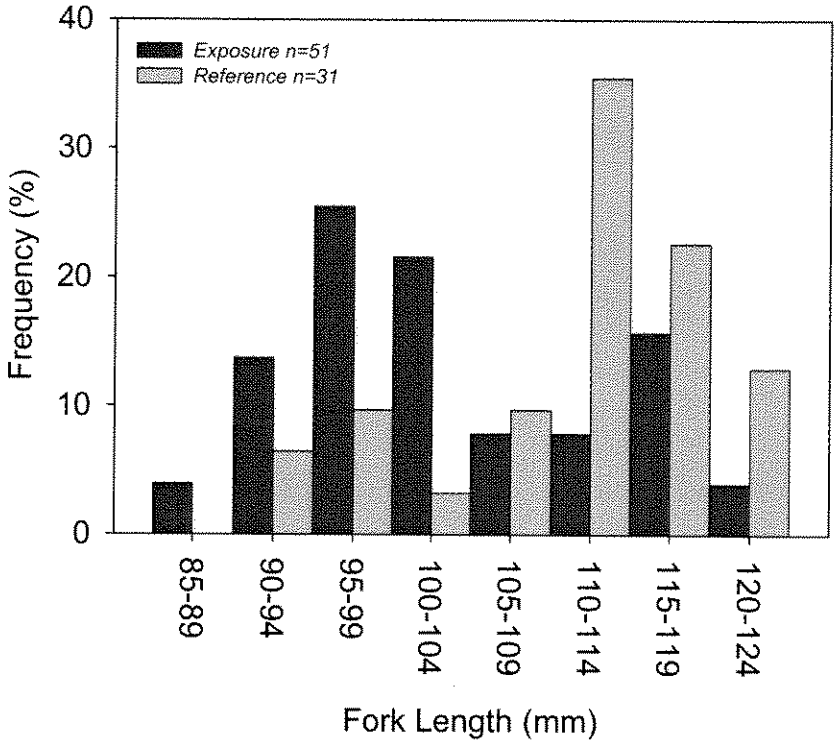
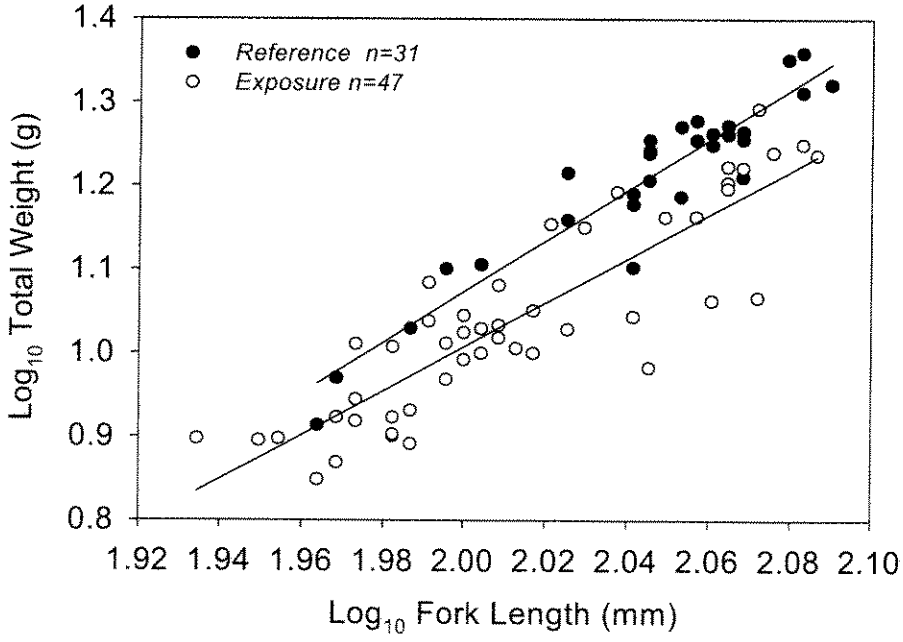


Figure 6.4. Relationship of Artic grayling total weight to fork length (condition)



The interaction term was not significant ($P>0.1$)for the GLM of liver weight-at-fork length, indicating that the slope of the regressions were similar between reference and exposure areas; however, the intercept was significantly higher in the reference area than in the exposure area (Table 6.5; Figure 6.6). The covariate was significant, though the r^2 values were very low, which suggests that the relationships between liver weight and fork length were weak. For a given length, fish from the reference area have 9.5% larger livers than those from the exposure area. The linear liver weight-at-length relationships were described by the following equations, where Liver is liver weight in grams and Length is fork length in millimetres:

Reference area: $\text{Log}_{10}\text{ Liver} = -5.30\ \text{Log}_{10}\text{ Length} + 2.20;\ r^2 = 0.48;\ n = 29$

Exposure area: $\text{Log}_{10}\text{ Liver} = -5.04\ \text{Log}_{10}\text{ Length} + 2.03;\ r^2 = 0.26;\ n = 37$

Figure 6.5. Relationship of Arctic grayling liver weight-at-carcass weight

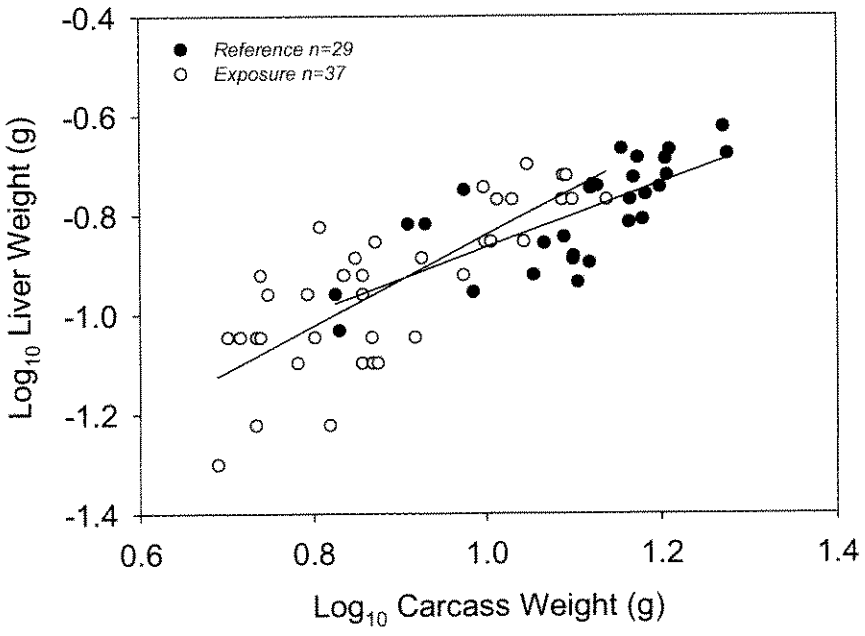
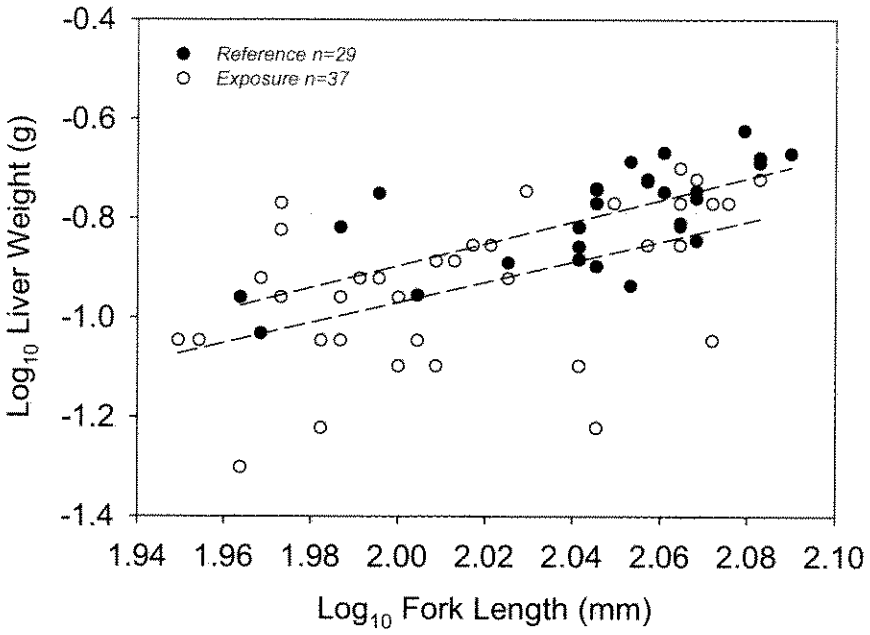


Figure 6.6. Relationship of Artic grayling liver weight-at-fork length



Note: dashed lines are used to represent weak regression relationships

6.3.3.1.5 Power Analysis

Currently, the MMER EEM program does not specify a critical effect size for fish endpoints (EC 2002); thus, the power of this study and the sample size required to detect a range of effect sizes was calculated for α and β equal at 0.05 and 0.10. The data in this study had sufficient power (greater than 90%) to detect a change of 10% in length, 20% in weight, 5% in condition and 10% in relative liver weight (Table 6.7).

6.3.3.2 Ninespine Stickleback

Despite considerable effort, only one ninespine stickleback was captured in the reference area. In total, 99 ninespine stickleback were captured in the exposure area. The lengths and weights were recorded for all captured fish, and internal and external examinations were conducted on the first 30 captured fish to assess abnormalities and parasite load. Though statistical analyses could not be conducted, data for the exposure area are presented here as background data for possible comparison in future monitoring programs.

Table 6.7. Power analysis for Arctic grayling.

Endpoint	Critical Effect Size	Estimated Power		Required *n* for desired power	
		α=β=0.1	α=β=0.05	1-β=0.9	1-β=0.95
Length	5%	0.7280	0.6105	52	79
	10%	0.9941	0.9979	14	21
	15%	1.0	1.0	7	10
Weight	5%	0.1971	0.1183	446	676
	10%	0.4539	0.3302	112	170
	15%	0.7408	0.6254	51	77
	20%	0.9206	0.8598	29	44
	25%	0.9851	0.9670	19	29
	30%	0.9983	0.9953	14	20
	35%	1.0	1.0	10	15
Condition	5%	1.0	1.0	11	13
Liver (at carcass weight)	5%	0.6450	0.5197	58	86
	10%	0.9950	0.9881	15	22
	15%	1.0000	1.0000	7	10

Note: *n*=sample size per area; critical effect size is a percentage of the reference mean; 1-β = power

6.3.3.2.1 General Health

The majority of the 30 sacrificed fish did not have any recordable abnormalities. Of the abnormalities that were recorded, one fish had severe skin aberrations, one fish had a small lesion on the opercule, one fish had a cream coloured liver, one had cysts on the liver, and low parasite loads were recorded in six fish.

6.3.3.2.2 Summary Statistics

Summary statistics were calculated for fork length, weight, liver weight and carcass weight for the exposure area (Table 6.8).

Table 6.8. Summary statistics for ninespine stickleback captured in the exposure area

Variable	*n*	Median	Mean	SD	SE	Minimum	Maximum
Total length (mm)	99	55	56	5.184	0.521	43	74
Total weight (g)	99	1.02	1.08	0.326	0.033	0.65	2.72
Carcass weight (g)	17	0.04	0.04	0.043	0.011	0.02	0.20
Liver weight (g)	17	0.52	0.60	0.238	0.060	0.34	1.23

Note: *n*=sample size; SD=standard deviation; SE=standard error

6.3.3.2.3 Energy Use and Energy Storage

The mean length (total length) of ninespine stickleback in the exposure area was 56 mm (range 43 to 74 mm), and the mean weight was 1.08 g (range 0.65 to 2.72 g). The length-frequency distribution shows that 86% of ninespine stickleback were between 50 and 64 mm (Figure 6.7).

The condition of ninespine stickleback in the exposure area was assessed using linear regression analysis on $\log_{10}$-transformed length and weight data (Figure 6.8). Three outliers were removed from the regression analysis due to errors in data recording. The length-weight relationship was described by the following equation, where W is total body weight in grams and L is total length in millimetres:

$$\text{Log}_{10}\, W = -4.17\, \text{Log}_{10}\, L + 2.40;\ r^2 = 0.80;\ n = 96$$

Relative liver weight was also assessed using linear regression analysis on the $\log_{10}$-transformed data. There was no relationship found between liver weight and carcass weight (r^2=0.003) or liver weight and total length (r^2=0.008).

Figure 6.7. Length-frequency distribution of ninespine stickleback in the exposure area.

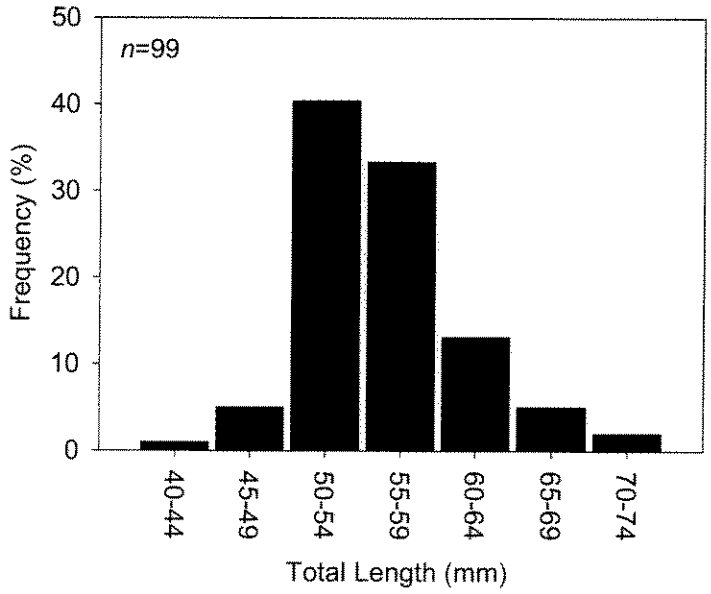
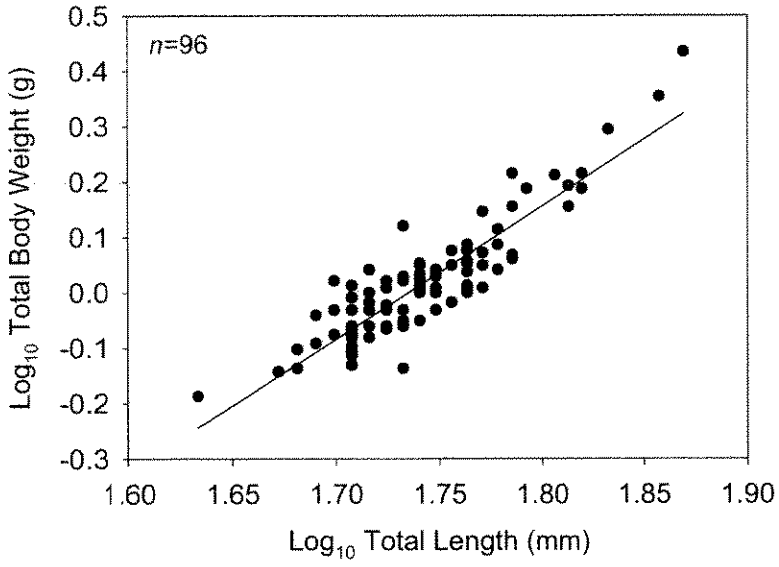


Figure 6.8. Relationship of ninespine stickleback total weight to total length (condition) in the exposure area



6.4 Summary

Fish were collected from a variety of habitats, including streams, lakes and ponds, in an attempt to increase fish capture. Conductivity was higher in the exposure area, which suggested the presence of mine effluent. Nitrate and ammonia were elevated in the exposure area, particularly in the stations nearest to the FDP. In the exposure area, aluminum, arsenic, cadmium, copper, lead, nickel and zinc concentrations exceeded the CWQG for the protection of aquatic life.

Overall, the state of general health (i.e., occurrence of abnormalities and parasites) of Arctic grayling was similar between the Fingers Lake system (reference) and the Seep Creek system (exposure).

Age 1 Arctic grayling were 31.0% heavier and 6.8% longer , on average, in the reference area than in the exposure. Condition was 7.5% greater in the reference fish than in exposure fish. Liver weight-at-carcass weight was not statistically different between reference and exposure fish. Although not considered an effect endpoint, the liver weight-at-fork length was 9.5% lower in exposure fish than in reference fish. Increased liver weight for fish exposed to metal pollution has been observed in field studies (Orlando et al. 1999); however, liver weight may decrease in response to exposure to specific metals.

For example, Norris et al. (2000) and Ricard et al. (1998) reported that long-term exposure to cadmium resulted in decreased liver weight and activity.

Power analysis indicated that there was sufficient sample size to detect a change of 10% in length, 20% in weight, 5% in condition and 10% in relative liver weight for Arctic grayling, with 90% power.

Although similar numbers of Arctic grayling in the exposure area were captured by backpack electrofishing and gill nets, all of the Arctic grayling in the reference area were captured by backpack electrofishing. Gill nets tend to be more selective than electrofishing, and are generally more effective at capturing smaller individuals (Hubert 1996). In contrast, electrofishing is generally more effective at capturing larger individuals (Reynolds 1996). Thus, the differences between exposure and reference areas may have been influenced by gear selectivity.

Of the ninespine stickleback that were examined for abnormalities and parasites, 13% had recordable abnormalities and 20% had parasites.

7.0 SUBLETHAL TOXICITY TESTING

Under the MMER regulations, sublethal toxicity tests on the final effluent must be conducted by the Mine twice each calendar year for the first three years; thereafter, the frequency may be reduced to once per calendar year. The purpose of sublethal toxicity testing is to determine if mine effluent may affect fish, invertebrates, and aquatic plants.

Sublethal toxicity test results can be used to estimate the potential for exposure area impacts (EC 2002). The regulations stipulate that if the sublethal toxicity results show an IC_{25} result of less than 30% concentration of effluent, the mine should calculate the geographic extent of the response in the exposure area and identify the zone where the concentration of effluent is comparable to the IC_{25} result. The IC_{25} is the effluent concentration where a 25% inhibition is observed in the exposed test organisms.

Sublethal toxicity test results can also be used to measure changes in effluent quality over time as a result of changes in water treatment, surface water management, acid rock drainage mitigation measures or mine process changes.

7.1 Methods

7.1.1 Field

Grab samples were collected by Mine staff from the final discharge point (SNP 925-10) in July and August of 2005. These samples were shipped to HydroQual Laboratories Ltd. in Calgary, Alberta, for toxicity testing.

7.1.2 Laboratory

The following toxicity tests were performed by HydroQual on each of the effluent samples collected in July and August according to established protocols:

- 7 day *Ceriodaphnia dubia* survival and reproduction (EC 1992a).
- 7 day Fathead minnow (*Pimephales promelas*) survival and growth (EC 1992b).
- 72 hour algal growth inhibition (*Selenastrum capricornutum*; EC 1992c).
- 7 day *Lemna minor* growth (EC 1999).

7.1.3 Data Analysis

The laboratory testing methods and QA/QC procedures are presented in the detailed reports from HydroQual in Appendix B.

7.2 Results

7.2.1 Survival and Growth Tests for the Fathead Minnow

Undiluted mine effluent was not acutely toxic to fathead minnows in either of the two samples tested (Table 7.1). Similarily, sublethal effects (growth) were not observed for the undiluted effluent samples.

Table 7.1. Acute and sublethal toxicity test results for fathead minnows (*Pimephales promelas)*

Date	Laboratory	Survival (LC_{50})	Growth (EC_{25} or IC_{25})
27-Jul-05	HydroQual	>100%	>100%
30-Aug-05	HydroQual	>100%	>100%

LC_{50} = lethal concentration at 50%; EC_{25} = effect concentration at 25%; IC_{25} =inhibition concentration at 25%

7.2.2 Survival and Reproduction of *Ceriodaphnia dubia*

Undiluted effluent was not acutely toxic to *Ceriodaphnia dubia* (Table 7.2). Reproductive impairment (IC_{25}) was observed at effluent concentrations ranging from 4.1% to 15%.

Table 7.2. Acute and sublethal toxicity test results for the water flea (*Ceriodaphnia dubia*)

	Laboratory	Survival (LC_{50})	Reproduction (EC_{25} or IC_{25})	EC_{25} or IC_{25} Lower	EC_{25} or IC_{25} Upper
27-July-05	HydroQual	>100%	>100%	n/a	n/a
30-Aug-05	HydroQual	>100%	12%	4.1%	15%

Notes: n/a=not applicable

7.2.3 Growth Inhibition Test for *Selanstrum capricornutum*

Undiluted effluent affected the growth of *Selanstrum capricornutum*. In July and August 2005, effluent concentrations of 17% and 27%, respectively, were found to inhibit growth in 25% of the test population (Table 7.3).

Table 7.3. Sublethal toxicity test results for *Selanstrum capricornutum*

Date	Laboratory	Growth (EC_{25} or IC_{25})	EC_{25} or IC_{25} Lower	EC_{25} or IC_{25} Upper
27-Jul-05	HydroQual	17%	15%	19%
30-Aug-05	HydroQual	27%	22%	32%

7.2.4 Growth and Reproduction of *Lemna minor*

Undiluted effluent collected from the mine in July and August 2005 demonstrated sublethal toxicity to *Lemna minor* (Table 7.4). The tests showed growth inhibition occurring at 10% (July) and 51% (August). Reproductive impairment was observed at effluent concentrations of <6.1% (July) and 7.8% (August).

Table 7.4. Sublethal toxicity test results for *Lemna minor*

Date	Laboratory	Growth (EC_{25} or IC_{25})	EC_{25} or IC_{25} Lower	EC_{25} or IC_{25} Upper	Reproduction (EC_{25} or IC_{25})	EC_{25} or IC_{25} Lower	EC_{25} or IC_{25} Upper
27-Jul-05	HydroQual	10%	3.5%	71%	<6.1	n/a	n/a
30-Aug-05	HydroQual	51%	n/a	n/a	7.8%	1.5%	12%

Notes: n/a=not applicable

7.3 Summary

While the biological surveys provide the direct measurement of ecological effects (if any), toxicity tests serve as a useful tool for predicting potential biological effects. However, predicting ecological significances in the field of a potential sub-lethal effect zone from single-species laboratory tests is difficult, at best, given the tools currently available under the EEM program. Laboratory measurements of physical, chemical, or biological properties of a substance or mixture cannot predict its exact behaviour in the

more variable and complicated environment found in nature. Translating potential (laboratory) into actual (field) toxicity requires consideration of the environmental factors that influence the expression of toxicity in the field. These confounding factors include:

- the nature of the toxicant;
- the chemical and physical environment,
- the timing and duration of exposure;
- the transport and fate mechanisms; and,
- the similarity of the test organisms to the organisms in the field.

All of these factors must be considered when toxicity data are used to estimate potential effect zones. Caution must be exercised when trying to extrapolate results from the laboratory to the receiving environment.

Sublethal and acute effects of mine effluent were not detected for fathead minnow. Effluent was not acutely toxic to *Ceriodaphnia dubia*, although reproductive inhibition was recorded at 12% effluent in August 2005. *Selenastrum capricornutum* growth was inhibited in effluent concentrations greater that 17%, and *Lemna minor* growth and reproduction were inhibited at concentrations greater than 10% and 6.1%, respectively.

The plume delineation modeling results (Section 2.7; Golder 2004) estimate that the 1% effluent zone could extend up to 1630 m into Outer Sun Bay. At the narrows between Inner and Outer Sun bays, the estimated effluent concentration ranged from 5.9 to 33%, depending on the stream and effluent flows. The sublethal toxicity test results indicate that biological effects may be detected at effluent concentrations as low as 6.1%. Based on the effluent plume model results and the sublethal toxicity results, the potential zone of biological effects could extend from the final discharge point downstream to the narrows, or Outer Sun Bay of Contwoyto Lake under worst case effluent mixing scenarios.

8.0 SYNOPSIS

The overall objective of the MMER EEM biological monitoring program was to assess whether or not the effluent from Lupin Mine had a negative effect on the aquatic receiving environment. The biological monitoring survey included a benthic invertebrate survey and fish survey. Fish tissue analysis was not conducted, but samples have been archived for examination if required by Environment Canada.

Effluent was discharged between 15 July and 11 August 2005. Effluent quality did not exceed the maximum allowable concentrations for deleterious substances as listed under the MMER. Effluent was not acutely lethal to fathead minnows or *Ceriodaphnia dubia*. Sublethal effects on growth and reproduction were recorded for *Ceriodaphnia dubia, Selenastrum capricornutum,* and *Lemna minor* in effluent concentrations ranging from less than 6.1% to 51%. Based on these effluent concentrations and the plume delineation model, the potential zone of biological effects could extend from the FDP as far as Outer Sun Bay.

Water quality variables were generally elevated in the exposure area relative to the reference area. Aluminum, arsenic, cadmium, copper, lead, nickel and zinc exceeded the CWQG in the exposure area.

Sediment quality variables were also elevated in the exposure area relative to the reference area. Arsenic exceeded the ISQG in the exposure area and in one reference sample. Chromium exceeded the ISQG in two of the five exposure area samples.

Benthic invertebrate communities in the exposure area have been negatively affected by Mine effluent. Statistically significant differences between the reference and exposure areas were not detected for total invertebrate density, family richness, or BCI. SDI and evenness were significantly lower in the exposure area than in the reference area. These impacts may be related to the elevated arsenic and chromium concentrations, which exceeded the ISQG for the protection of aquatic life, or the elevated metal concentrations in the water column, including several metals that exceeded CWQG for the protection of aquatic life.

Arctic grayling in the exposure area have been negatively impacted by Mine effluent. The general health (i.e., abnormalities and parasites) were similar between reference and exposure areas; however, Arctic grayling were heavier, longer and in better condition in the reference area compared to the exposure area. Liver weight relative to carcass weight was not significantly different between reference and exposure fish. Although not

considered an effect endpoint, liver weight relative to fork length was greater in reference fish than in exposure fish. Decreased liver weight may be related to exposure to elevated concentrations of specific metals, such as cadmium (Norris et al. 2000; Ricard et al. 1998).

Results of the present study indicated that the benthic invertebrate community and the fish population have been affected by mine effluent. Therefore, Lupin Mine is required to continue with the subsequent phase of EEM, Periodic Monitoring – Surveillance.

9.0 RECOMMENDATIONS FOR SUBSEQUENT BIOLOGICAL MONITORING

Based on the results obtained from the Initial EEM Biological Monitoring program, the MMGD (EC 2002) suggests the appropriate subsequent EEM study would be a Periodic Monitoring – Surveillance study. Table 9.1 outlines the schedule of the second EEM program at Lupin mine.

Table 9.1. Schedule of the second Lupin EEM program

Component	Execution/Submission Date
EEM Study Design	6 months prior to the biological monitoring field program (2008)
Field program	Late Summer 2008
Second Interpretive Report	06-Jun-09

In addition to the information included in the First EEM Study Design, the second study design will include a summary of the results from the First EEM Biological Monitoring Program. Fingers Creek has provided appropriate data for use as a reference comparison and should remain as the reference area for the second EEM program.

Although adequate biological information was collected in the Initial EEM Biological Monitoring Program to determine if there was an effect, it is recommended that the following changes be made to the EEM study design:

- standardize fishing gear use between reference and exposure areas to eliminate the potential confounding influence of differential size-selectivity between gear types;
- increase efforts to capture ninespine stickleback in the reference area;
- sample fish when the Mine is discharging effluent into the exposure area if possible; and,
- sample the BIC when the Mine is discharging effluent into the aquatic receiving environment, if possible.

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11.0 CLOSURE

This report was prepared by Golder Associates Ltd. (Golder) on behalf of Kinross Gold Corporation. We trust that the information provided meets the requirements of the MMER submission for the Lupin Mine. Should there be any further requirements or clarification, please do not hesitate to contact the undersigned individuals.

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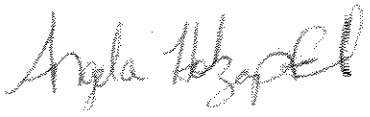

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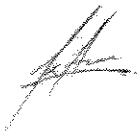

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