National Assessment of Phase 1 Data from the Metal Mining Environmental Effects Monitoring Program

R.B. Lowell, C. Tessier, S.L. Walker, A. Willsie, M. Bowerman, and D. Gautron

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Executive Summary

Under the *Fisheries Act*, the 2002 *Metal Mining Effluent Regulations* (MMER) require the owners or operators of metal mines to conduct environmental effects monitoring (EEM) to assess effects potentially caused by mine effluents. The EEM Program focuses particular attention on biological monitoring studies comprised of the following three core components: a fish population survey to assess fish health, a benthic invertebrate community survey to assess effects on fish habitat, and a study of mercury levels in fish tissue to assess effects on the usability of fisheries resources. These studies are used to evaluate the effects of metal mining effluents on the environment and the adequacy of regulated limits for environmental protection. Study results determine the need for more focused monitoring or further investigation at some mine sites. To aid in interpreting effects at individual mines, supporting measurements (including effluent characterization, water and sediment quality monitoring, and sublethal toxicity) are taken to contribute to the program in areas such as assessing effluent quality and field conditions at individual mines.

The Metal Mining EEM Program is structured into "phases", whereby a mine conducts an EEM study every two to six years. The EEM Program uses a tiered approach to monitoring, where standard periodic monitoring is followed by targeted or focused studies to determine the extent, magnitude, and cause of effects where effects are detected and confirmed or by a reduced level of monitoring where effects are not found. The purpose of this report is to present and discuss the major findings, at a national scale, of the three core components of the EEM Program, using data collected from the receiving environments of metal mines across Canada for Phase 1 of the program. More detailed information, including supporting measurements and local site-specific considerations, are available in the Phase 1 interpretive reports submitted in 2005 and 2006 by each mine.

A series of complementary approaches were used to provide a national assessment of measured effects. These included tabulations of the results of individual mine comparisons for each of the major fish and invertebrate endpoints. In addition, meta-analyses, together with bivariate and multivariate plotting and analyses, were used to investigate national patterns of effects. Response patterns were further evaluated with respect to the potential influence of habitat, ore type, fish gender and species, effluent concentration, and continuous versus intermittent effluent discharge.

At a national scale, when effects were measured, they tended to be more inhibitory than stimulatory, although both kinds of effects occurred. Several lines of analysis showed this. For fish, meta-analyses revealed significant reductions in condition and relative liver size when looking at all mines nationally. For benthic invertebrates, meta-analyses across all mines showed significant reductions in density and taxon richness, contributing to significant changes in community structure, as measured by the Bray-Curtis and Simpson's evenness endpoints. For both fish and invertebrates, these conclusions were further reinforced by inspection of the national distribution of measured effects shown in the endpoint histograms, as well as the multivariate and bivariate analyses.

The tendency for more inhibitory effects was particularly evident when comparing these results with the one other industry (pulp and paper) that has been studied at this scale in Canada. Similar analyses of pulp and paper EEM data have repeatedly revealed more stimulatory effects at a national scale, such as significant increases in fish condition, growth rate, and relative liver size, as well as increases in benthic invertebrate density, although metabolic disruption in gonad growth was also observed. For pulp and paper mills, these stimulatory effects are thought to result from the input of excess nutrients into receiving waters. The greater incidence of inhibitory effects at metal mines could be due to a variety of causes, ranging from the direct effects of toxicity and habitat alteration to indirect effects such as food limitation and dietary toxicant exposure due to effluent effects on prey organisms.

It should be noted, however, that the metal mining industry is fairly heterogeneous, and analyses of smaller subgroupings of data help to provide a more complete picture of mine effluent effects. For example, for fish, condition and relative liver size were significantly reduced in lake habitats and for precious metal, uranium, and/or ferrous mine effluents. On the other hand, relative gonad size was significantly increased in river habitats, together with a nearly significant increase for relative liver size, showing the influence of habitat type on response patterns. Effects were also influenced by fish gender and species. Benthic invertebrates showed a similar diversity of effects. For example, reductions in density and taxon richness were more pronounced in lake habitats and for ferrous mines than for most other subgroupings. The fish and benthic invertebrate responses were consistent with one another.

As expected, greater effects on benthic invertebrates were observed at higher concentrations of effluent in the receiving environment. Nevertheless, the concentration of effluent only accounted for a small proportion of the heterogeneity in measured effects, indicating that concentration did not have an overwhelming influence on the magnitude of effects. None of the nine fish and invertebrate core endpoints was significantly correlated with the number of months during which mines discharged during the year. Thus, effluent effects did not appear to be greatly influenced by the degree to which mines discharge intermittently versus continuously.

Only one mine detected tissue mercury levels greater than the 0.45 µg/g "effect" level (as defined in the MMER) in exposure area fish and significantly greater than reference area levels. Thus, based on the EEM findings so far, the available data do not suggest that metal mine effluents were broadly linked to high mercury levels in fish tissue.

Given that the metal mining EEM Program is still relatively new, these findings represent a preliminary look at mine effluent effects in Canada. Although a substantial amount of data for a large number of mines is summarized in this report, these data represent one point in time. Further rounds of data collection will measure how constant or variable these response patterns are through time. For example, future EEM studies will help determine where effluent effects are improving, worsening, or staying the same in terms of both individual mines, as well as larger groupings of mines. Thus, as ongoing EEM data collection progresses, future analyses are expected to provide a more comprehensive picture of metal mining effluent effects in Canada.

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1.0 Introduction

1.1 The Metal Mining Effluent Regulations and the National EEM Program

Under the *Fisheries Act*, the 2002 *Metal Mining Effluent Regulations* (MMER) prescribe discharge limits for arsenic, copper, cyanide, lead, nickel, zinc, total suspended solids and radium 226, and require the effluent to be non-acutely lethal to rainbow trout. These end-of-pipe limits reflect the level of effluent treatment that mines can currently achieve, and provide a national technology-based standard that is intended to protect fish, fish habitat and the use of fisheries resources. At the time the regulations were developed, it was acknowledged that while these more stringent discharge limits would improve environmental protection, there was uncertainty about the effectiveness of the new limits for protecting the broad variety of aquatic environments receiving metal mining effluents in Canada.

In order to evaluate the effects of mine effluent on fish, fish habitat and the use of fisheries resources, the 2002 MMER require the owners or operators of all Canadian metal mines to conduct environmental effects monitoring (EEM). This information helps determine any effects in aquatic ecosystems that may be caused by mine effluent, and the effectiveness of the regulation for aquatic environmental protection. The EEM Program requires biological monitoring studies undertaken in the aquatic recipient environment on the following components:

- a fish population survey to assess fish health;
- a benthic invertebrate community survey to assess effects on fish habitat; and
- a study of mercury levels in fish tissue to assess the effects on the usability of fisheries resources.

A select group of “effect” endpoints are used to assess the fish population and benthic invertebrate communities, the results of which help determine future monitoring needs, and also contribute to an understanding of the real nature of impacts from metal mining effluent discharges. In EEM, an “effect” is defined as a statistically significant response in at least one of the select endpoints in comparisons between biological samples taken downstream of a mine (exposure area) and samples taken from a reference area. The reference area is a sampling area as similar as possible in all aspects to the exposure area (e.g., same habitat, hydrological features, etc.), but without the presence of mining effluent. The EEM effect endpoints used in the fish population and benthic invertebrate community surveys are as follows (see Section 8.0 and Lowell *et al.* 2002, 2003, 2005 for further descriptions):

Fish population survey endpoints:
Condition
Relative liver weight
Relative gonad weight
Weight at age

Age

Benthic invertebrate community survey endpoints:
Total density
Taxon richness
Bray-Curtis index of dissimilarity
Simpson's evenness

In addition, supporting measurements (including effluent characterization, water and sediment quality monitoring, and sublethal toxicity) are taken to contribute to the program in areas such as assessing effluent quality and field conditions at individual mines. Unlike the biological field data, these supporting variables are not used to determine whether or not environmental "effects" are occurring at mine sites. Instead, they are meant to provide further information that may help to evaluate effects on a site-specific basis. This national assessment report is not intended to cover all data submitted to fulfill the requirements of the program nor to cover all analyses conducted. Rather, it focuses on the core components of the EEM Program used for decision making and interpreting major patterns of effects (i.e., fish population survey, benthic invertebrate community survey, and study of mercury levels in fish). Further site-specific information on the supporting variables is available in the interpretive reports produced for each mine.

The Metal Mining EEM Program is structured into "phases", whereby a mine conducts an EEM study every two to six years with both monitoring and interpretation components. At the beginning of each phase, each mine is required to develop a site-specific study design in consultation with Environment Canada regional staff. At the end of each phase, each mine must submit an interpretive report that summarizes its monitoring results. The EEM Program uses a tiered approach to monitoring, with initial studies carried out to characterize and assess the condition of the receiving environment. These are followed by targeted or focused studies to determine the extent, magnitude, and cause of effects where effects are detected and confirmed, or by a reduced level of monitoring where effects are not found. Technical guidance has been developed by Environment Canada on all aspects of EEM studies, including study design, as well as analyses and interpretation of data. Additional information on the EEM Program is available at http://www.ec.gc.ca/eem/.

To ensure the program continues to evolve with our scientific understanding and offers technical expertise needed for effective design and implementation of the program, support is provided by scientific experts from the EEM Science Committee. In addition, the Metal Mining EEM Review Team, which included experts from the federal government (Environment Canada, Fisheries and Oceans Canada, Natural Resources Canada, Canadian Nuclear Safety Commission), the mining industry, and the environmental and aboriginal communities, was created in fulfillment of an Environment Canada commitment to review the first phase of the program and to provide recommendations to Environment Canada for improving the program. Extensive information on the Review Team's evaluation of the program and its recommendations are available in the Review Team's Report (Metal Mining EEM Review Team, 2007).

1.2 Objectives of the Report

The purpose of this report is to present and discuss the major findings of a national assessment of EEM data collected in 2004 and 2005 from the receiving environments of metal mines across Canada for Phase 1 of the program. Both the EEM Science Committee and the Metal Mining EEM Review Team were given the opportunity to recommend particular areas of analyses to be included in this national assessment. Based on their recommendations, as well as on resources and data available, the data analyses focused on the following questions:

1) What are the types and magnitudes of effects of mine effluents on adult fish and benthic invertebrate communities?
2) How are the effects influenced by habitat, ore type, fish gender and species, concentration of effluent, and continuous versus intermittent discharge?
3) What are the effects of mine effluent on the usability of fisheries resources with respect to mercury concentrations in fish tissue?

2.0 Overview of Studies Conducted in Phase 1

In the first phase of monitoring for the Metal Mining EEM Program, 70 mines conducted EEM studies. Approximately half of the mines submitted their biological interpretive reports in June of 2005 and the remaining mines submitted their reports in June of 2006. This difference in the timing of the submission of the reports arose as mines that chose to submit a historical report were granted a one year extension for the submission of their first interpretive report. Table 1 provides a regional summary of the numbers and types of field surveys undertaken. All of these mines conducted studies in freshwater with the exception of two mines in the Prairie and Northern Region that discharged to marine environments. One of these marine mines was exempt from monitoring due to untenable local conditions but did conduct sublethal toxicity testing. The second marine mine conducted the benthic invertebrate survey in a freshwater stream and the fish survey in the marine environment. The majority of mines (39 mines or 56%) conducted a regular fish survey, 11 mines (or 16%) conducted non-lethal surveys, and 13 mines (19%) conducted both a regular fish survey and a non-lethal survey. One mine was exempt from the fish survey since the effluent concentration was <1% at 250 m, and one mine, discussed above, located in the arctic was exempt due to untenable local conditions that rendered sampling unsafe. Very few of the mines conducted alternatives for the benthic and fish surveys. In the Ontario Region, two mines conducted a joint mesocosm study as an alternative to the fish survey. Two mines in the Pacific and Yukon Region conducted alternatives to the fish survey including a fish hatchbox study and an alternative mussel study.

Table 1: General summary of Phase 1 metal mining EEM studies.

Region	Number of Mines that conducted EEM Studies	Fish surveys			Benthic invertebrate surveys	Use of Alternatives	Mines that received an exemption
		Lethal surveys	Non-lethal surveys	Lethal and non-lethal surveys			
Atlantic	4	2	2	0	4	0	0
Quebec	19	19	0	0	19	0	0
Ontario	20	9[a]	2	6[a]	20[b]	2[c]	1[d]
Prairie and Northern	22	9[e]	4	7	21	0	2[f]
Pacific and Yukon	5	0	3	0	5	2[g]	0
		39	11	13			
Total	70	63			69	4	3

[a] Three mines conducted a joint study
[b] Includes one set of two mines and one set of three mines that conducted a joint study
[c] Two mines conducted a joint fish mesocosm study
[d] Mine was expected to conduct fish mesocosm study but was not discharging effluent at the time of study
[e] Two mines conducted a joint study
[f] One mine exempt from conducting both fish and benthic studies due to untenable conditions and one exempt from conducting fish survey (effluent <1% at 250 m)
[g] One mine – fish hatchbox study, one mine – alternative mussel study

3.0 General Methods

3.1 Data Preparation and Analysis

This section describes the general methodologies used to carry out the national assessment of data from the fish and benthic invertebrate community surveys conducted in Phase 1 of the Metal Mining EEM Program. The methodologies employed were similar to those used in the national assessments of Cycles 2 and 3 of the Pulp and Paper EEM Program (see Lowell *et al.* 2003, 2005 for further details). As for these previous national assessments, this assessment is based on two quantitative approaches: 1) tabulation of results of individual mine comparisons and 2) meta-analyses. The tabulations are presented in this study as frequency distributions of magnitudes of effects (exposure vs. reference percent differences) and as histograms of the number of significant or non significant differences at the level of the individual mine. Interpretation of these latter histograms was partly limited by the fact that the significance level was dependent not only on the magnitude of effect, but also on sample size. Meta-analysis does not have the same limitations as individual study tabulations. Meta-analysis is a technique used to statistically examine the magnitude of effects in a way that loses less information due to constraints of individual study sample size and scale of measurement (Hedges and Olkin 1985, Rosenberg *et al.* 2000, Gurevitch and Hedges, 2001). In this case, the analysis treats the individual studies essentially as replicates; as such, it is possible to look at questions that are difficult to examine at the individual mine level (e.g., the influence of fish gender/species or habitat/ore type on effluent effects in the field). A full description of how meta-analysis was used for the Pulp and Paper Cycle 2 national assessment can be found in Lowell *et al.* (2003).

Sampling designs for the fish surveys as well as most of the benthic invertebrate community surveys were based on the control/impact approach, where sampling stations were located in reference and exposure areas. ANOVA (Analysis of Variance) or ANCOVA (Analysis of Covariance) was used to compare calculated endpoints between each reference versus exposure area. Of the mines included in this national assessment, one transformed a benthic invertebrate gradient design into a simple control/impact design by grouping stations according to abiotic conditions (e.g., conductivity), and we have followed their method of analysis here. Further information on EEM study designs and respective analyses for the fish and benthic invertebrate surveys is provided in Glozier *et al.* (2002), Lowell *et al.* (2002, 2003) and the Metal Mining Guidance Document for Aquatic EEM (Environment Canada, 2002).

The national assessment focused on near-field effects in order to investigate the more pronounced effects that were occurring nationally for the fish and benthic invertebrate community surveys. Some mines collected data from multiple areas. Data from more than one near-field area were pooled only if warranted based on inspection of pooling procedures used in the interpretative reports. The Statistical Assessment Tool (SAT), a program developed initially by the National Water Research Institute of Environment Canada, was used to calculate the magnitude and statistical significance of effects for the five fish and four benthic invertebrate community endpoints.

The primary steps common to both the fish and benthic invertebrate data analyses were as follows. First, submitted electronic data were screened for obvious errors (e.g., missing data fields, obvious data entry errors, misnamed stations or areas). SAT aided with the selection of the appropriate data for analysis, including removal of outliers (fish analysis). All data were analyzed in SAT, via ANOVA or ANCOVA, to statistically compare exposure and reference areas for each of the endpoints for each mine. The ANOVA and ANCOVA analyses provided area means (adjusted means for ANCOVA) and standard deviations, which were required for subsequent tabulations and meta-analyses of measured effects. The significance level (α) used for ANOVA and ANCOVA was set at 0.05 for the purposes of the tabulations and statistical analyses presented here.

The fish data were log-transformed and analyzed using ANCOVA (all endpoints except age); fish age data were non-transformed and were analyzed using ANOVA. The invertebrate data were also analyzed using ANOVA and were non-transformed, with the exception of density, which was log-transformed. Further discussion regarding data transformation and methods of analysis can be found in the Metal Mining Guidance Document for Aquatic EEM (Environment Canada, 2002) and in Lowell *et al.* (2005).

3.2 Procedure for Determining National Response Patterns

Meta-analysis is a set of statistical procedures used to quantitatively synthesize the results of a large number of independent studies. Further, it permits overall response patterns to be determined. The meta-analyses required determination of a standardized magnitude of effect, the Hedges' d effect size, which was calculated as the difference between the exposure and reference means, divided by the pooled standard deviation (this value is multiplied by a correction factor that accounts for the effect of small sample sizes) (Rosenberg *et al.* 2000).

The main meta-analytical results are presented in the following summary format (Fig. 1). The standardized effect size is on the x-axis, with the vertical line representing a zero effect. The result for each mine grouping (e.g., grouped by ore type) is presented as a horizontal 95% confidence interval about a vertical tick mark indicating the average effect size for that grouping of mines. Mine distributions to the right of the zero effect line indicate that the average effect associated with effluent exposure was an increase in the measured endpoint. Similarly, mine distributions to the left of the zero effect line indicate an effluent-associated decrease in the measured endpoint. The increase or decrease is statistically significant for the group as a whole if the 95% confidence interval does not overlap the zero effect line. Larger mine groupings (that are non-significant as a whole) can be composed of smaller subgroups, some or all of which may be significantly different from zero. Most of the meta-analysis results in the following sections will use this graphical representation of the data.

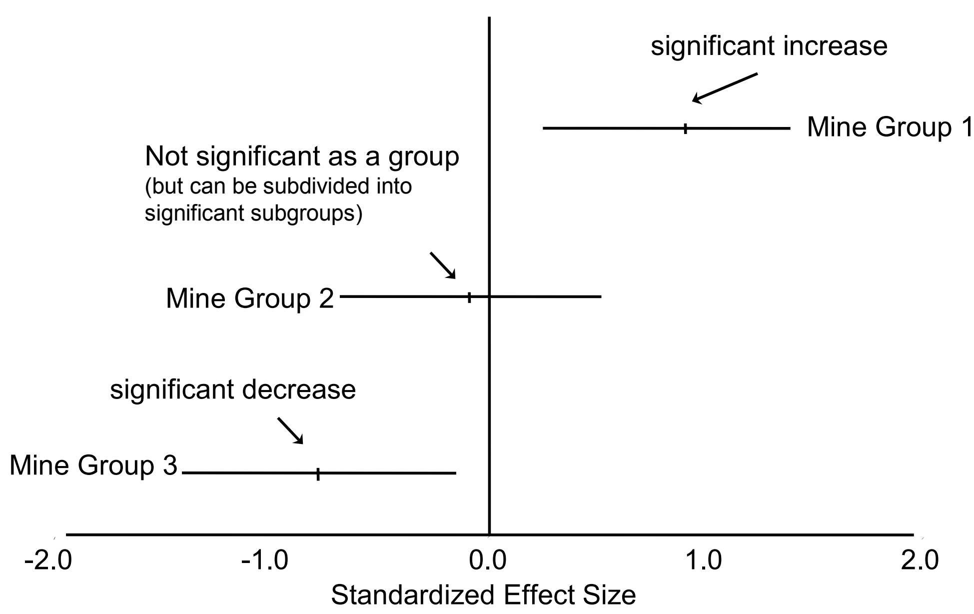

Figure 1: Example of a meta-analysis summary figure. The effect size was measured as Hedges' d (see text).

A variant form of meta-analysis was used for two of the central questions addressed in this assessment dealing with the influence of effluent concentration and of continuous versus intermittent discharge. These analyses used a regression approach where standardized effect size (Hedges' d) was regressed against the continuous variable of interest using meta-analytical procedures (Rosenberg *et al.* 2000).

Response patterns were further investigated using a third form of analysis, where the outputs of the meta-analyses were fed into either multivariate analyses or bivariate plots (Faith *et al.* 1987, Belbin 1992, Lowell and Culp 2002, Lowell *et al.* 2003). The patterns in mine distributions within the resulting plots reflected certain patterns in effluent effects, as outlined in the following sections. Interpretation of the fish multivariate plot was enhanced by also plotting principal axis correlation vectors (Belbin 1992). These were calculated using a multiple linear regression technique that determined the linear relationship between each of the standardized endpoints and the ordination space. Each vector gave the direction of best fit within the ordination space for a particular endpoint. This was an improvement over correlating the endpoints to the ordination axes, because it provided the direction of best fit and was not dependent on the positioning of the axes.

4.0 Fish Survey

The adult fish survey is used to determine if the mine effluent is affecting fish populations by comparing effluent-exposed fish with those from reference areas. The survey uses fish growth, reproduction, condition, and age structure to assess the overall health of exposed fish. These are assessed via measurements of five core fish endpoints: weight at age, relative gonad and liver weights, condition (body weight relative to length), and age. The Metal Mining Guidance Document for Aquatic EEM (Environment Canada, 2002) recommends that mines sample adults of two sentinel fish species and conduct analyses of the five core endpoints on both species.

4.1 Data Processing and Study Designs

Data for 48 of the 52 lethal fish surveys were submitted electronically. A total of 35 of these surveys, including three joint studies (each of two mines) contained adult fish data that had sufficient replication (i.e., at least 12 fish of same sex and species per area) to conduct statistical analysis. In addition, 24 mines conducted non-lethal fish surveys and 4 mines carried out alternative fish surveys including a fish mesocosm study, a caged bivalve study and a fish hatchbox study. Due to the different nature of their endpoints, the non-lethal and alternative studies were not included in these summary analyses. Prior to analysis, the electronically submitted fish data were screened for errors and incomplete data. Although the majority of submitted data were of good quality, several mines encountered problems during fish field surveys (Table 2).

Table 2: Examples of problems encountered with fish field surveys in Phase 1.

Problems	**Number of mines**
Only one sentinel species reported	9
Too few fish (<12) in one or both sexes of one or both sentinels	34[a]
Immature fish captured	10
Ageing problems / not aged	6[b]
No fish caught due to untenable climate	1
Pooling of fish caught with multiple fishing techniques[c]	14[d]

[a] Twenty-one cases where too few fish caught in both the reference and the exposure area, seven cases in reference area only and six cases in exposure area only.
[b] Five mines did not attempt or were unsuccessful in determining the age of brook stickleback (*Culaea inconstans*) and one mine could not age the threespine stickleback (*Gasterosteus aculeatus*) because the annuli were not clearly visible.
[c] Multiple fishing techniques were used to attain sufficient number of fish.
[d] Twelve mines used different fishing techniques in exposure and reference area.

Thirty-two fish species were used as sentinel species by mines that conducted lethal fish studies across the country. Of these 32 species, 21 were included in the national assessment. The frequencies of species used in lethal surveys are presented in Table 3.

Table 3: List and frequencies of sentinel species used in lethal fish surveys.

Species	**Scientific name**	**Number of studies[a]**	**Number of studies in national assessment[b]**
Large-bodied fish			
White sucker	*Catostomus commersoni*	19	10
Northern pike	*Esox lucius*	10	4
Walleye	*Sander vitreus*	7	0
Lake whitefish	*Coregonus clupeaformis*	6	2
Yellow perch	*Perca flavescens*	6	1
Burbot	*Lota lota*	5	1
Lake trout	*Salvelinus namaycush*	4	0
Brown bullhead	*Ameiurus nebulosus*	3	2
Round whitefish	*Prosopium cylindraceum*	3	0
Arctic char	*Salvelinus alpinus*	2	1
Brook trout	*Salvelinus fontinalis*	2	0
Cisco	*Coregonus artedii*	2	0
Arctic grayling	*Thymallus arcticus*	1	0
Longnose sucker	*Catastomus catastomus*	1	1
Rock bass	*Ambloplites rupestris*	1	0
Goldeye	*Hiodon alosoides*	1	1
Shorthead redhorse	*Moxostoma macrolepidotum*	1	0
Fallfish	*Semotilus corporalis*	1	0
Total number of studies that used large-bodied fish		***75***	***23***

Small-bodied fish			
Brook stickleback	*Culaea inconstans*	7	6
Lake chub	*Couesius plumbeus*	6	3
Pearl dace	*Margariscus margarita*	6	4
Spottail shiner	*Notropis hudsonius*	4	2
Trout perch	*Percopsis omiscomaycus*	4	3
Golden shiner	*Notemigonus crysoleucas*	2	0
Logperch	*Percina caprodes*	2	1
Fathead minnow	*Pimephales promelas*	2	2
Ninespine stickleback	*Pungitius pungitius*	2[c]	1
Longnose dace	*Rhinichthys cataractae*	2	2
Mottled sculpin	*Cottus bairdi*	1	1
Slimy sculpin	*Cottus cognatus*	1	1
Threespine stickleback	*Gasterosteus aculeatus*	1	1
Common shiner	*Luxilis cornutus*	1	0
Total number of studies that used small-bodied fish		***41***	***27***

[a] Includes all species and studies for which at least partial data were submitted.
[b] Includes only those studies for which sufficient electronic data were available to include in the national assessment (e.g., excludes studies that did not capture sufficient numbers of adult fish).
[c] Includes one freshwater and one marine ninespine stickleback study. Electronic data sufficient for the national assessment were available only for the freshwater study. Note that all other fish studies were conducted in a freshwater environment.

4.2 Summary of Effect Sizes

For each of the five core fish endpoints, Fig. 2 provides the frequency distribution of the magnitudes of measured differences. Measured difference was calculated as exposure minus reference area mean, expressed as a percentage of the reference area mean (adjusted means for ANCOVA). All measured differences (i.e., significant and non-significant) were taken into consideration. Figure 2 focuses on those comparisons where exposure versus reference ANCOVA slopes were parallel (the majority of comparisons). For a given mine and endpoint, a maximum of four comparisons were possible (i.e., two fish species and two genders). Condition showed the narrowest range of percent differences (-25% to 35%), followed by liver weight (-45% to 60%). Fish condition is an inherently less variable endpoint, and a similarly narrow range has been observed for fish exposed to pulp and paper mill effluent (Lowell *et al.* 2003, 2005). Gonad weight showed the widest range of measured effects (-55% to 350%). The other two fish endpoints showed an intermediate range (-70% to 120% for weight at age; -45% to 105% for age).

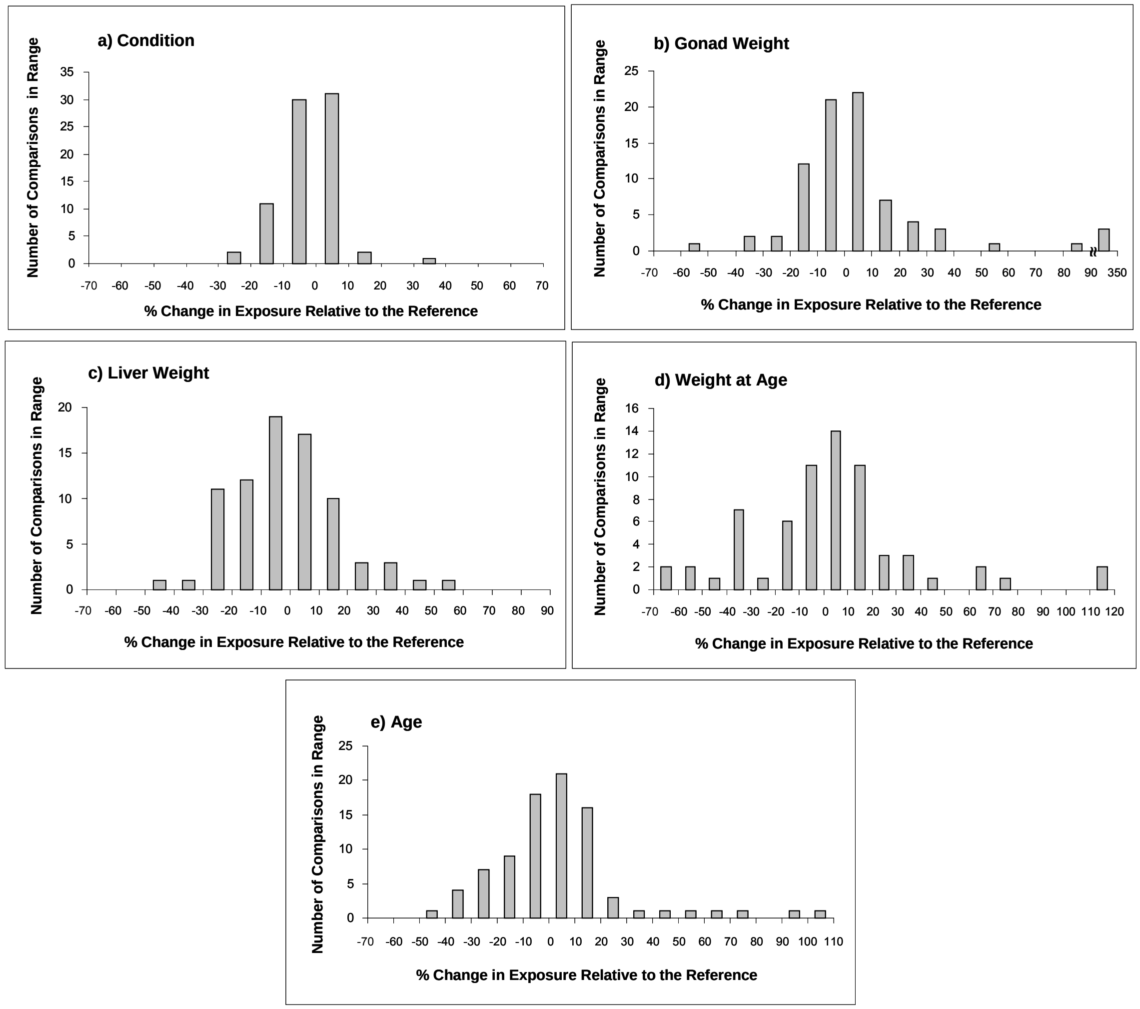

Figure 2: Distribution of measured percent differences between exposure and reference area fish in Metal Mining EEM Phase 1 for a) condition, b) gonad weight, c) liver weight, d) weight at age and e) age.

Figure 3 illustrates the number of comparisons that showed non significant differences, significant differences in means (adjusted means for ANCOVA), or significant interactions for each of the five fish endpoints. Significant interaction occurs when the exposure versus reference area slopes are statistically different in the ANCOVA analysis; that is, when the slopes can be considered to be non-parallel. For example, non-parallel exposure versus reference slopes for an ANCOVA regression of gonad weight against body weight could indicate that fish exposed to effluent allocate resources to gonad weight differently for fish of different size, relative to fish in the reference area. Both significant differences in means and significant interactions are considered to be significant effects. Note that age data were analyzed with ANOVA and therefore did not produce interactions. See Environment Canada (2002) for further information on ANCOVA procedures and interpretation.

For the five endpoints, between 33% (age) and 60% (condition) of the comparisons were significant (including both significant differences in means and significant interactions; Fig. 3). The number of significant interactions was fairly similar among the four ANCOVA endpoints (approximately 15-20% of the comparisons).

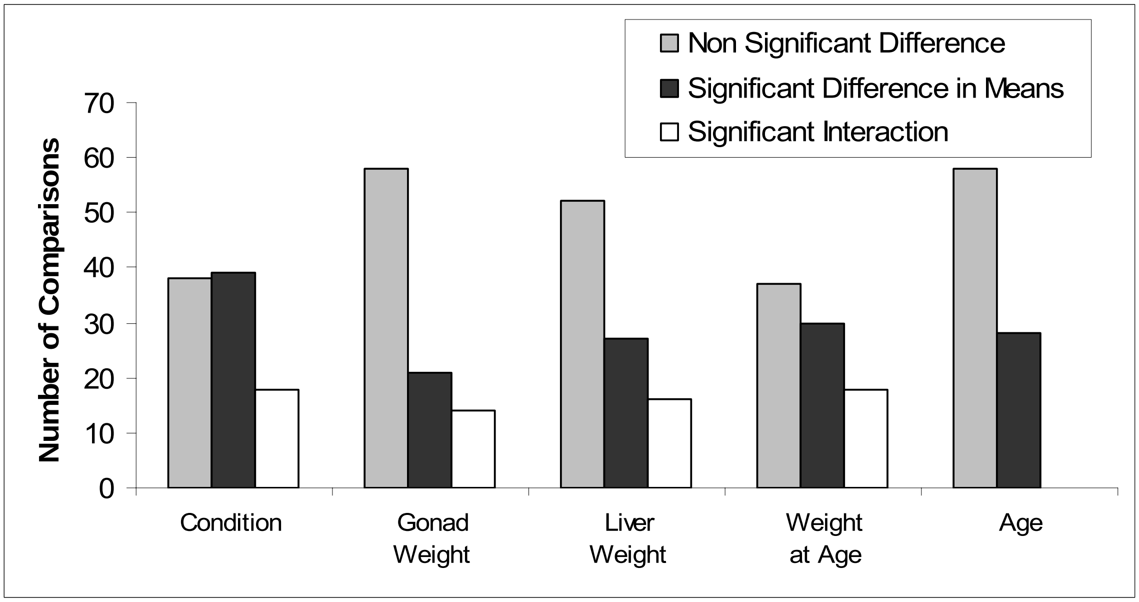

Figure 3: Number of exposure versus reference fish comparisons showing non significant differences, significant differences in means, or significant interactions.

4.3 Response Patterns – National Averages

The national average response patterns for fish exposed to mine effluent can be seen by plotting the grand means and 95% confidence intervals from the meta-analyses of all the mines across the country (Fig. 4). These analyses showed that, on average, effluent exposed fish exhibited significantly lowered condition and relative liver size – that is, they were thinner and had smaller livers. A similar national level effect was not seen for relative gonad size, weight-at-age, or age (measures related to reproduction, growth rate, and survival, respectively), with the 95% confidence intervals overlapping zero for these latter three variables.

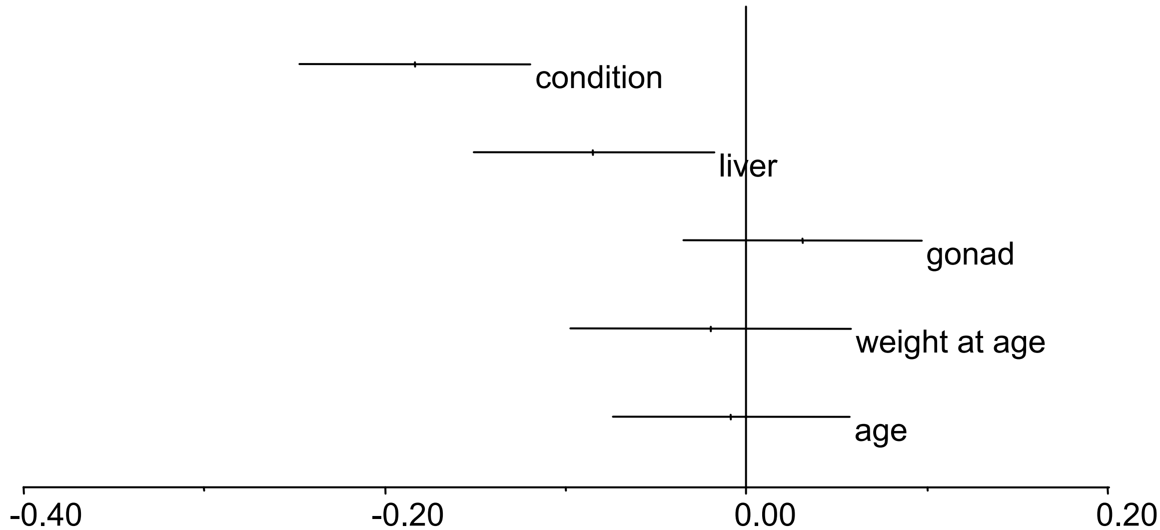

Figure 4: Metal mine grand means for fish endpoints. Error bars represent 95% confidence intervals. Number of comparisons: condition = 77, liver = 79, gonad = 79, weight at age = 67, age = 86.

This national average response pattern for mines differed markedly from the broad-scale response pattern that has been repeatedly observed for fish exposed to pulp and paper mill effluent (Lowell *et al.* 2003, 2004, 2005). Fish exposed to pulp and paper mill effluents are frequently fatter and faster growing, with bigger livers, but smaller gonads (Fig. 5). This latter response pattern is generally indicative of nutrient enrichment coupled with metabolic disruption (Munkittrick *et al.* 2000) and is an area of active research (Hewitt *et al.* 2005, McMaster *et al.* 2005, Parrott 2005). Pulp and paper mills tend to add organics and other nutrients to receiving waters (nutrient enrichment), resulting in overall stimulatory effects on fish (fatter fish), with the exception of disruption of allocation of resources to gonads. In comparison, the national average mine effects shown in Fig. 4 were more in the inhibitory direction (thinner fish with smaller livers). Similar types of inhibitory effects have been reported in a number of earlier studies of fish exposed to metal contaminants (e.g., Eastwood and Couture 2002, Rajotte and Couture 2002, Hansen *et al.* 2004, Rickwood *et al.* 2006). Effluent-induced inhibitory effects in general can have a variety of causes (see Munkittrick and Dixon 1988, Munkittrick *et al.* 1991, 1994, 2000 for reviews). For example, they may be due to direct inhibitory effects of the effluent on fish and/or to food limitation resulting from habitat alteration and inhibitory effects on prey items, such as benthic invertebrates.

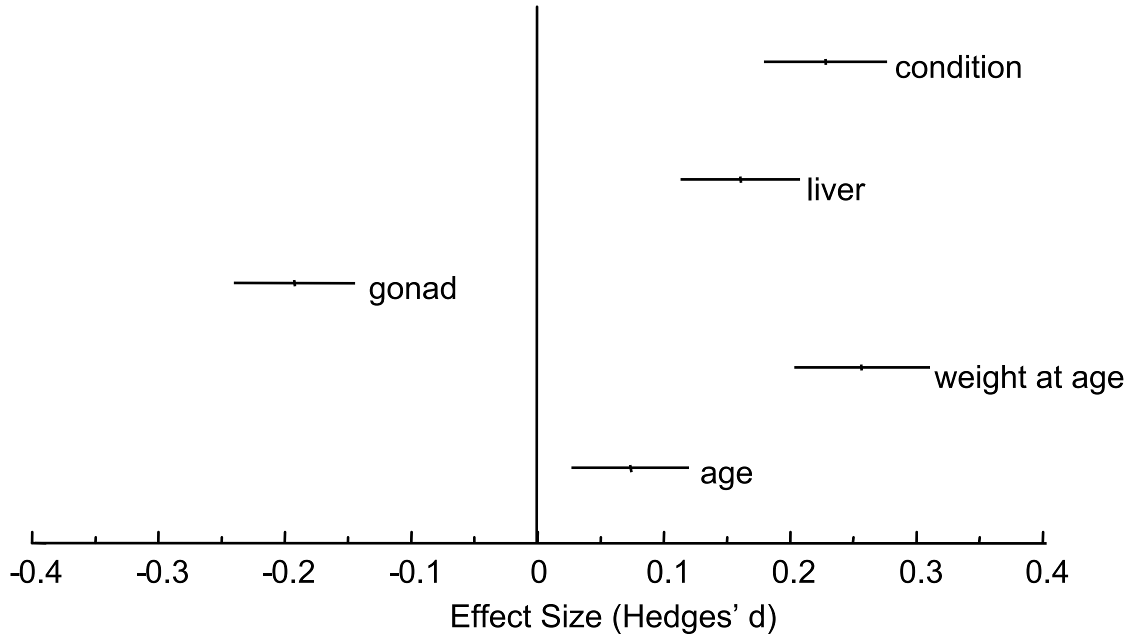

Figure 5: Pulp and paper Cycle 2 grand means for fish endpoints. Error bars represent 95% confidence intervals. Number of comparisons: condition = 123, liver = 128, gonad = 126, weight at age = 100, age = 133.

4.4 Response Patterns – Other Meta- and Multivariate Analyses

It should be noted that the mining industry in Canada is quite heterogeneous. Thus, it is instructive to break the meta-analysis results down by dividing the mines into smaller subgroups. When subdividing by major receiving water habitat types (as defined by the mines submitting data), more detailed response patterns became apparent (Fig. 6, which also includes the national average grand mean for condition from Fig. 4). The two most common habitat types were lakes and rivers. Erosional and depositional river habitats were pooled because the more mobile nature of fish makes separating the two problematic (cf. the benthic invertebrate meta-analyses). Condition was significantly reduced in lake but not river habitats. Condition was also prominently reduced in creek habitats, but the low sample size increases the odds that this could have occurred due to factors other than habitat type. Therefore, more confidence can be assigned to conclusions based on the lake and river results. It should be noted that sample size for the fish meta-analyses refers to the number of exposure versus reference area comparisons (or studies), not to the number of fish captured within a study.

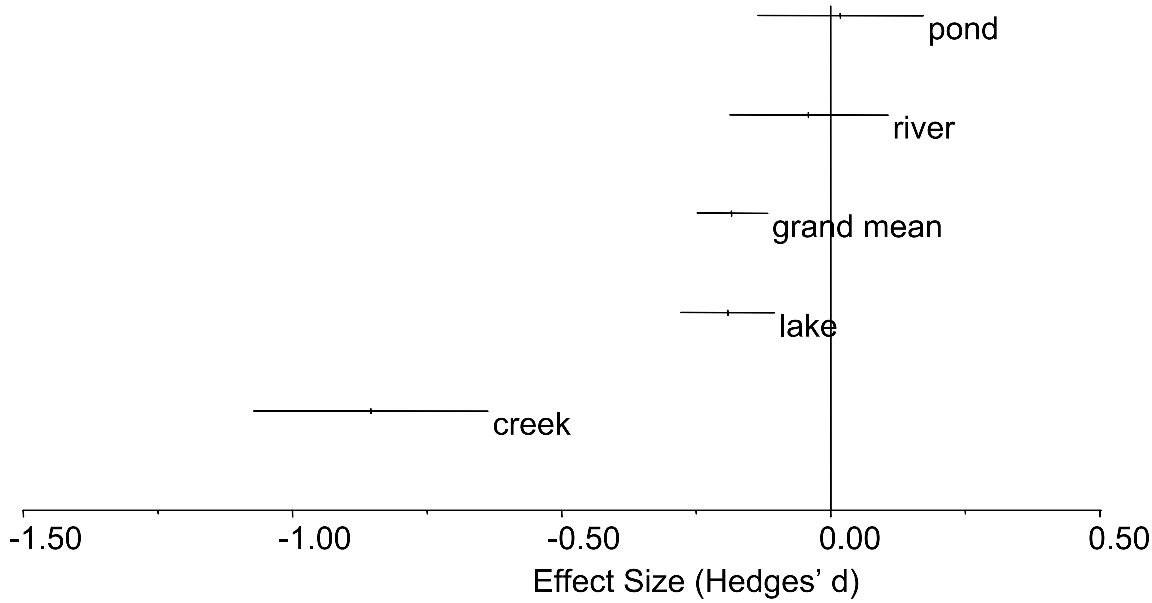

Figure 6: Fish condition by habitat type. Error bars represent 95% confidence intervals. Number of comparisons: pond = 6, river = 16, lake = 49, creek = 6.

Relative liver size was significantly reduced in lake habitats, but was increased, although not significantly, in river habitats (Fig. 7). Thus, mine effluent appears to have different effects on fish in these two habitat types. Although, as noted elsewhere in this report, mining effects are frequently associated with inhibitory effects, increases in liver size have been noted in at least one previous study (Dubé *et al*. 2005). Liver size was also significantly increased in pond habitats, but the sample size was very low. Although the relative gonad size grand mean was not significantly different from zero, subdividing by habitat type showed that gonad size was significantly increased in river habitats (Fig. 8). Further meta-analyses (not detailed here), indicated that growth rate and age were significantly increased in lake habitats and decreased in river habitats, but this was driven by different subsets of mines than those causing the main response patterns for condition and liver (unpublished analyses). That is, large increases in growth rate were not associated with large decreases in condition and liver size at the same mines.

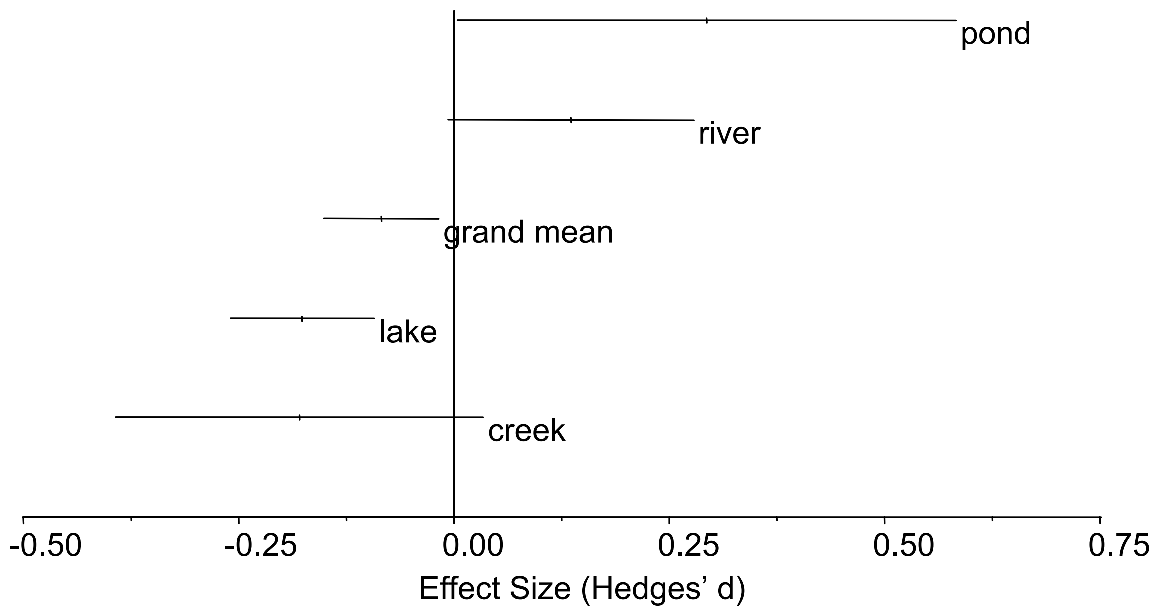

Figure 7: Fish liver by habitat type. Error bars represent 95% confidence intervals. Number of comparisons: pond = 3, river = 17, lake = 53, creek = 6.

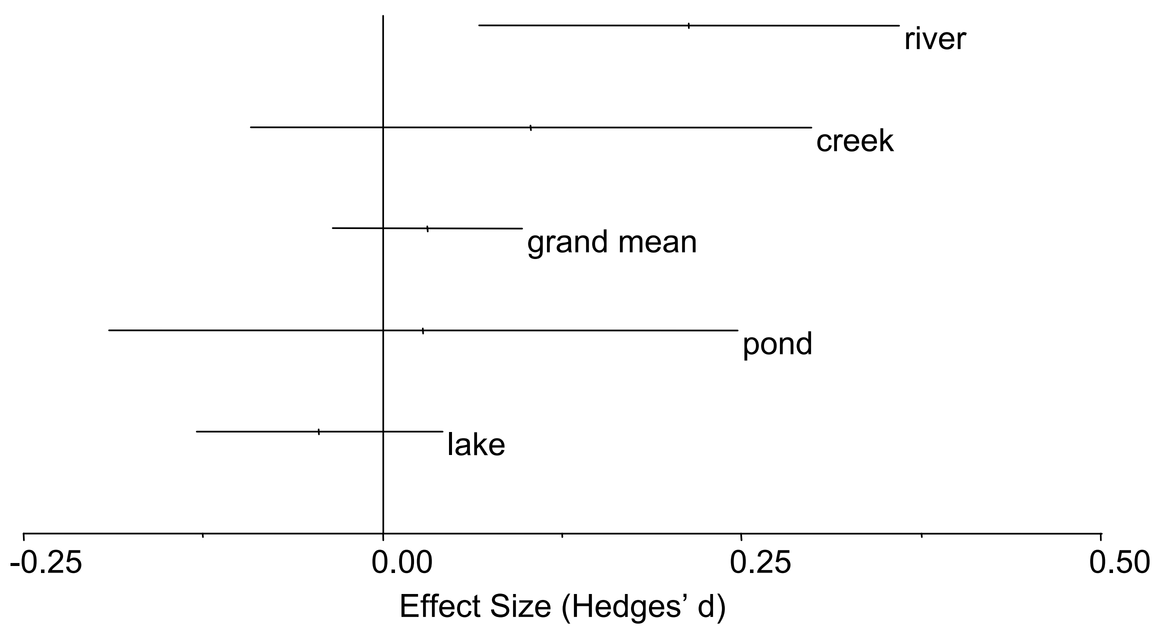

Figure 8: Fish gonad by habitat type. Error bars represent 95% confidence intervals. Number of comparisons: river = 17, creek = 7, pond = 5, lake = 50.

Subdividing mines by ore type (as defined by the mines submitting data) revealed more detailed response patterns. The two most common ore types were precious metal and base metal, and fish condition was significantly reduced for waters receiving precious metal effluents (Fig. 9). The uranium subgroup also showed significantly reduced condition, although the sample size was not large. The ferrous and uranium subgroups together helped reduce the grand mean for relative liver size, although the sample size was not large for either subgroup alone (Fig. 10). Relative gonad size was not significant for any of the ore type subgroups (Fig. 11). Further meta-analyses (not presented here) showed significantly reduced growth rate and age for the precious metal subgroup and significantly increased growth rate for the base metal subgroup (unpublished analyses).

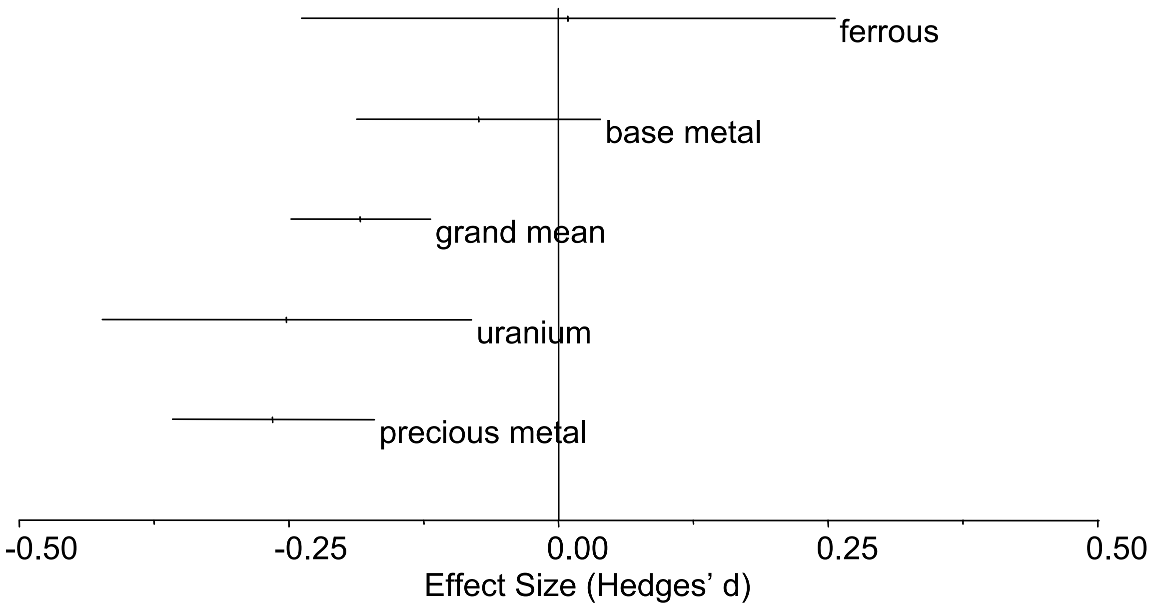

Figure 9: Fish condition by ore type. Error bars represent 95% confidence intervals. Number of comparisons: ferrous = 7, base metal = 25, uranium = 11, precious metal = 34.

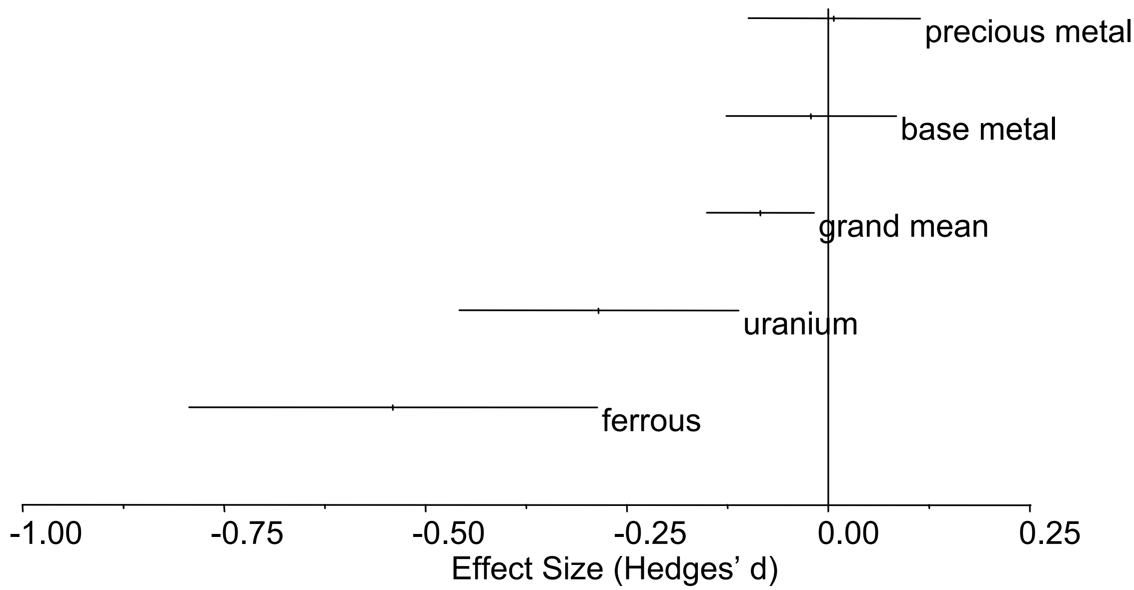

Figure 10: Fish liver by ore type. Error bars represent 95% confidence intervals. Number of comparisons: precious metal = 31, base metal = 29, uranium = 12, ferrous = 7.

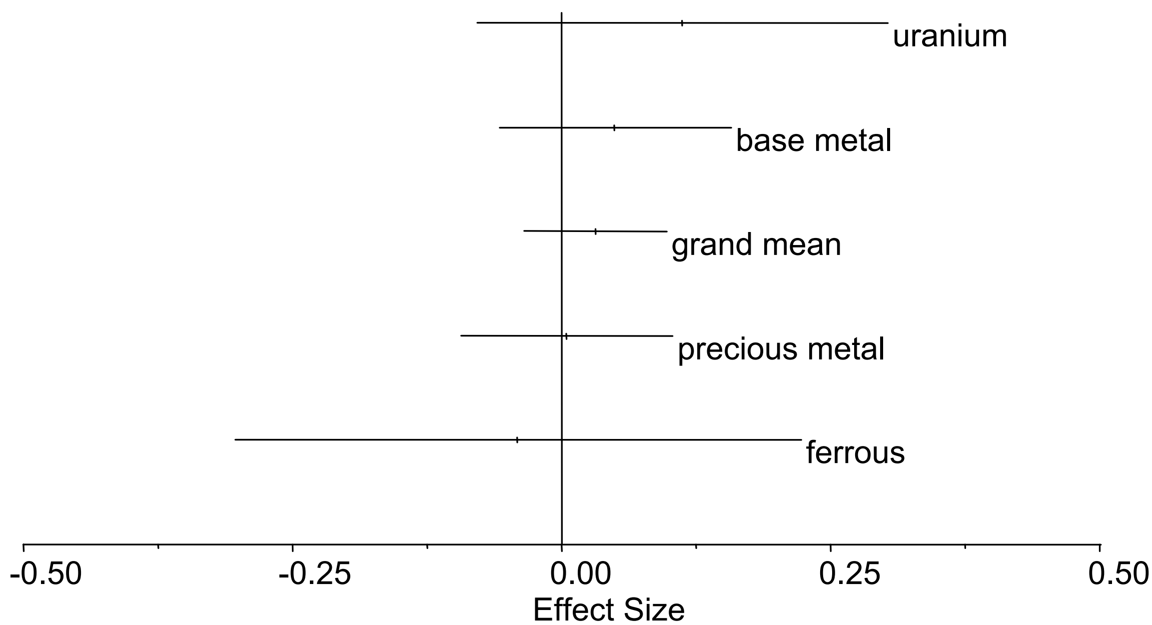

Figure 11: Fish gonad by ore type. Error bars represent 95% confidence intervals. Number of comparisons: uranium = 10, base metal = 27, precious metal = 36, ferrous = 6.

Response patterns were sometimes, but not always, influenced by fish gender and species. For example, relative gonad size was significantly increased for males, but not for females (Fig. 12). Males were also significantly different than females in the age response (greater tendency toward increased age), although neither gender was quite significantly different than zero (Fig. 13). Males and females did not differ significantly for the other three endpoints. Significant differences were also observed among fish species (e.g., relative gonad size; Fig. 14). Nevertheless, most species were only used in a small number of studies (i.e., low sample size for meta-analytical comparisons), so the observed differences could have been due to co-occurring factors other than fish species.

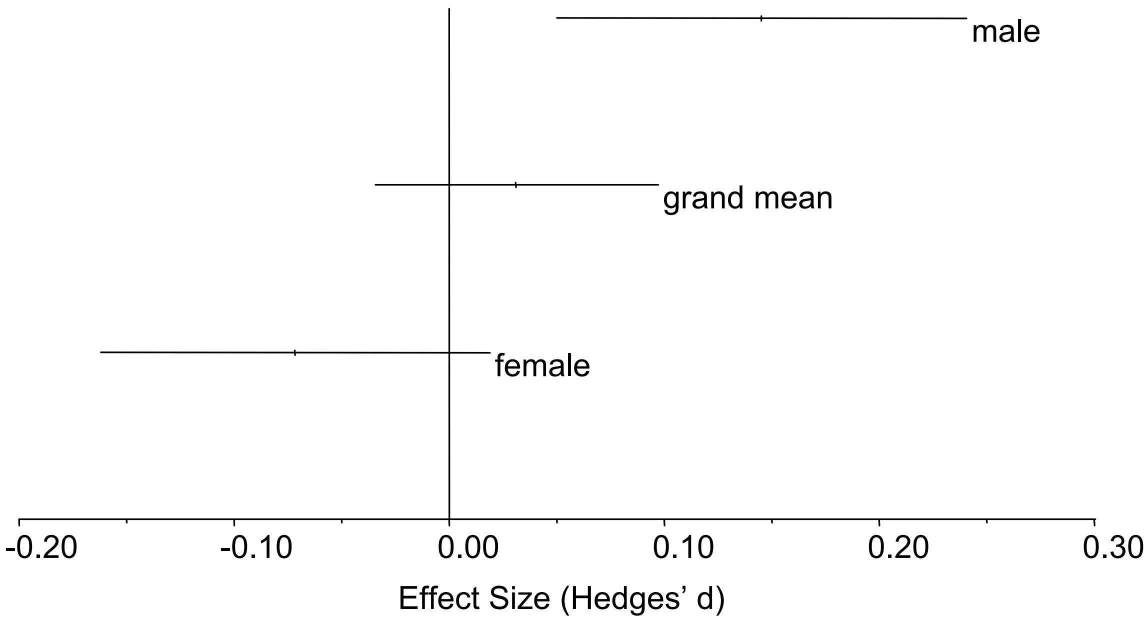

Figure 12: Fish gonad by gender. Error bars represent 95% confidence intervals. Number of comparisons: male = 38, female = 41.

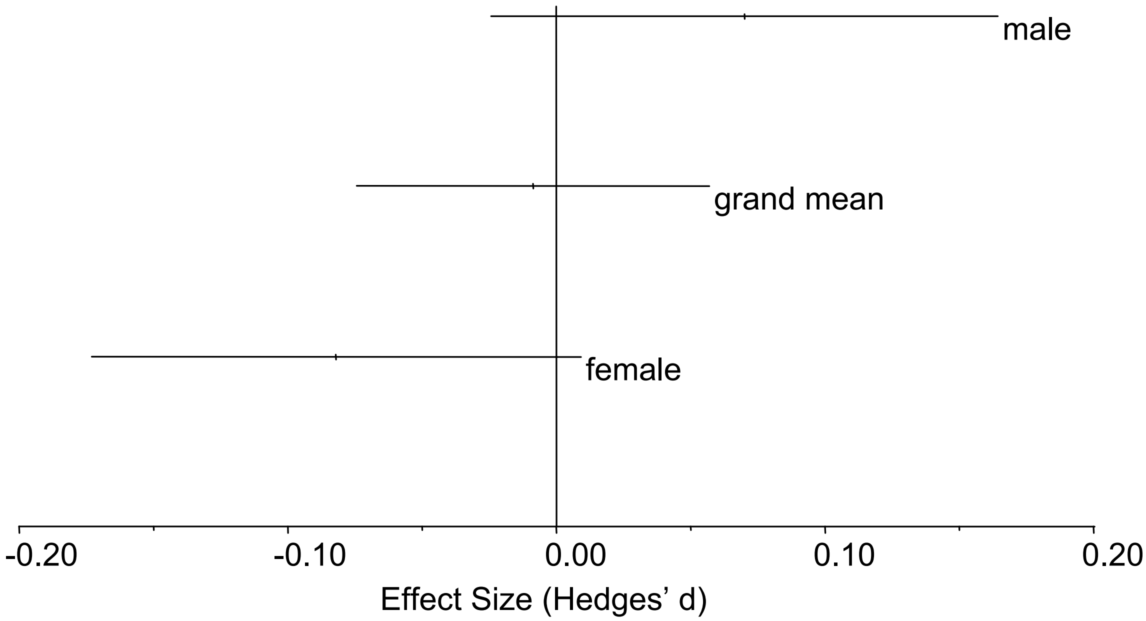

Figure 13: Fish age by gender. Error bars represent 95% confidence intervals. Number of comparisons: male = 42, female = 44.

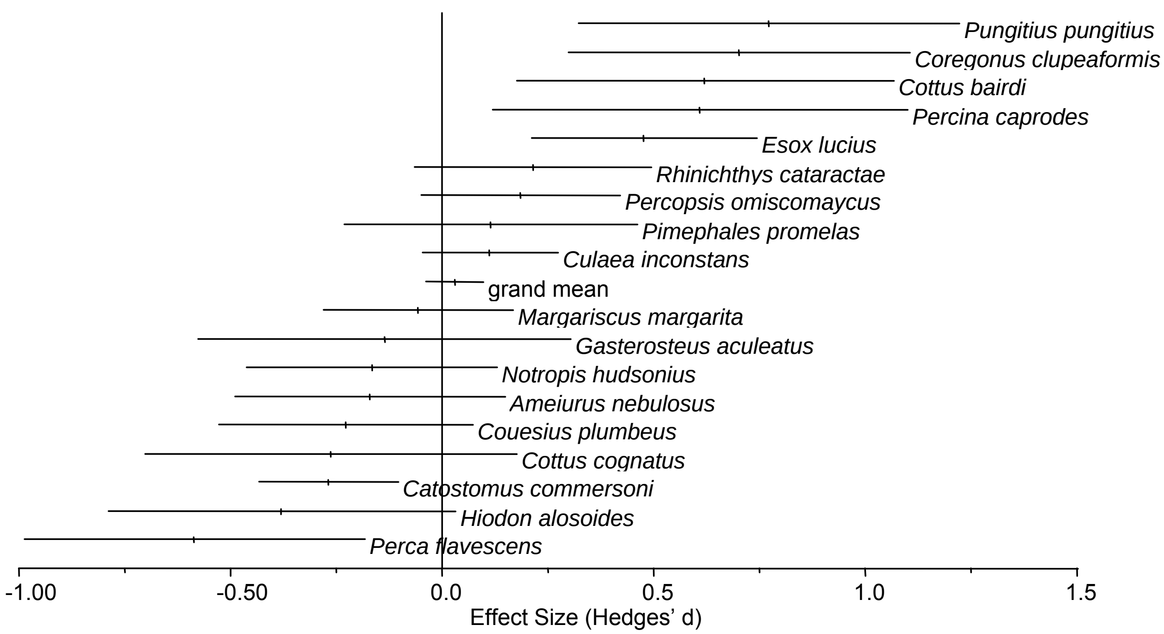

Figure 14: Fish gonad by species. Error bars represent 95% confidence intervals. Number of comparisons = 2 to 5, except *Culaea inconstans* = 10, *Margariscus margarita* = 7, *Catostomus commersoni* = 15. See Table 3 for the common English fish names.

Regression meta-analyses were performed to determine whether fish (or benthic invertebrate) responses were influenced by the degree to which mines discharged intermittently versus continuously. To address this question, standardized effect size (Hedges' d) was regressed against number of months during which mines discharged effluent during the year, using meta-analytical regression. For the primary discharge point of almost all mines nationally, the number of months of discharge during a year was relatively consistent during recent years, so the analyses were performed using 2004 data, for which the most complete data set was available. Regressions were run for all five core fish endpoints and all four core benthic invertebrate endpoints. Number of months of discharge spanned a wide range (1-12 months). Despite this wide range, none of the regressions were statistically significant, indicating a lack of correlation between effluent effects and the number of months of discharge during the year (e.g., Fig. 15). Note that each point in Fig. 15 represents a different exposure versus reference comparison, and the best-fit regression line from the meta-analysis is also included. Thus, the data suggest that effluent effects are not greatly influenced by whether mines discharge effluent intermittently or continuously.

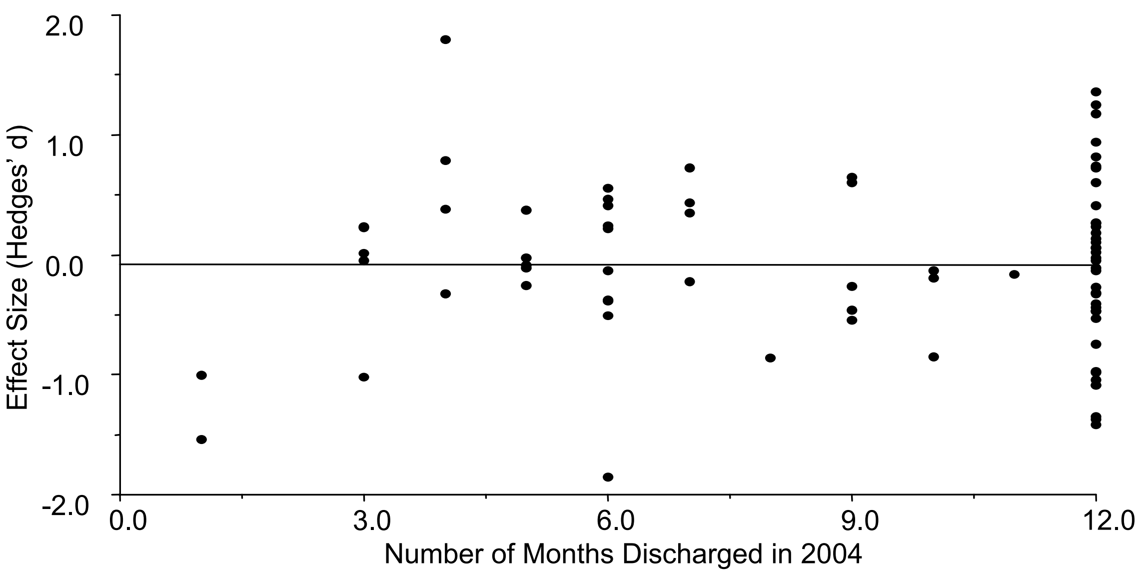

Figure 15: Fish liver regression meta-analysis of standardized effect size versus number of months of effluent discharge.

National response patterns for fish were further examined using multivariate analyses of the output of the meta-analyses. Multidimensional scaling ordination was used to fit the standardized effect sizes (Hedges' d) for all five core endpoints (five data dimensions) into a two-dimensional ordination space (Fig. 16; Faith *et al.* 1987, Belbin 1992, Lowell and Culp 2002). Each data point in Fig. 16 corresponds to a separate exposure versus reference comparison. Because the fish studies incorporated as many as two species and two genders per mine, each mine contributed up to four points to the figure. Only mines reporting valid data for all five of the endpoints could be included in the analyses. Mines (data points) that are closer together in Fig. 16 were more similar in terms of measured effects than mines that are farther apart. Principal axis correlation vectors are also plotted to show the direction of best fit within the ordination space for each of the five endpoints. The correlation coefficients for all the vectors were statistically significant (r>0.34, P<0.05).

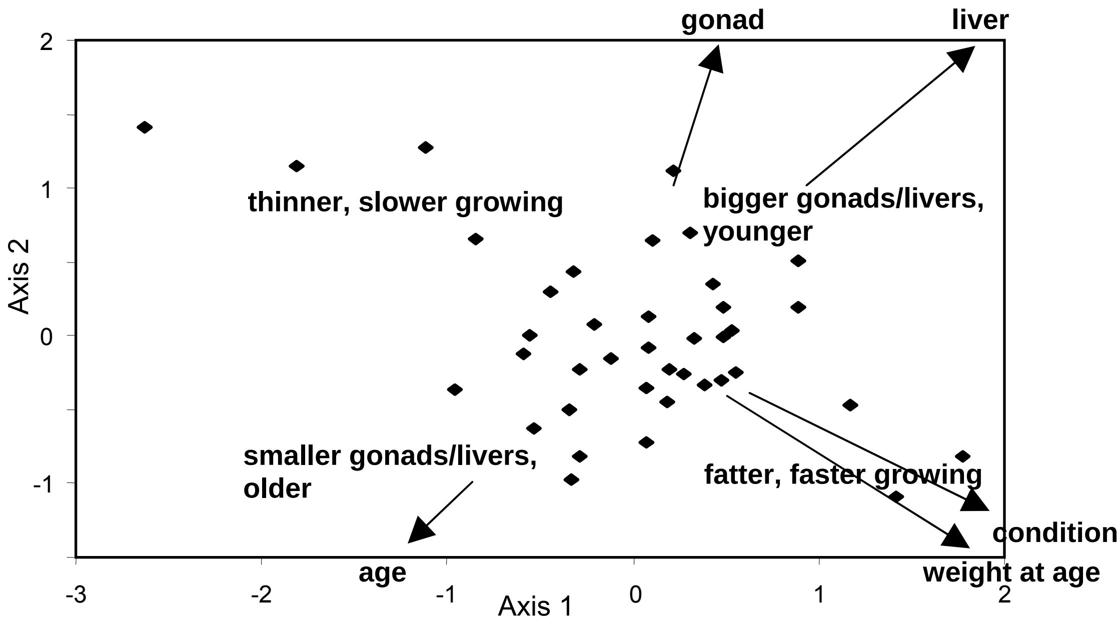

Figure 16: Multidimensional scaling analysis of output of fish meta-analyses.

Some distortion is inevitable when collapsing five dimensions of data into two dimensions, and this distortion is measured as “stress” in multidimensional scaling ordination. The goal is to maintain stress below 0.2 to facilitate reliable interpretation of the analyses. The stress level for these analyses was 0.16, indicating that distortion fell within acceptable limits for data interpretation.

Thus, the patterns in data point distribution in Fig. 16 reflect the different response patterns that were measured nationally for effluent-exposed fish. The positioning of the five endpoint vectors helps to identify sectors of the ordination space corresponding to the different main response patterns. Exposed fish for mines falling into the ordination space ranging from the center of the cluster of points to the upper left of the figure were thinner and slower growing. Those in the lower right of the figure were fatter and faster growing. Those in the lower left of the figure were older and had relatively smaller gonads and livers. Those in the upper right were younger and had relatively bigger gonads and livers. At a coarser scale, fish at mines ranging from the center of the cluster of points to the left portion of the ordination space were exhibiting more inhibitory responses to effluent exposure, while those in a crescent along the right side of the ordination space were showing more stimulatory responses.

5.0 Usability of Fisheries Resources: Mercury Analyses in Fish Tissue

Under the MMER, effects on fish usability, as part of the EEM Program, are evaluated by measuring concentrations of mercury in tissue from fish in the exposure and reference areas. If a mine is detecting levels of total mercury greater than or equal to 0.1 µg/L as part of effluent characterization, then the mine is required to do a fish tissue analysis. The Metal Mining Guidance Document for Aquatic EEM (Environment Canada, 2002) recommends that tissue analysis be conducted on a minimum of eight samples (to achieve 95% power) of a single species from the exposure area and the reference area. An effect on fish tissue is defined in the MMER as "measurements of total mercury that exceed 0.45 µg/g wet weight in fish tissue taken in an exposure area and that are statistically different from the measurements of total mercury in fish tissue taken in a reference area."

In the first phase of monitoring, 16 mines completed a fish tissue analysis. Of these 16 mines, ten reported mercury concentrations in their effluent ≥ 0.1 µg/L whereas six mines had mercury effluent concentrations < 0.1 µg/L but chose to undertake the mercury analysis regardless. These mines were all located in Quebec, where mines voluntarily participated in a joint study organized by the Quebec Mining Association to proactively go beyond the basic requirement regarding mercury.

A national summary of the results of the mercury in fish tissue analyses are presented in Fig. 17. One study was excluded from the national analysis since different species were compared in the near-field exposure area and reference area making the comparison invalid. There was only one study that found concentrations of mercury in fish tissue greater than 0.45 µg/g wet weight in fish tissue taken in the exposure area and statistically different from the measurements taken in the reference area. This mine reported the incidence of effluent concentrations of 0.1 and 0.2 µg/L total mercury. All other measurements in fish were below the "effect" level of 0.45 µg/g total mercury or were not significantly different from reference.

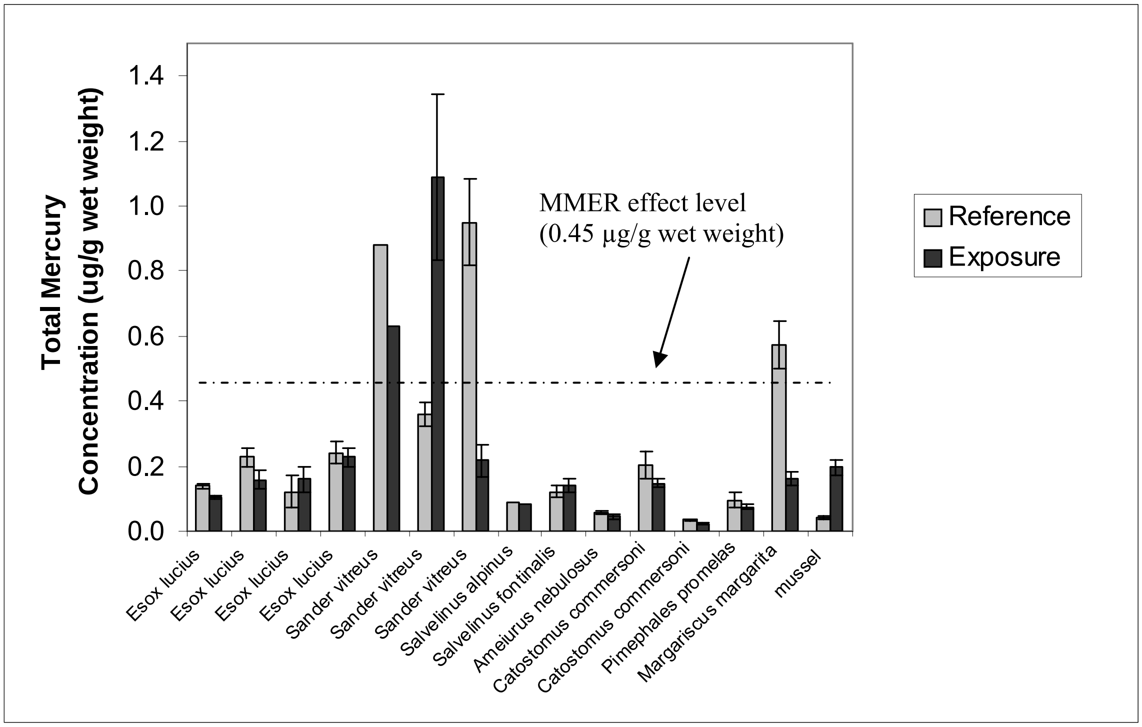

Figure 17: National summary of mercury analyses in fish tissue for phase 1 of the Metal Mining EEM Program (means ±1 standard error; n = 5 to 33).

Note: Each pair of bars represents one study. Fish species are ordered from large bodied to small bodied from left to right, with one study on a mussel included on the far right side.

6.0 Benthic Invertebrate Community Survey

The third primary component of the EEM Program is the benthic invertebrate community survey, which assesses the impacts of mine effluent on fish habitat. The benthic invertebrate survey provides information on the aquatic food resources available for fish and on the degree of habitat degradation due to physical and chemical contamination. The four endpoints used to assess changes in benthic invertebrate communities are total density, taxon richness (number of taxa), Simpson's evenness and the Bray-Curtis index of dissimilarity. In this national assessment, taxa were analyzed at the family level (or above, for a few taxa that were reported only at higher taxonomic levels). See Bowman and Bailey (1997), Bailey *et al.* (2001), Lenat and Resh (2001), and Culp *et al.* (2003) for further discussion of the taxonomic level of resolution.

6.1 Data Processing and Study Designs

A total of 69 mines conducted a benthic invertebrate community survey. Data from 57 surveys, including one joint study, were provided in an electronic format sufficient to be included in the national assessment (Table 4). Of these 57 surveys, 38 used a control/impact design, 18 used a multiple control/impact design, and one used a gradient design. As outlined in Section 3.1, all 57 studies used sampling station groupings such that reference versus near-field comparisons (the focus of this national assessment) could be made using ANOVA. Three mines conducted a joint study using the Reference Condition Approach (RCA) and one mine used artificial substrates; these were excluded from the analyses. One mine received an exemption due to untenable conditions.

Table 4: Frequencies of studies done by all mines and frequencies of studies included in the national assessment, by design type.

Study design type	Number of studies	Number of studies in national assessment
Control / Impact	41	38
Multiple Control /Impact	22[a]	18[b]
Gradient	1	1[b]
Multiple Gradient/artificial substrata	1	0
Reference Condition Approach	1[c]	0
Total	66	57

[a] Includes one study that was conducted jointly by two mines.
[b] The data submitted for these studies were analyzed using reference versus near-field ANOVA comparisons for the national assessment.
[c] Study conducted jointly by three mines.

Overall, most of the benthic invertebrate data submitted electronically were of good quality. As was done for the fish survey, the invertebrate data were initially screened for errors. Five benthic surveys were excluded from the national assessment because the data were not provided or were incomplete and two other surveys were excluded due to errors in the density data. Some mines submitted density data that were not adjusted to number of invertebrates per square meter, but it was possible to correct these manually. It was noted that some mines had difficulty finding suitable reference and exposure sites and that, in some cases, there were differences in habitats sampled among stations and areas. For example, some mines found differences in grain size between the exposure and reference areas and therefore the results for some individual mines should be interpreted with caution. One mine also used different sampling techniques for the reference and the exposure areas; benthic data from this mine were excluded from the analysis.

6.2 Summary of Effect Sizes

The Phase 1 distribution and range in measured exposure versus reference area percent differences for density, taxon richness, the Bray-Curtis index of dissimilarity and Simpson's evenness are shown in Fig. 18. Measured differences were calculated as the exposure area mean minus the reference area mean, expressed as a percentage of the reference area mean.

Of the four benthic invertebrate endpoints, density is known to typically show the greatest range in measured effects (Lowell *et al.* 2003, 2005), and this was observed in for the metal mining survey results (ranged from -99% to 1070% ; Fig. 18a). Taxon richness effects ranged from -85% to 125%. The Bray-Curtis and Simpson's evenness effects ranged from -35% to 430% and -65% to 400%, respectively. Note that, due to the method of calculation, Bray-Curtis measured effects are usually in the positive direction. The three negative values for this endpoint were due to unusual data distributions.

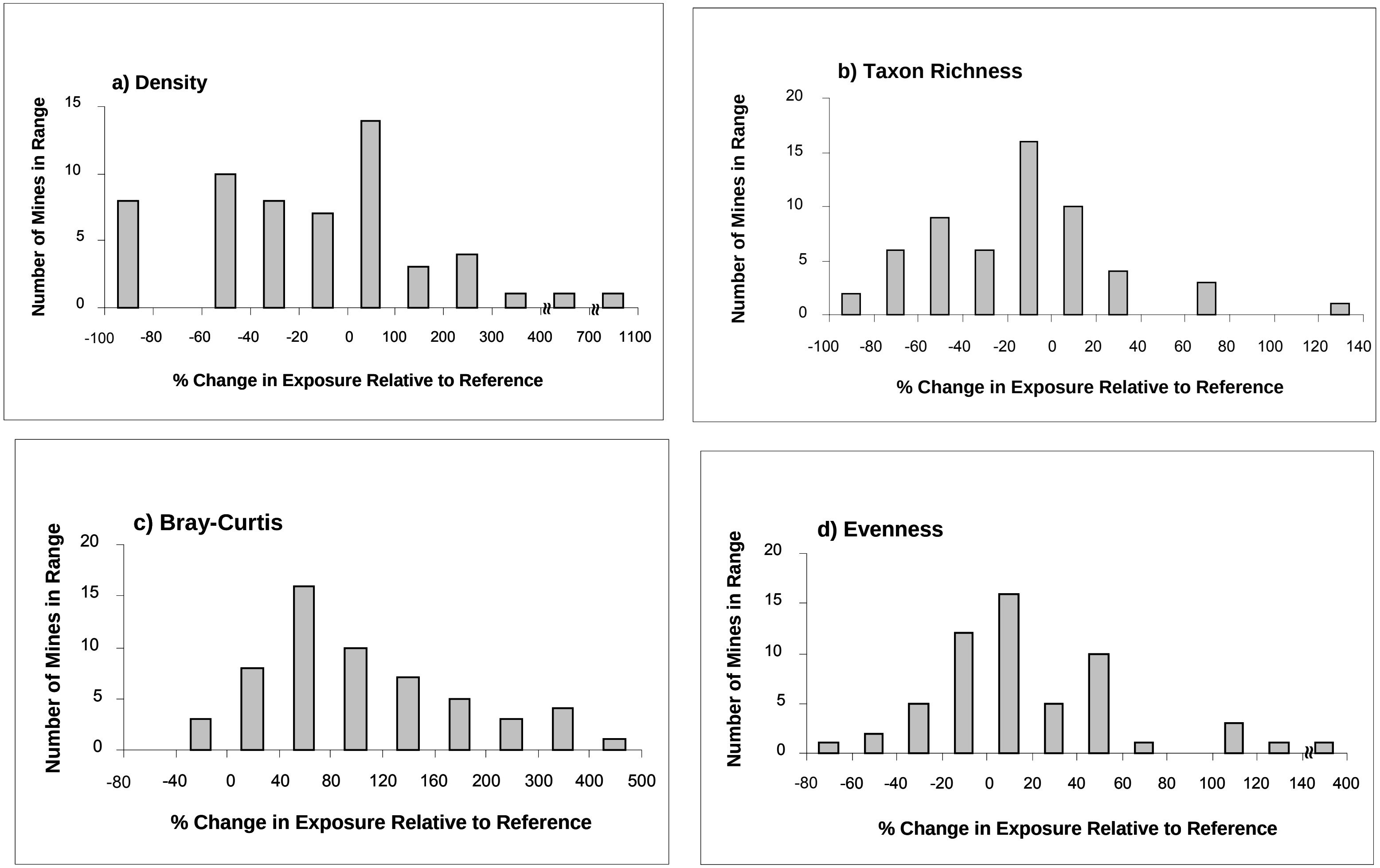

Figure 18: Distribution of measured percent differences between exposure and reference areas for the benthic invertebrate survey for a) density, b) taxon richness, c) Bray-Curtis dissimilarity and d) Simpson's evenness.

Figure 19 shows the number of mines measuring statistically significant or non significant differences in means for each of the four endpoints. For density, taxon richness, and Simpson's evenness, the percentage of mines showing significant differences were 40%, 42%, and 23%, respectively. Bray-Curtis is known to be the most sensitive of the four benthic invertebrate endpoints (Lowell *et al.* 2003, 2005), and 67% of the comparisons were significant for that endpoint (comparable to the condition endpoint for fish).

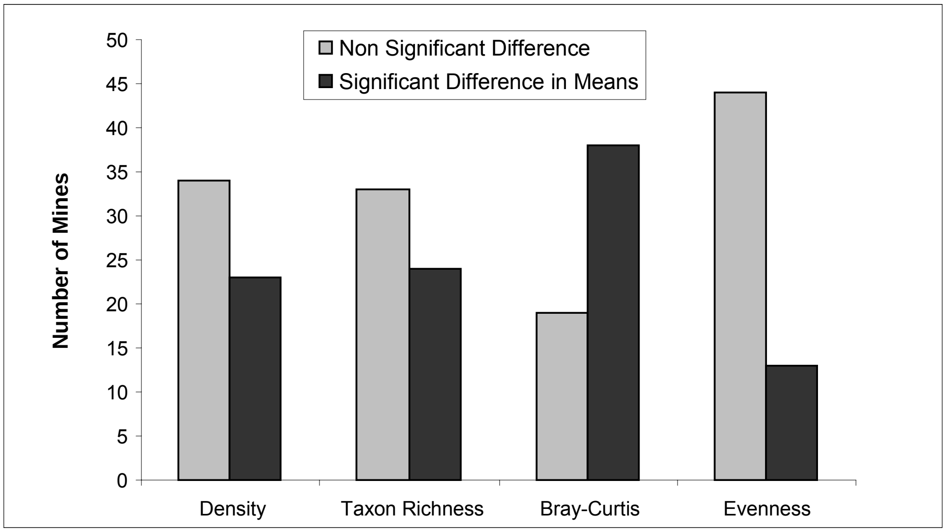

Figure 19: Number of mines showing non-significant and significant differences for the benthic invertebrate community endpoints.

6.3 Response Patterns – National Averages

On a national basis, benthic invertebrates showed significant changes in effluent exposure areas for all four of the core endpoints (Fig. 20). On average, both density and taxon richness were reduced in exposure areas relative to reference areas, reflecting overall inhibitory effects on effluent exposed invertebrates. Similar to the national average fish responses, such inhibitory effects could be due to a variety of causes, including direct toxicity and/or habitat alteration (Lowell *et al.* 1995, 2000, 2003). Due to the way it is calculated, the Bray-Curtis endpoint measures effects in the positive direction. It is known to be the most sensitive of the four endpoints (Lowell *et al.* 2003; also see Fig. 19), and showed the greatest national average effect (farthest from the zero effect line; Fig. 20). This reflected significant changes in community structure in effluent exposed areas. The Simpson's evenness endpoint also showed significant changes in community structure for exposed invertebrates.

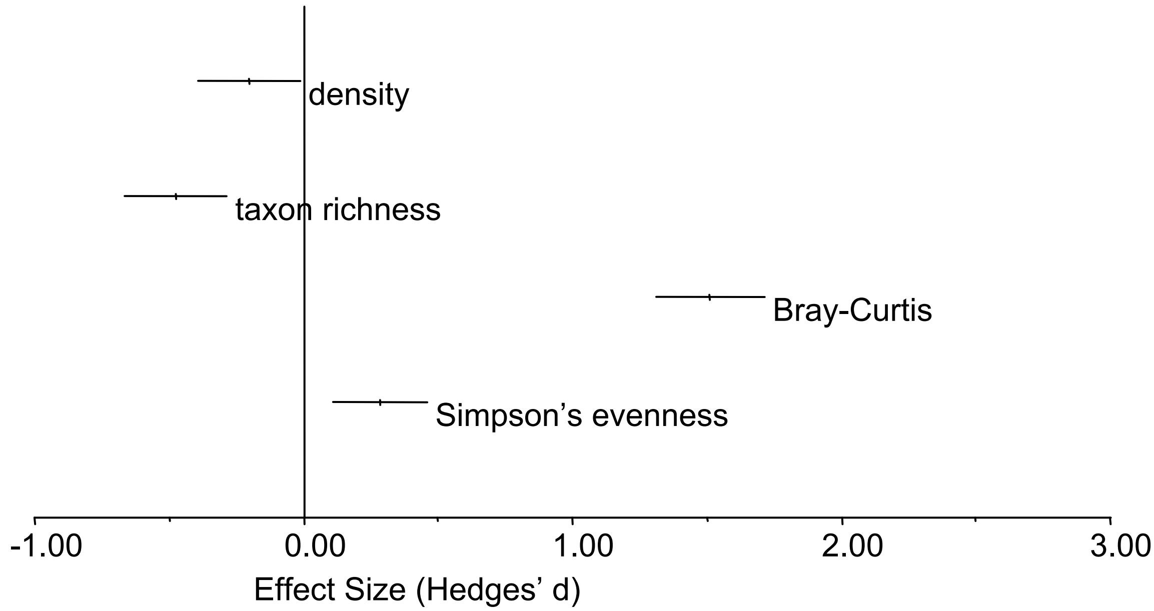

Figure 20: Metal mine grand means for benthic invertebrate community endpoints. Error bars represent 95% confidence intervals. Number of mines = 57.

As was seen for fish (Section 4.3), the national average response pattern for benthic invertebrates exposed to mine effluents differed noticeably from the response patterns that have been repeatedly observed for invertebrates exposed to pulp and paper mill effluents (Lowell *et al.* 2003, 2004, 2005). Pulp and paper mills tend to have a more stimulatory effect, due to the eutrophication effects of nutrient addition (Chambers *et al.* 2000, Culp *et al.* 2000, Lowell *et al.* 1995, 2000). This effect of pulp mill exposure has resulted in a national average increase in density (Fig. 21). Individual pulp mills may show either increases or decreases in taxon richness (or no significant change), with the national average overlapping the zero effect line in Cycle 2. These overall pulp mill effects contrast with the more inhibitory effects of mine effluent exposure.

Figure 21: Pulp and paper Cycle 2 grand means for benthic invertebrate community endpoints. Error bars represent 95% confidence intervals. Number of mills = 62.

6.4 Response Patterns – Other Meta- and Bivariate Analyses

As was done for fish, the benthic invertebrate response patterns can be better understood by dividing the meta-analyses into smaller sub-groupings. Breaking the analyses down by habitat type showed that density was significantly reduced in effluent receiving lake and creek habitats (Fig. 22), although sample size was low for creeks. Note that sample size for the invertebrate meta-analyses refers to the number of mines (or studies), not to the number of sampling stations within a study. Similarly, taxon richness was also significantly reduced in lake habitats (Fig. 23). These invertebrate responses paralleled the significant inhibitory effects seen for fish in lake habitats (Section 4.4). More detailed meta-analyses (not presented here) of the Bray-Curtis endpoint showed that similar significant changes in community structure occurred in all habitat types. Significant changes (increases) in evenness were observed only in lake and creek habitats (with low sample size for creeks) (unpublished analyses).

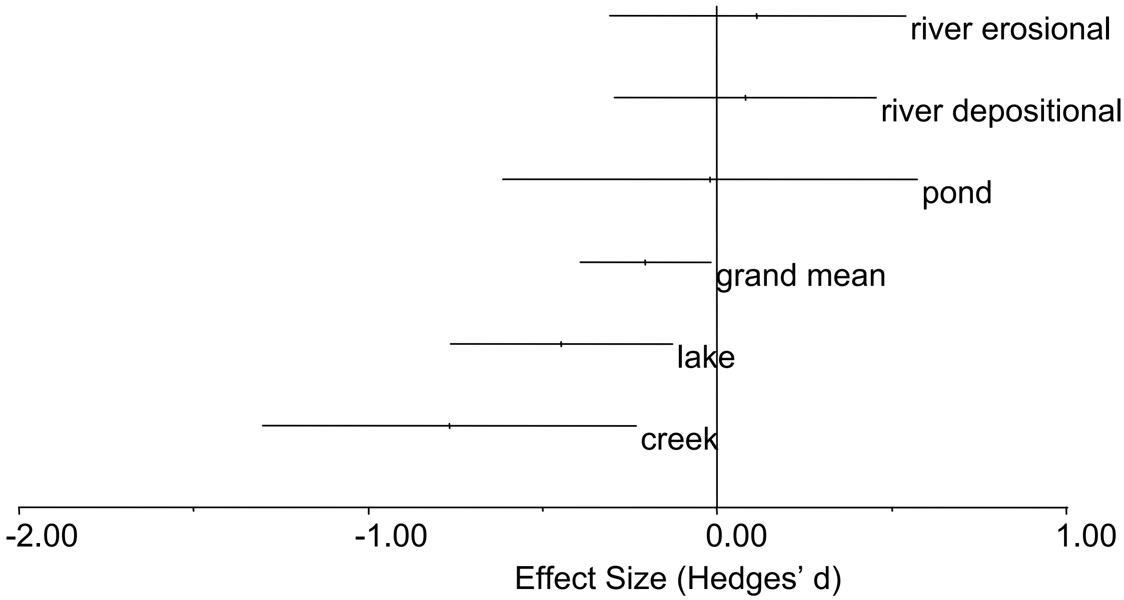

Figure 22: Benthic invertebrate density by habitat type. Error bars represent 95% confidence intervals. Number of mines: river erosional = 12, river depositional = 13, pond = 5, lake = 20, creek = 7.

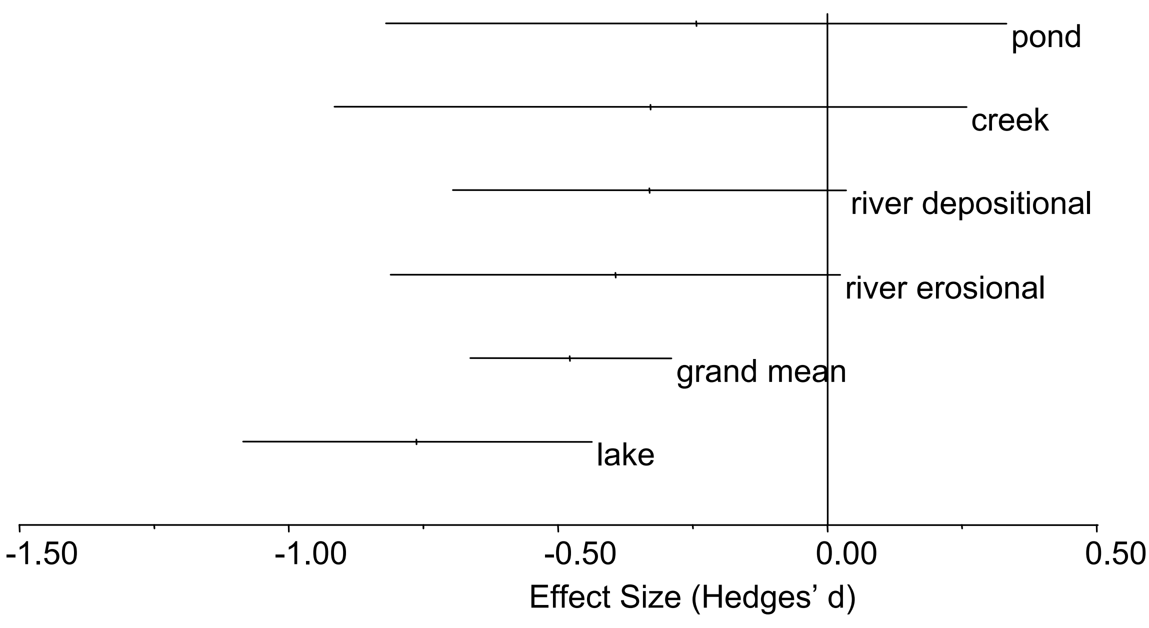

Figure 23: Benthic invertebrate taxon richness by habitat type. Error bars represent 95% confidence intervals. Number of mines: pond = 5, creek = 7, river depositional = 13, river erosional = 12, lake = 20.

More detailed response patterns were also observed when subdividing the benthic invertebrate analyses by ore type, with the two most common types being precious and base metals. Density was significantly reduced for ferrous mines, although the sample size was low (Fig. 24). It should be noted that the significant reduction in the national grand mean for density was also influenced by the larger sample size of precious metal mines showing reduced density, though the precious metal grouping was not statistically significant as a whole. Taxon richness was significantly reduced for both base metal and ferrous mines, with a low sample size for the latter (Fig. 25). All ore types showed similar significant changes in community structure as measured by the Bray-Curtis endpoint (not detailed here). Evenness was significantly increased for both precious metal and ferrous mines (unpublished analyses).

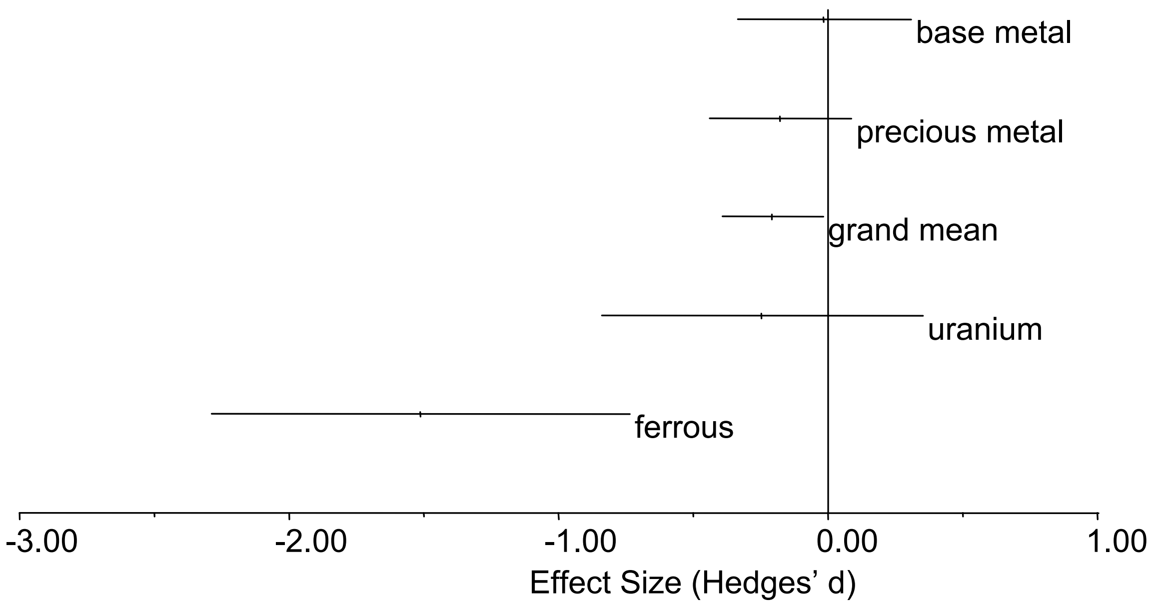

Figure 24: Benthic invertebrate density by ore type. Error bars represent 95% confidence intervals. Number of mines: base metal = 20, precious metal = 27, uranium = 6, ferrous = 4.

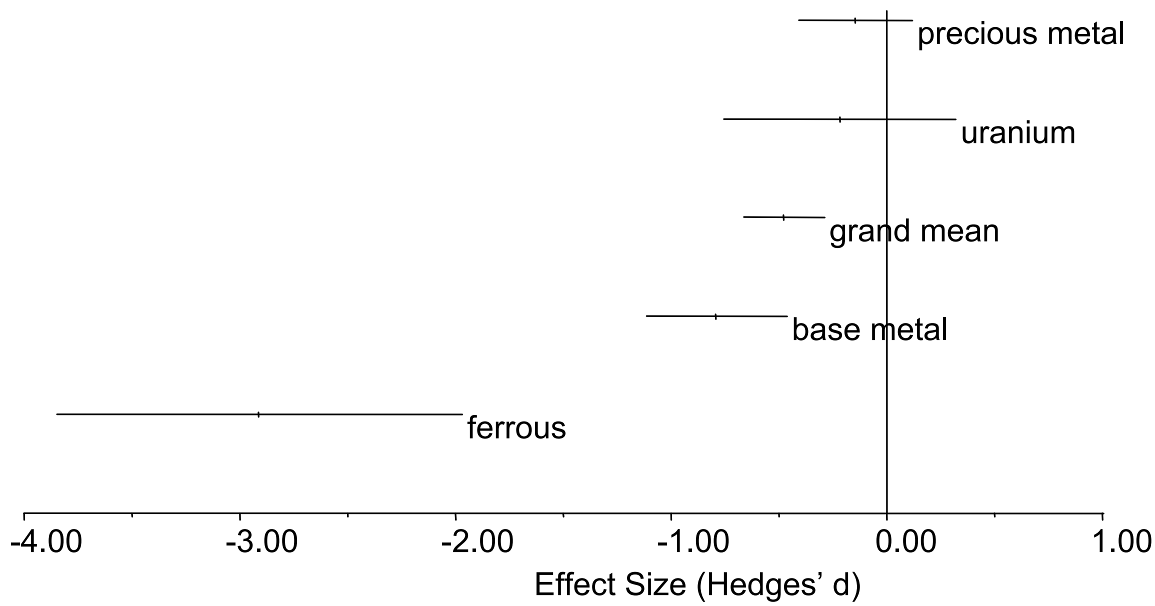

Figure 25: Benthic invertebrate taxon richness by ore type. Error bars represent 95% confidence intervals. Number of mines: precious metal = 27, uranium = 6, base metal = 20, ferrous = 4.

The influence of the concentration of effluent on the magnitude of effects was analyzed with regression meta-analyses. Benthic invertebrates were chosen for these analyses because their more sessile nature facilitates assigning more precise estimates of effluent concentration than is feasible for fish, which are characteristically more mobile and, as such, may potentially be exposed to more variable effluent concentrations than invertebrates. Concentration of effluent significantly influenced the magnitude of response for the density, taxon richness, and evenness endpoints (Figs. 26 through 29). Each point in these figures represents a different mine study; the best-fit regression lines from the meta-analyses are also included. On average, greater effects occurred at higher concentrations, as expected. A perhaps surprising result, however, was the finding that concentration of effluent only accounted for a small proportion of the heterogeneity in the data (1 to 16%), despite the very wide range in concentrations (1 to 100%) that were present in the sampling areas for the various mines across the country. This shows that concentration of effluent does not have an overwhelming influence and is only one of several factors that can influence the magnitude and pattern of effects.

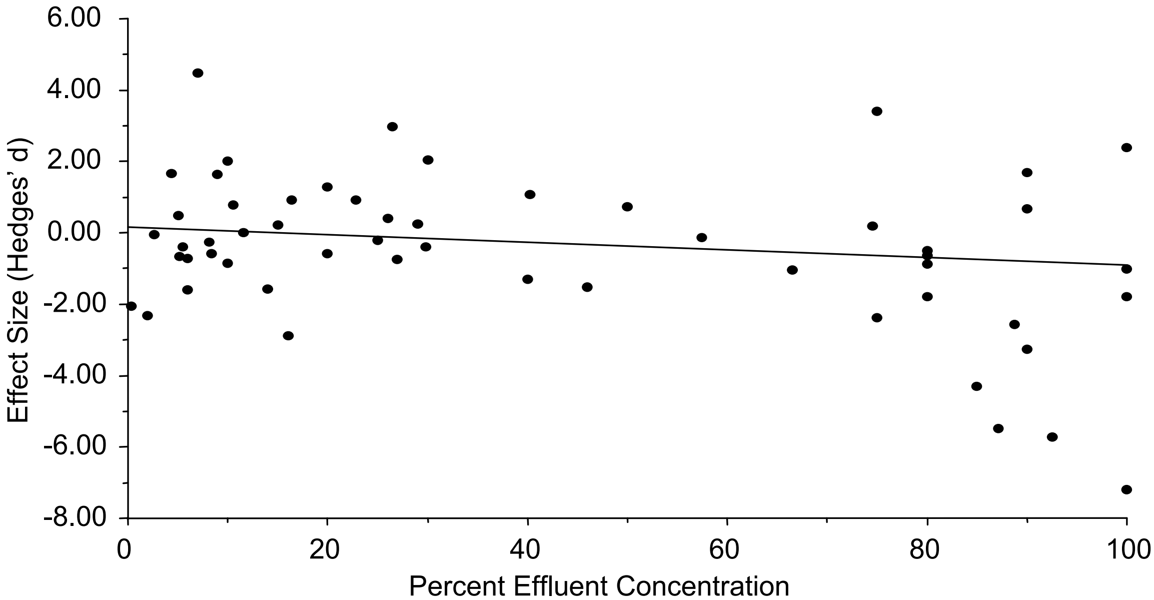

Figure 26: Benthic invertebrate density regression meta-analysis of standardized effect size versus effluent concentration. Proportion of heterogeneity explained by concentration of effluent = 5%, *P*<0.001.

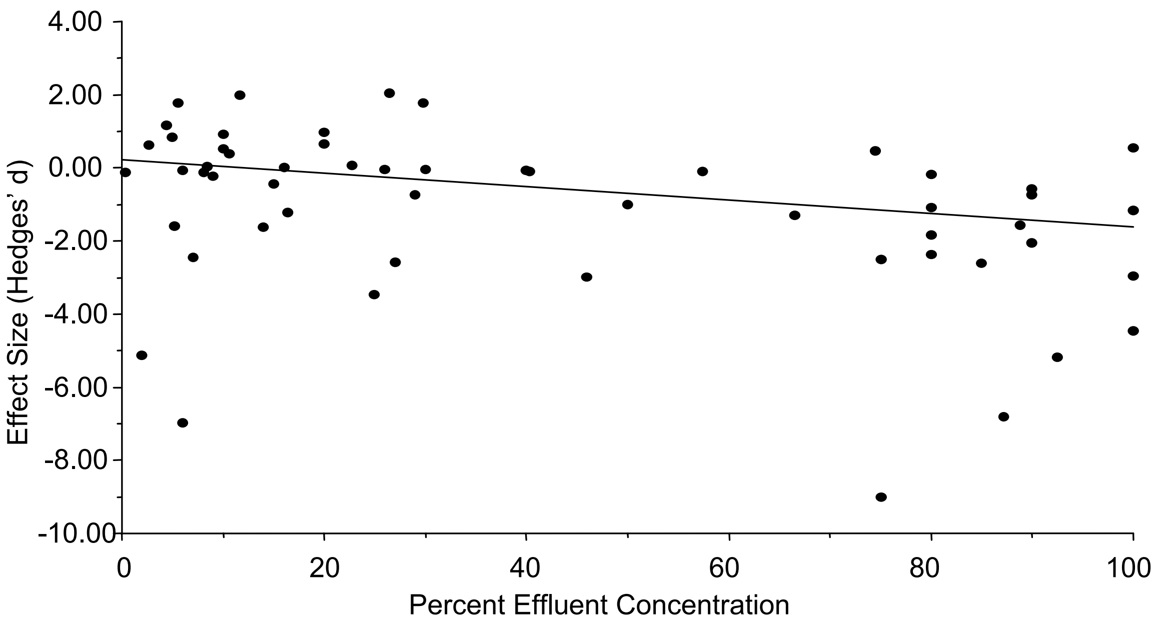

Figure 27: Benthic invertebrate taxon richness regression meta-analysis of standardized effect size versus effluent concentration. Proportion of heterogeneity explained by concentration of effluent = 16%, *P*<0.001.

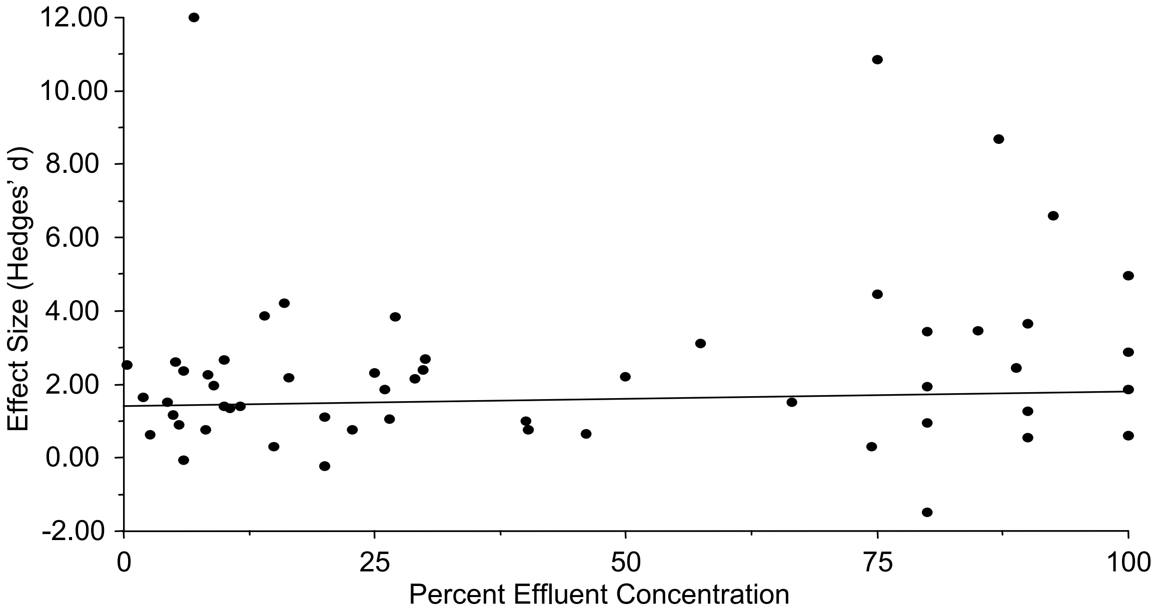

Figure 28: Benthic invertebrate Bray-Curtis regression meta-analysis of standardized effect size versus effluent concentration. Proportion of heterogeneity explained by concentration of effluent = 1%, *P*=0.183.

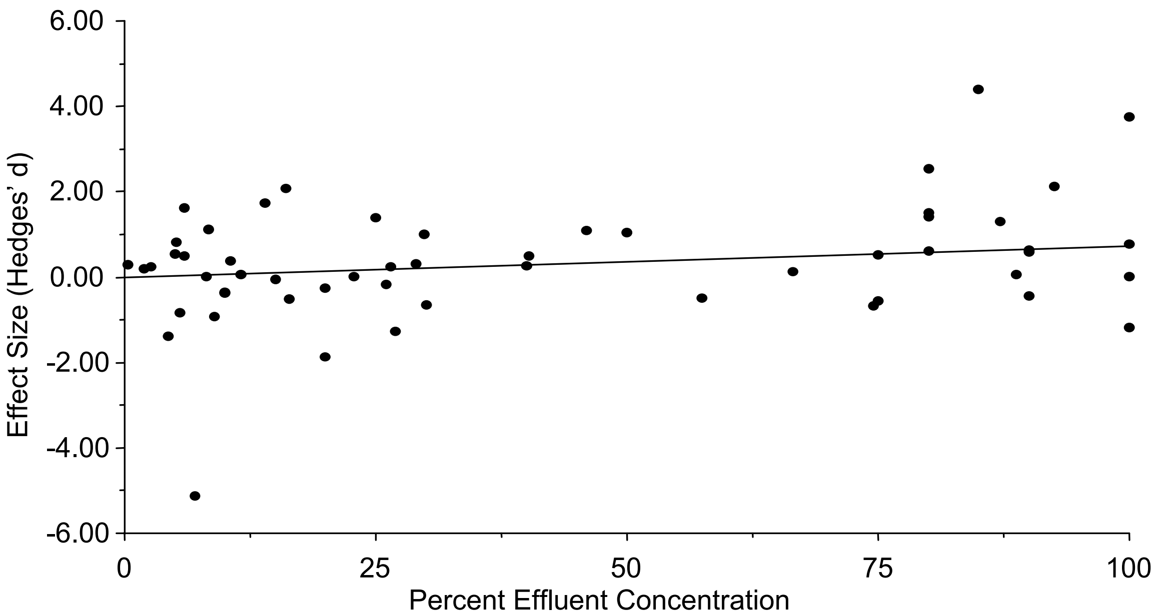

Figure 29: Benthic invertebrate evenness regression meta-analysis of standardized effect size versus effluent concentration. Proportion of heterogeneity explained by concentration of effluent = 6%, *P*=0.007.

National response patterns for benthic invertebrates were further investigated via bivariate plotting of the output of the meta-analyses for density and taxon richness

(Fig. 30). Each data point corresponds to a separate mine study. Exposure areas for mines falling into the two quadrants on the right (increased density) were showing invertebrate responses typically associated with various levels of eutrophication. Mine exposure areas in the lower right showed increases in density and decreases in taxon richness, characteristic of more pronounced eutrophication. Those in the lower left quadrant showed decreases in both density and taxon richness, a more inhibitory response often associated with toxicity effects and/or habitat alteration. As expected, few points fell within the upper left quadrant (reduced density, increased taxon richness). Usually, when impacts are great enough to cause decreases in density, taxon richness is also reduced (Lowell *et al.* 2003).

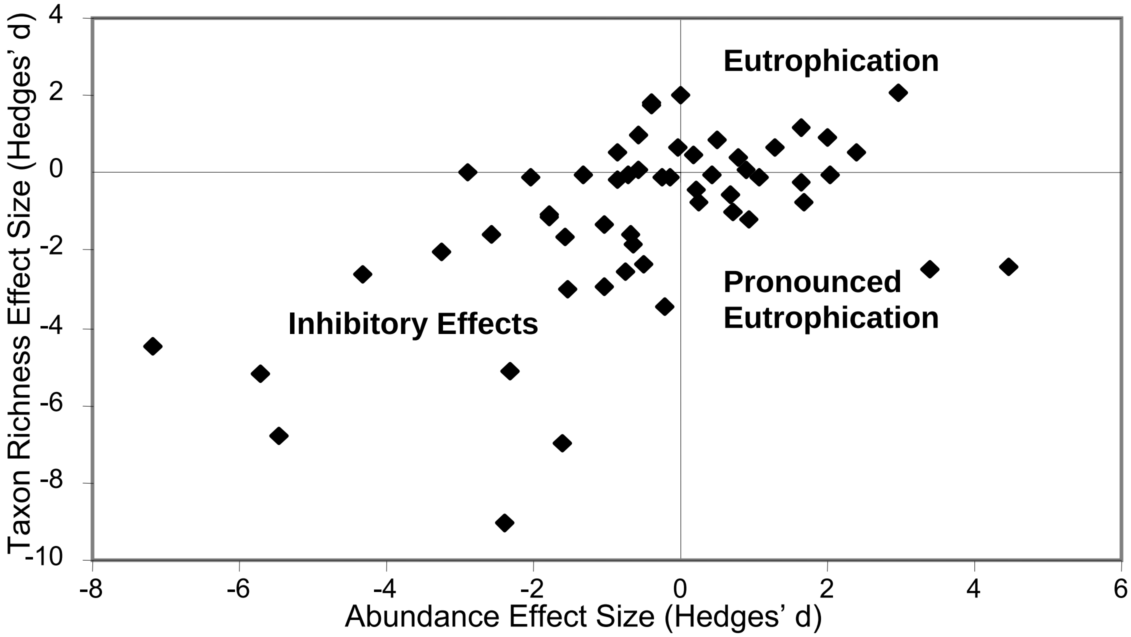

Figure 30: Metal mine bivariate plot of output of benthic invertebrate meta-analyses.

As also described in Section 6.3, these mine response patterns differed markedly from those that have been measured for invertebrates exposed to pulp and paper mill effluents. As noted above, pulp and paper mill effluents frequently have nutrient enrichment effects, resulting in a large number of data points falling into the two eutrophication quadrants on the right (Fig. 31). In contrast, the mine data points were distributed much more heavily toward the lower left quadrant (Fig. 30), again reflecting the more inhibitory effects for mine effluent-exposed invertebrates.

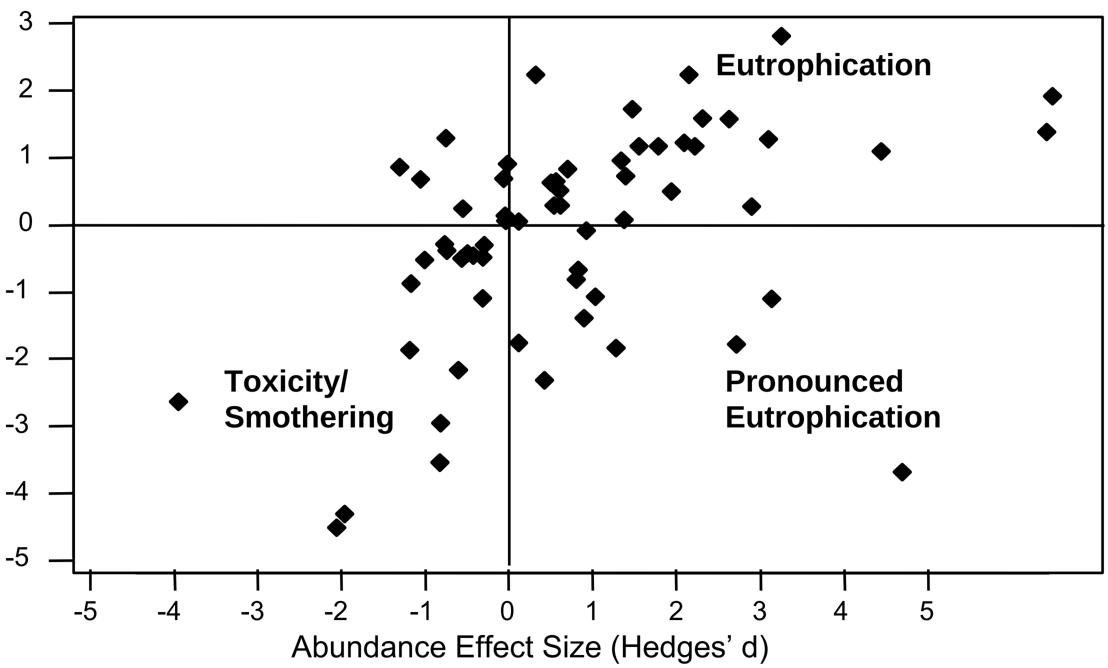

Figure 31: Pulp and paper Cycle 2 bivariate plot of output of benthic invertebrate meta-analyses.

7.0 Summary and Conclusions

The first round of data collection for the metal mining EEM Program has produced a geographically extensive database for evaluating the effects of mine effluents across the country. Nationally integrated analyses of the fish and benthic data have revealed a number of response patterns in receiving water biota, as summarized in this report.

At a national scale, several lines of analysis showed that mine effluent effects tended to be more inhibitory than stimulatory. For effluent exposed fish, meta-analyses revealed significant reductions in condition and relative liver size when looking at all mines nationally. For benthic invertebrates, meta-analyses across all mines showed significant reductions in density and taxon richness, contributing to significant changes in community structure, as measured by the Bray-Curtis and Simpson's evenness endpoints. For both fish and invertebrates, these conclusions were further reinforced by inspection of the national distribution of measured effects shown in the histograms in Sections 4.2 and 6.2, as well as the multivariate and bivariate analyses in Sections 4.4 and 6.4. Of note, however, is that these latter analyses showed that stimulatory effects were also observed at a smaller number of mines.

The tendency for inhibitory effects was particularly evident when comparing these results with the one other industry (pulp and paper) that has been studied at this scale in Canada. Similar analyses of pulp and paper EEM data have repeatedly revealed more stimulatory effects at a national scale, such as significant increases in fish condition, growth rate, and relative liver size, as well as increases in benthic invertebrate density, although metabolic disruption in gonad growth was also observed (Lowell *et al.* 2003, 2005). For pulp and paper mills, these stimulatory effects are thought to result from the input of excess nutrients into receiving waters. Thus, the data suggest that inhibitory effects are comparatively more common for biota exposed to metal mine effluents. This could be due to a variety of causes, ranging from the direct effects of toxicity (Hruska and Dubé 2004) and habitat alteration to indirect effects such as food limitation due to effluent effects on prey organisms (Munkittrick and Dixon 1988) and toxicity to fish through a dietary exposure pathway, i.e., metal-contaminated invertebrates (Hansen *et al.* 2004, Woodward *et al.* 1994, 1995).

It should be noted, however, that the metal mining industry is fairly heterogeneous, and analyses of smaller subgroupings of data help to provide a more complete picture of mine effluent effects. For example, for fish, condition and relative liver size were significantly reduced in effluent-receiving lake habitats and for precious metal, uranium, and/or ferrous mine effluents. On the other hand, relative gonad size was significantly increased in river habitats, together with a nearly significant increase for relative liver size, showing the influence of habitat type on response patterns. Furthermore, effluent exposure had significantly different effects on relative gonad size and age for female versus male fish. Different fish species also responded differently, although interpretation was difficult because most species were only used in a small number of studies. Benthic invertebrates showed a similar diversity of effects. For example, reductions in density and taxon richness were more pronounced in effluent-receiving lake habitats and for ferrous mines than for most other subgroupings.

Two factors that have been hypothesized to further influence mine effluent effects are concentration of effluent and whether mines discharge intermittently versus continuously. As expected, greater effects on benthic invertebrates were observed at higher concentrations of effluent in the receiving environment. Nevertheless, concentration of effluent only accounted for a small proportion of the heterogeneity in measured effects, demonstrating that it does not have an overwhelming influence on the magnitude of effects. None of the nine fish and invertebrate core endpoints was significantly correlated with the number of months during which mines discharged during the year. Thus, effluent effects did not appear to be greatly influenced by whether mines discharge effluent intermittently or continuously.

Fish and benthic invertebrate responses showed good agreement, not just in terms of the national averages, but also when dividing the data into smaller subgroups. For example, inhibitory effects often co-occurred for fish and invertebrates within habitat type (e.g., lakes, creeks) and within ore type (e.g., ferrous). This might have been due to direct effects on both fish and invertebrates, as well as to indirect effects, such as food limitation for fish due to reductions in their invertebrate prey (Munkittrick and Dixon 1988).

Effluent effects on fish usability were also evaluated via measurements of mercury levels in fish tissue. These measurements were required when mercury concentration in the effluent exceeded 0.1 µg/L. Only one mine detected tissue mercury concentrations exceeding the 0.45 µg/g "effect" level in exposure area fish which were significantly greater than reference area levels. Thus, at this time, the available data do not suggest that metal mine effluents were broadly linked to high mercury levels in fish tissue.

Much effort has been expended in the EEM Program to design studies that distinguish effects due to recent discharges versus effects caused by older historical discharges or other factors that may influence measured responses (e.g., multiple land uses, other industrial or municipal effluent sources, etc.). Even so, uncertainties remain at some mines. As the EEM Program progresses through future rounds of data collection, continuous improvements in study design and analysis, as well as ongoing research at selected mines, are expected to help better understand how such factors may contribute to the effects that are measured.

Given that the Metal Mining EEM Program has only completed one phase of data collection, these findings necessarily represent a preliminary look at mine effluent effects in Canada. Although a substantial amount of data for a large number of mines is summarized in this report, these data represent one point in time. Further rounds of data collection will measure how constant or variable these response patterns are through time. For example, future EEM studies will help determine where effluent effects are improving, worsening, or staying the same in terms of both individual mines, as well as larger groupings of mines. Thus, as ongoing EEM data collection progresses, future analyses are expected to provide a more comprehensive picture of metal mining effluent effects in Canada.

8.0 Glossary

Benthic invertebrate community – The interacting populations of small animals (excluding fish and other vertebrates), living at the bottom of a water body, on which fish may feed. Measuring changes in invertebrate communities helps to understand changes in aquatic habitats and provides an evaluation of the aquatic food resources available to fish.

Bray-Curtis index – An index that measures the degree of difference in community structure (especially community composition) between sites. This measure helps to evaluate the amount of dissimilarity between benthic invertebrate communities at different sites.

Condition – A measure of the physical condition of fish that describes the relationship between body weight and body length. Essentially, condition measures how “fat” fish are at each area.

Control/impact design – A study design consisting of no less than one reference area, usually upstream from the mine or situated in a different watershed, and one or a series of exposure areas that are often downstream from the mine.

Density – The total number of individuals of all taxonomic categories collected at the sampling station, expressed per unit area (i.e., total abundance).

Depositional – Section of a riverine (or other) habitat where the flow of water tends to be slower and therefore where sediment tends to deposit. The bottom substrate in these areas tends to be softer and more silty or granular in nature.

Effect – In the context of the EEM Program, an effect is a statistically significant difference between measurements taken from the exposure area and from the reference area or measurements taken from sampling areas that have gradually decreasing effluent concentrations.

Endpoint – A particular measurement that is used as an indicator of potentially important effluent effects on receiving water biota. Examples of endpoints are gonad weight, liver weight, condition, age and weight at age for fish or density, taxon richness, Simpson’s evenness index and Bray-Curtis index of dissimilarity for benthic invertebrates.

Erosional – Section of a riverine (or other) habitat where the flow of water tends to be fast and turbulent. In these areas, sediments are usually carried downstream. Generally, the bottom substrate in these sections tends to be made up of larger sediments, rocks and boulders.

Eutrophication – The process of over fertilization of a body of water by nutrients that often results in excessive production of organic biomass and is typified by large numbers of organisms and, when pronounced, few species. Eutrophication can be a natural process, or it can be accelerated by an increase of nutrient loading to a water body by human activity.

Exposure area – A sampling area where fish and benthic invertebrates are exposed to mine effluent. This area may extend through a number of receiving environments and contain a variety of habitat types.

Gradient design – Generally, sampling is done along a gradient of decreasing effluent concentration, starting with exposure areas close to the mine and progressing towards less exposed areas farther from the mine. This study design was sometimes used in situations where rapid effluent dilution was a factor.

Metabolic disruption – Metabolism is a mechanism used by the body whereby complex substances are synthesized from simple ones or complex substances are broken down. The disruption of this system can occur from exposure to deleterious substances in the environment and can cause important imbalances in the maturation, sexual behaviour, growth, etc. of the organism.

Nutrient enrichment – The effect of adding large quantities of organic and inorganic nutrients to the environment.

Reference area – A sampling area that has no effluent exposure from the mine in question and natural habitat features that are similar to those of the exposure area, including anthropogenic impacts.

Relative gonad weight – A measure of fish reproductive investment that describes the relationship between gonad weight and body weight.

Relative liver weight – A measure of fish energy storage and response to toxicant exposure that describes the relationship between liver weight and body weight.

Simpson’s evenness index – A measure of how evenly individuals are distributed among taxa. This measure helps to evaluate changes in the relative abundance of taxa.

Smothering – The overaccumulation of organic matter derived from pulp mill effluent at the bottom of a water body, impeding the functioning of organisms and sometimes causing death.

Sublethal toxicity – In the context of EEM, sublethal toxicity tests usually measure the proportion of organisms affected by their exposure to specific concentrations of mine effluent in a laboratory setting. A sublethal toxicity test measures what is detrimental to the organism (e.g., effects on growth or reproduction), but below the level that directly causes death within the test period.

Taxon – Organisms are classified into categories based on similarities and evolutionary relationships between them. Each of these categories (e.g., species, genus, family, phylum, etc.) is called a taxon (plural taxa).

Taxon richness – The total number of different taxonomic categories collected at a sampling station.

Weight at age – A measurement of the rate of growth of fish described by the relationship of size (weight) to age. Over the entire life span of a fish, the rate of increase in size may decline as the fish ages.

9.0 References

Bailey, R.C., R.H. Norris, and T.B. Reynoldson. 2001. Taxonomic resolution of benthic macroinvertebrate communities in bioassessments. J.N. Am. Benthol. Soc. 20: 280-286.

Belbin, L. 1992. PATN pattern analysis package: technical reference. CSIRO, Lyneham, Australia.

Bowman, J.F. and R.C. Bailey. 1997. Does taxonomic resolution affect the multivariate description of the structure of freshwater benthic macroinvertebrate communities? Can. J. Fish. Aquat. Sci. 54: 1802-1807.

Chambers, P.A., A.R. Dale, G.J. Scrimgeour, and M.L. Bothwell. 2000. Nutrient enrichment of northern rivers in response to pulp mill and municipal discharges. J. Aquat. Ecosyst. Stress Recov. 8: 53-66.

Culp, J.M., R.B. Lowell and K.J. Cash. 2000. Integrating mesocosm experiments with field and laboratory studies to generate weight-of-evidence risk assessments for large rivers. Environ. Toxicol. Chem. 19: 1167–1173.

Culp, J.M., M.E. Wiseman, R.C. Bailey, N.E. Glozier, R.B. Lowell, T.B. Reynoldson, L. Trudel, and G.D. Watson. 2003. New requirements for benthic community assessments at Canadian metal mines are progressive and robust: reply to Orr *et al.* SETAC Globe 4: 31 32.

Dubé, M.G., D.L. MacLatchy, J.D. Kieffer, N.E. Glozier, J.M. Culp and K.J. Cash. 2005. Effects of metal mining effluent on Atlantic salmon (Salmo salar) and slimy sculpin (Cottus cognatus): using artificial streams to assess existing effects and predict future consequences. Science of the Total Environment 343: 135-154.

Eastwood, S and P. Couture. 2002. Seasonal variations in condition and liver metal concentrations of yellow perch (*Perca flavescens*) from a metal-contaminated environment. Aquatic Toxicology 58: 43-56.

Environment Canada. 2002. Metal mining guidance document for aquatic environmental effects monitoring. National EEM Office, Environment Canada, Gatineau, QC.

Faith, D.P., P.R. Minchin, and L. Belbin. 1987. Compositional dissimilarity as a robust measure of ecological distance. Vegetatio 69: 57-68.

Glozier, N.E., J.M. Culp, T.B. Reynoldson, R.C. Bailey, R.B. Lowell and L. Trudel. 2002. Assessing metal mine effects using benthic invertebrates for Canada's Environmental Effects Program. Water Qual. Res. J. Can. 37: 251–278.

Gurevitch, J. and L.V. Hedges. 2001. Meta-analysis. Combining the results of independent experiments. Pages 347-369 in *Design and Analysis of Ecological Experiments*, S.M. Scheiner and J. Gurevitch (eds.), Oxford University Press, New York.

Hansen, J.S., J. Lipton, P.G. Welsh, D. Cacela, and B. MacConnell. 2004. Reduced growth of Rainbow Trout (*Oncorhynchus mykiss*) fed a live invertebrate diet pre-exposed to metal-contaminated sediments. Environ. Toxicol. Chem. 23: 1902-1911.

Hedges, L.V. and I. Olkin. 1985. Statistical methods for meta-analysis. Academic Press, New York, N.Y.

Hewitt, L.M., M.G. Dube, S.C. Ribey, J.M. Culp, R. Lowell, K. Hedley, B. Kilgour, C. Portt, D.L. MacLatchy, and K.R. Munkittrick. 2005. Investigation of cause in pulp and paper environmental effects monitoring. Water Qual. Res. J. Can. 40:261-274.

Hruska, K.A., and M.G. Dubé. 2004. Using artificial streams to assess the effects of metal-mining effluent on the life cycle of the freshwater midge (*Chironomus tentans*) in situ. Environ. Toxicol. Chem. 23: 2709-2718.

Lenat, D.R. and V.H. Resh. 2001. Taxonomy and stream ecology – The benefits of genus- and species- level identifications. J.N. Am. Benthol. Soc. 20: 287-298.

Lowell, R.B. and J.M. Culp. 2002. Implications of sampling frequency for detecting temporal patterns during environmental effects monitoring. Water Qual. Res. J. Can. 37: 119–132.

Lowell, R.B., J.M. Culp, and M.G. Dubé. 2000. A weight-of-evidence approach for northern river risk assessment: integrating the effects of multiple stressors. Environ. Toxicol. Chem. 19: 1182–1190.

Lowell, R.B., J.M. Culp and F.J. Wrona. 1995. Stimulation of increased short-term growth and development of mayflies by pulp mill effluent. Environ. Toxicol. Chem. 14: 1529–1541.

Lowell, R.B., K. Hedley and E. Porter. 2002. Data interpretation issues for Canada's Environmental Effects Monitoring Program. Water Qual. Res. J. Can. 37: 101–117.

Lowell, R.B., K.R. Munkittrick, J.M. Culp, M.E. McMaster, and L.C. Grapentine. 2004. National response patterns of fish and invertebrates exposed to pulp and paper mill effluents: metabolic disruption in combination with eutrophication and other effects. Pages 147-155 in *Pulp and Paper Mill Effluent Environmental Fate and Effects* , D.L. Borton, T.J. Hall, R.P. Fisher, J.F. Thomas, eds., DEStech Publications, Lancaster, PA.

Lowell, R.B., S.C. Ribey, I.K. Ellis, E.L. Porter, J.M. Culp, L.C. Grapentine, M.E. McMaster, K.R. Munkittrick and R.P. Scroggins. 2003. National Assessment of the Pulp and Paper Environmental Effects Monitoring Data. National Water Research Institute, Environment Canada, Gatineau, QC. NWRI Contribution 03-521.

Lowell, R.B., B. Ring, G. Pastershank, S. Walker, L. Trudel and K. Hedley. 2005. National Assessment of Pulp and Paper Environmental Effects Monitoring Data: Findings from Cycles 1 through 3. National Water Research Institute, Burlington, Ontario. NWRI Scientific Assessment Report Series No. 5. 40 p.

Metal Mining Environmental Effects Monitoring Review Team. 2007. Metal Mining Environmental Effects Monitoring Review Team Report. National EEM Office, Environment Canada, Gatineau, QC. http://www.ec.gc.ca/eem/English/Publications/web_publication/mm_review_team/default.cfm

McMaster, M.E., L.M. Hewitt, G.R. Tetreault, L. Peters, J.L. Parrott, G.J. Van Der Kraak, C.B. Portt, K. Kroll, and N. Denslow. 2005. Detailed endocrine assessments of wild fish in the northern river basins, Alberta in comparison to EEM monitored endpoints. Water Qual. Res. J. Can. 40:299-314.

Munkittrick, K.R. and D.G. Dixon. 1988. Growth, fecundity, and energy stores of white sucker *(Catostomus commersoni)* from lakes containing elevated levels of copper and zinc. Can. J. Fish. Aquat. Sci. 45:1355-1365.

Munkittrick, K. R., M.E. McMaster, G. Van Der Kraak, C. Portt, W.N. Gibbons, A. Farwell, and M. Gray. 2000. Development of methods for effects-based cumulative effects assessment using fish populations: Moose River Project. Society of Environmental Toxicology and Chemistry (SETAC) Press, Pensacola, Florida.

Munkittrick, K. R., C. Portt, G.J. Van Der Kraak, I. Smith and D. Rokosh. 1991. Impact of bleached kraft mill effluent on population characteristics, liver MFO activity and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. *Can. J. Fish. Aquat. Sci*. 48: 1371-1380.

Munkittrick, K.R., G.J. Van Der Kraak, M.E. McMaster, C.B. Portt, M.R. van den Heuvel, and M.R. Servos. 1994. Survey of receiving water environmental impacts associated with discharges from pulp mills. 2. Gonad size, liver size, hepatic EROD activity and plasma sex steroid levels in white sucker. Environ. Toxicol. Chem. 13: 1089 1101.

Parrott, J.L. 2005. Overview of methodology and endpoints in fathead minnow lifecycle tests assessing pulp and paper mill effluents. Water Qual. Res. J. Can. 40:334-346.

Rajotte, J. W. and Couture, P. 2002. Effects of environmental metal contamination on the condition, swimming performance, and tissue metabolic capacities of wild yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 59: 1296-1304.

Rickwood, C.J., M.G. Dubé, L.P. Weber, K.L. Driedger, and D.M. Janz. 2006. Assessing effects of metal mining effluent on fathead minnow (*Pimephales promelas*) reproduction in a trophic-transfer exposure system. Environ. Sci. Technol. 40: 6489-6497.

Rosenberg, M.S., D.C. Adams, and J. Gurevitch. 2000. MetaWin: Statistical software for meta-analysis. Version 2.0. Sinauer Associates, Sunderland, Massachusetts.

Woodward, D.F., W.G. Brumbaugh, A.J. DeLonay, E.E. Little, and C.E. Smith. 1994. Effects of rainbow trout fry of a metals-contaminated diet of benthic invertebrates from the Clark Fork River, Montana. Transactions of the American Fisheries Society 123: 51-62.

Woodward, D.F., A.M. Farag, H.L. Bergman, A.J. DeLonay, E.E. Little, C.E. Smith, and F.T. Barrows. 1995. Metal-contaminated benthic invertebrates in the Clark Fork River, Montana: effects on age-0 Brown Trout and Rainbow Trout. Can. J. Fish. Aquat. Sci. 52: 1994-2004.