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Second National Assessment of Environmental Effects Monitoring Data from Metal Mines Subjected to the *Metal Mining Effluent Regulations*

National Environmental Effects Monitoring Office, Forestry, Agriculture and Aquaculture Division, Environment Canada

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Canada

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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 metal mine effluents. Specifically, the EEM comprises

- a fish population survey to assess fish health,
- a benthic (bottom-dwelling) 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 when conditions specified in the MMER are met.

Metal mines also collect supporting data through sublethal toxicity testing, water and sediment quality monitoring, and effluent characterization. Results of EEM are used to evaluate the effectiveness of the MMER, including the effects of metal mine effluents on the environment. EEM information provides a basis for current and future water pollution prevention and control technologies, practices and programs within the mining sector.

The MMER EEM reporting period is structured into "phases," whereby a mine conducts an EEM study every two to six years according to conditions specified in the Regulations. The sequence of EEM studies is based on a tiered approach to monitoring from one phase to another. Initial field surveys are followed by 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 metal mining EEM program has now completed two national assessment periods of monitoring. The purpose of this report is to present and discuss the major findings of the MMER EEM results, using data collected by metal mines across Canada. Several lines of analysis, based now on two national assessments, indicate that effects related to the discharge of effluent from metal mines tend to be more inhibitory than stimulatory. That is, effluent exposure was more often associated with reductions rather than increases in the indicators (endpoints) such as growth rate of fish, which are used to assess effects.

During at least one of the national assessment periods, fish collected in areas exposed to effluent, referred to as exposure areas, showed significantly[1] reduced condition, relative liver size, and growth rate. Other effects included some reductions in gonad (reproductive organ) size and a significantly increased age structure. In other words, fish collected in areas exposed to effluent were, on average, older, thinner and slower-growing, with smaller

[1] In this context, significance refers to a statistically significant difference at a national level when comparing monitoring results collected in effluent exposure areas to results collected in reference areas not exposed to effluent.

livers and with more of a tendency toward reduced gonad size. These generally inhibitory response patterns may reflect direct inhibitory effects of the effluent on fish, and/or food limitation resulting from habitat alteration and inhibitory effects on prey organisms, such as benthic invertebrates.

Data for benthic invertebrates collected in exposure areas from both national assessment periods showed significantly reduced taxon richness. That is, there were fewer kinds of benthic invertebrates found in exposure areas. The Bray-Curtis endpoint, which measures differences in community structure, revealed different groupings of benthic invertebrates in exposure areas compared to reference areas. Relative to the first assessment period, a national average increase in benthic invertebrate density (number of individuals per unit area) was observed during the second national assessment period, which could indicate that some mine effluents may have stimulatory effects on benthic invertebrates.

Reduced growth rate of fish collected in exposure areas was most strongly associated with metal mines that discharge effluent to lake and river habitats, and with base metal and iron ore mine types. Smaller gonads in fish collected in exposure areas were mostly associated with mines that discharge effluent directly into river habitats, as well as precious and base metal mines. The presence of older fish in exposure areas was associated with metal mines that discharge effluent to lake and river habitats, as well as precious metal and uranium mine types. Increased benthic invertebrate density was associated with all habitat and ore types, with the exception of river erosional habitat types.

Analyses of benthic invertebrate community data in relation to effluent flow data generally did not indicate that changes in effluent flow had an influence on changes in magnitudes and patterns of effect for either density or taxon richness, although site-specific exceptions may exist.

At this time, the available data do not suggest that metal mine effluents were broadly linked to high mercury levels in fish tissue.

To supplement the primary field surveys, sublethal toxicity testing is conducted on effluent from the mine’s final discharge point. This testing monitors effluent quality by measuring survival, growth and/or reproduction endpoints for organisms in a controlled laboratory environment. The tests showed fairly similar effluent quality over the two national assessment periods, though future tests may prove useful in determining whether effluent quality is improving.

Although a substantial amount of data for a large number of mines is summarized in this report, these data represent just two monitoring periods, and some of the variations between phases may have been partly due to factors other than effluent exposure. Further rounds of data collection and analysis will help to shed light on how constant or variable these response patterns are through time. Of particular interest, some mines are entering the investigation of cause phase, which is expected to further elucidate the nature of metal mining effluent effects on receiving waters in Canada.

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1.0 Introduction

1.1 The Metal Mining Effluent Regulations *and EEM*

The *Metal Mining Effluent Regulations* (MMER) came into force in 2002 under the *Fisheries Act*. The Regulations prescribe discharge limits for arsenic, copper, cyanide, lead, nickel, zinc, total suspended solids, radium 226 and pH, and require the effluent to be non-acutely lethal to rainbow trout. These end-of-pipe limits provide a national technology-based standard that is intended to protect fish, fish habitat and the use of fisheries resources. A map illustrating the location of metal mines subject to the MMER is included in Appendix A (figure A1).

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

- 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.

Indicators (endpoints) prescribed in the MMER are used to assess the fish population and benthic invertebrate communities. The results of these assessments help determine future monitoring needs and contribute to an understanding of the types of effect profiles from metal mining effluent discharges.

In the context of the MMER, an “effect” is defined as a statistically significant difference in at least one of the select endpoints in comparisons between biological samples taken from an area exposed to a mine discharge (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), but without the presence of mining effluent (e.g., upstream of the mine, or a nearby water body). The EEM effect endpoints used are as follows:

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 Index[2]

The MMER EEM is structured into "phases" (or rounds of monitoring) whereby a mine conducts an EEM study every two to six years, including the monitoring and interpretation components. At the beginning of each phase, each metal mine is required to develop a site-specific study design. At the end of each phase, each mine must submit an interpretative report that summarizes its monitoring results. EEM uses a tiered approach, with initial studies carried out to characterize and assess the existence of effects, followed by 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. Environment Canada has developed technical guidance on all aspects of EEM studies, including study design, as well as analyses and interpretation of data (see Environment Canada, 2012).

EEM practitioners agreed that not all statistically significant differences were of the same risk, and developed a critical effect size (CES) for the core parameters. A CES is a threshold above which an effect may be indicative of a higher risk to the environment. In cases of confirmed effects, the level of effort of further studies is based on whether the effect magnitude is above or equal to, or below, the CES (see Environment Canada, 2012). The CESs are described here to aid in the understanding of the histograms in sections 4.2 and 6.2. CESs were initially developed for the pulp and paper EEM program after EEM data showed that most mills observed an effect in at least one of the effect endpoints. These CESs were adopted from the pulp and paper EEM and used for the metal mining EEM on an interim basis until they were subsequently validated in June 2011 (Table 1).

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[2] A definition can be found in the glossary

Table 1. Critical effect sizes for metal mining environmental effects monitoring program.

Fish Effect Endpoints	**CES[1]**	**Benthic Invertebrate Effect Endpoints**	**CES[1]**
Relative fish gonad weight	± 25%	Density	± 2SD
Relative liver weight	± 25%	Richness	± 2SD
Condition	± 10%	Simpson's Evenness	± 2SD
Weight-at-age	± 25%	Bray-Curtis Index	+ 2SD
Age	± 25%		

[1] Differences in fish population effect endpoints are expressed as percent (%) of reference mean, while differences in benthic invertebrate effect endpoints are expressed as multiples of within-reference-area standard deviations (SDs).

CESs are a non-regulatory management tool used to determine the level of effort of investigative studies (e.g., extent, magnitude and cause of effects) to be conducted by regulated facilities.

In addition to biological data obtained from field studies, support variables are measured (MMER, schedule 5: effluent characterization, sublethal toxicity testing [SLT], water and sediment quality monitoring) in order to contribute to the assessment of effluent quality and field conditions at individual mines. Support variables 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 EEM requirements of the MMER, nor is it intended to cover all analyses conducted. Rather, it focuses on the core EEM components 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). In addition, it summarizes the SLT data reported by metal mines for phases 1 and 2, and discusses test method sensitivity (or responsiveness).

Currently, most metal mines in Canada have completed their second phase of monitoring and reporting, although some new mines that became subject to the MMER more recently have just completed their first phase of monitoring and reporting. The first national assessment of metal mine EEM data was completed in 2008 (Lowell et al. 2008), and examined the results of metal mines that had completed their first phase of monitoring. At that time, all metal mines were in the process of conducting Phase 1 monitoring. Since then, all the metal mines included in the first national assessment either moved on to their second phase of monitoring or became recognized closed mines as per conditions specified in the Regulations and were no longer required to conduct EEM. This second national assessment therefore allows a temporal examination of metal mine effluent effects in Canada. It also examines other metal mines that were required to conduct EEM studies for the first time. Meta-analysis (a set of statistical procedures used to quantitatively synthesize the results of a large number of independent studies) of Phase 2 study results on a national scale has helped to measure how constant or variable the response patterns identified in the first national assessment have been through time.

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 over the first two phases (first and second national assessment periods) of monitoring of metal mine receiving environments across Canada. The data analyses focused on the following questions:

1) What are the types and magnitudes of effects of metal mine effluents on adult fish and benthic invertebrate communities?
2) How constant or variable are these effects through time?
3) How are effects influenced by habitat, ore type and effluent flow rates?
4) What are the effects of metal mine effluent on the usability of fisheries resources with respect to mercury concentrations in fish tissue?
5) What do the sublethal toxicity tests reflect in terms of effluent quality?

2.0 Overview of Studies Conducted in Phases 1 and 2

The second national assessment includes the results of 78 metal mines that conducted EEM studies (Table 2). Of 70 metal mines that were included in the first national assessment, 62 facilities completed their Phase 2 monitoring studies and submitted their biological interpretative reports in 2008 and 2009. The other 8 metal mines from the first national assessment are either mines that subsequently became recognized closed mines and are no longer required to do EEM or mines that subsequently applied to become recognized closed mines and were in the process of completing their final EEM studies but were not required to submit their final interpretive reports within the time frame covered by the second national assessment.

The 62 metal mines that completed their Phase 2 studies submitted their first interpretative reports (Phase 1) in 2005 and 2006. Differences in the timing of the submission of the reports are the result of a one-year extension for the submission of the first interpretative report that was granted to mines that chose to submit a report containing historical biological monitoring data.

Table 2. Number of first and second metal mining national assessment studies.

			Atlantic	Quebec	Ontario	Prairie Northern	Pacific Yukon	Total
Mines conducting EEM studies								
	1st Assessment	Phase 1	4	19	20	22	5	70
	2nd Assessment	Phase 1	2	6	6	1	1	16
		Phase 2	4	16	20	18	4	62
		Total	6	22	26	19	5	78
Benthic invertebrate surveys conducted								
	1st Assessment		4	19	20[a]	21	5	69
	2nd Assessment		6	22	26[b]	19	5	78
Fish surveys conducted								
	1st Assessment	Fish surveys	4	19	19	20	5	67
		Lethal	2	19	9[c]	9[d]	0	39
		Non lethal	2	0	2	4	3	11
		Both	0	0	6[c]	7	0	13
	2nd Assessment	Fish surveys	5	22	25	18	5	75
		Lethal	2	21	9[e]	6	0	38
		Non lethal	1	1	6	1	4	13
		Both	1	0	7[f]	11	0	19
Alternative fish studies								
	1st Assessment		0	0	2[g]	0	2[h]	4
	2nd Assessment		1[i]	0	3[j]	0	1[k]	5
Data not available								
	1st Assessment		0	0	1[l]	2[m]	0	3
	2nd Assessment		1[n]	0	1[o]	1[p]	0	3

[a] Includes one set of two mines and one set of three mines that conducted a joint study.
[b] Two mines conducted a joint study.
[c] Three mines conducted a joint study.
[d] Two mines conducted a joint study.
[e] Two mines conducted a joint study.
[f] Four mines conducted a joint study.
[g] Two mines conducted a joint fish mesocosm study.
[h] One mine – fish hatchbox study, one mine – alternative mussel study.
[i] Caged bivalves.
[j] Three mines conducted a joint fish mesocosm study.
[k] Fish hatchbox study.
[l] Mine was expected to conduct fish mesocosm study but was not discharging effluent at the time of study.
[m] One mine exempt from conducting both fish and benthic invertebrate studies due to untenable conditions, and one not required to conduct a fish study (proportion of effluent in the receiving environment < 1% at 250 m from the final discharge point).
[n] Delayed fish studies until summer 2009.
[o] Lethal survey attempted but insufficient number of fish caught due to extreme dry weather.
[p] Not required to conduct fish studies (proportion of effluent in the receiving environment < 1% at 250 m from the final discharge point).

Following the first national assessment period, 16 additional metal mines completed Phase 1 monitoring studies, and they are included as part of this national assessment. Three of these metal mines submitted their first interpretative reports in 2007 and the other 13 metal mines submitted their reports in 2008 and 2009. Table 2 provides a regional summary of the numbers and types of field surveys undertaken in both the previous and current national assessments.

In the period covered by the first national assessment, all metal mines conducted studies in freshwater with the exception of two facilities in the Prairie and Northern Region[3] that discharged to marine environments. One of these marine mines was exempt from monitoring due to untenable local conditions but did conduct SLT. The second mine conducted the benthic invertebrate survey in a freshwater stream and the fish survey in the marine environment. In the period covered by the second national assessment, all mines conducted studies in freshwater, with the exception of a mine from the Atlantic Region that conducted an alternative caged bivalve study in lieu of a standard fish survey.

In the current national assessment, the majority of metal mines conducted a regular fish survey. One mine was not required to conduct a fish survey, since the proportion of the effluent in the receiving environment was < 1% at 250 m from the final discharge point; one mine attempted to conduct a lethal fish survey but did not catch sufficient fish due to extreme dry weather; and one mine had delayed its sampling, hence its results were not available for inclusion in this assessment.

Very few of the mines conducted alternative studies for the benthic invertebrate and fish surveys. In the Ontario Region, three mines conducted a joint mesocosm study as an alternative to the fish survey. Two mines—one from the Pacific and Yukon Region and one from the Atlantic Region—conducted alternatives to the fish survey, using a fish hatchbox study and a caged bivalve study, respectively. Several metal mines that became subject to the MMER or became a recognized closed mine only conducted SLT and did not conduct a biological monitoring study during the same period: these mines were therefore not included in the biological results of this report, but existing SLT data were included in section 7.0.

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

[3] Environment Canada divides facilities conducting EEM studies into five regions: Atlantic, Quebec, Ontario, Prairie Northern, and Pacific Yukon.

surveys conducted in the second national assessment period. The methodologies employed were similar to those used in previous national assessments of EEM data (metal mine EEM: Lowell et al. 2008; pulp and paper mill EEM: Lowell et al. 2003, 2005; Tessier et al. 2009). As was the case with the 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). To facilitate comparisons of results between the two national assessments, the frequency distributions were categorized into distributions based on the first national analysis and the second national analysis, which is further divided into mines that conducted Phase 1 and Phase 2 studies. Similarly, histograms of the number of significant and non-significant differences were also prepared, and significant differences were further categorized into differences that are below or that exceed (or are equal to) the CES for the first and second national assessment periods.

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 habitat, ore type, or effluent flow rates on effluent effects in the receiving environment). 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. Analysis of variance (ANOVA) or analysis of covariance (ANCOVA) was used to compare calculated endpoints between each reference and exposure area. 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 Environment Canada (2012).

This 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 metal 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 Environment Canada (Booty et al. 2009), was used to calculate the magnitude and statistical significance of effects for the five fish and four benthic invertebrate community endpoints.

Submitted electronic data were screened for obvious errors (e.g., missing data fields, obvious data entry errors, misnamed stations or areas). The use of SAT aided in selecting the appropriate data for analysis, including removing outliers (fish analysis) prior to performing ANOVA or ANCOVA, with SAT, 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 benthic 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 Environment Canada (2012) 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 (e.g., a meta-analysis of multiple studies of the effects of smoking to determine larger trends in the health impacts of smoking). Furthermore, 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 (Figure 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.

In this context, significance refers to a statistically significant difference at a national level when comparing effluent exposure areas to reference areas not exposed to effluent. These national-level analyses were carried out across all mines. Statistical significance therefore reflects repeated effects in the same direction over a large number of mines — a result that is also of biological significance.

It should be noted that for the fish meta-analyses results, the sample sizes indicated in the corresponding figures refer to the number of exposure versus reference area comparisons (or studies), not the number of fish captured within a study.

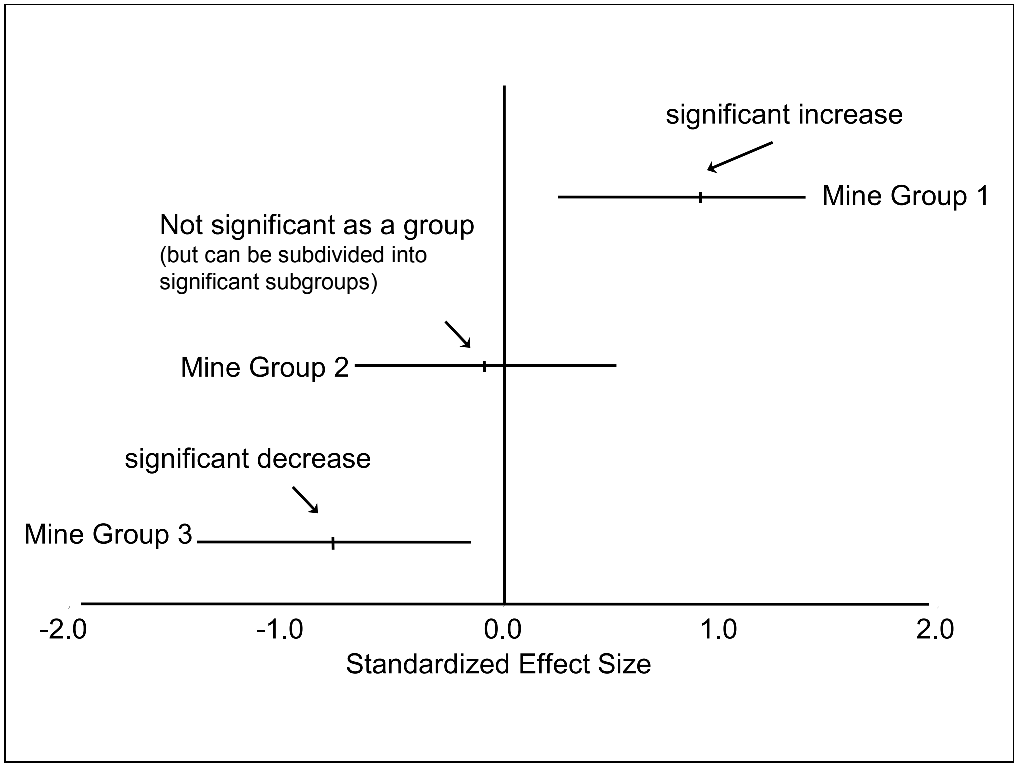

Figure 1. Example of a meta-analysis summary figure. The effect size was measured as Hedges' d (see text).

4.0 Fish Survey

The adult fish survey compares exposure-area fish with those from reference areas to determine if the metal mine effluent is affecting fish populations. The survey uses fish growth, reproduction, condition and age structure to assess the overall health of exposure-area 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 Environmental Effects Monitoring (EEM) Technical Guidance Document (Environment Canada 2012) 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 were available for 54 lethal fish surveys during the second national assessment period. A total of 41 of these surveys, including two joint studies (one study involving four mines and one involving 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, 13 mines conducted only non-lethal fish surveys and 5 mines carried out alternative fish surveys including a fish mesocosm study (joint study involving three mines), 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. The majority of submitted data were of good quality.

Twenty-six fish species were used as sentinel species by metal mines that conducted lethal fish studies during the second national assessment period. Of these 26 species, 24 were included in the national assessment. The frequencies of species used in lethal surveys are presented in Table 3. All fish studies included in the national assessment were conducted in a freshwater environment.

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		1st National Assessment	2nd National Assessment	1st National Assessment	2nd National Assessment
White Sucker	*Catostomus commersoni*	19	16	10	10
Yellow Perch	*Perca flavescens*	6	3	1	3
Brown Bullhead	*Ameiurus nebulosus*	3	3	2	3
Brook Trout	*Salvelinus fontinalis*	2	3	0	2
Northern Pike	*Esox lucius*	10	2	4	2
Walleye	*Sander vitreus*	7	2	0	2
Lake Whitefish	*Coregonus clupeaformis*	6	2	2	2
Burbot	*Lota lota*	5	2	1	2
Brown Bullhead Catfish	*Ictalurus nebulosus*	0	1	0	0
Arctic Grayling	*Thymallus arcticus*	1	1	0	0
Longnose Sucker	*Catostomus catostomus*	1	1	1	1
Mooneye	*Hiodon tergisus*	0	1	0	1
Lake Trout	*Salvelinus namaycush*	4	0	0	0
Round Whitefish	*Prosopium cylindraceum*	3	0	0	0
Arctic Char	*Salvelinus alpinus*	2	0	1	0
Cisco	*Coregonus artedii*	2	0	0	0
Rock Bass	*Ambloplites rupestris*	1	0	0	0
Goldeye	*Hiodon alosoides*	1	0	1	0
Shorthead Redhorse	*Moxostoma macrolepidotum*	1	0	0	0
Fallfish	*Semotilus corporalis*	1	0	0	0
Total number of studies that used large-bodied fish		***75***	***37***	***23***	***28***

[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.

Table 3 (cont'd). 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]	
Small-bodied fish		1st National Assessment	2nd National Assessment	1st National Assessment	2nd National Assessment
Brook Stickleback	*Culaea inconstans*	7	7	6	7
Fathead Minnow	*Pimephales promelas*	2	6	2	3
Pearl Dace	*Margariscus margarita*	6	5	4	4
Lake Chub	*Couesius plumbeus*	6	5	3	5
Spottail Shiner	*Notropis hudsonius*	4	3	2	3
Trout Perch	*Percopsis omiscomaycus*	4	3	3	3
Emerald Shiner	*Notropis atherinoides*	0	3	0	2
Slimy Sculpin	*Cottus cognatus*	1	3	1	2
Mottled Sculpin	*Cottus bairdi*	1	3	1	1
Longnose Dace	*Rhinichthys cataractae*	2	2	2	2
Finescale Dace	*Phoxinus neogaeus*	0	1	0	1
Ninespine Stickleback	*Pungitius pungitius*	2[c]	2	1	1
Brassy Minnow	*Hybognathus hankinsoni*	0	1	0	1
Golden Shiner	*Notemigonus crysoleucas*	2	1	0	1
Logperch	*Percina caprodes*	2	0	1	0
Threespine Stickleback	*Gasterosteus aculeatus*	1	0	1	0
Common Shiner	*Luxilus cornutus*	1	0	0	0
Total number of studies that used small-bodied fish		***41***	***45***	***27***	***36***

[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 Measured Effect Sizes

For each of the five core fish endpoints, Figure 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 and 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). For each endpoint, frequency distributions are shown for each of the following categories: 1) mines conducting Phase 1 studies during the first national assessment period; 2) mines conducting Phase 2 studies during the second national assessment period (these are the same mines that were in Phase 1 during the first national assessment period); and 3) newly regulated metal mines conducting their Phase 1 studies during the second national assessment period.

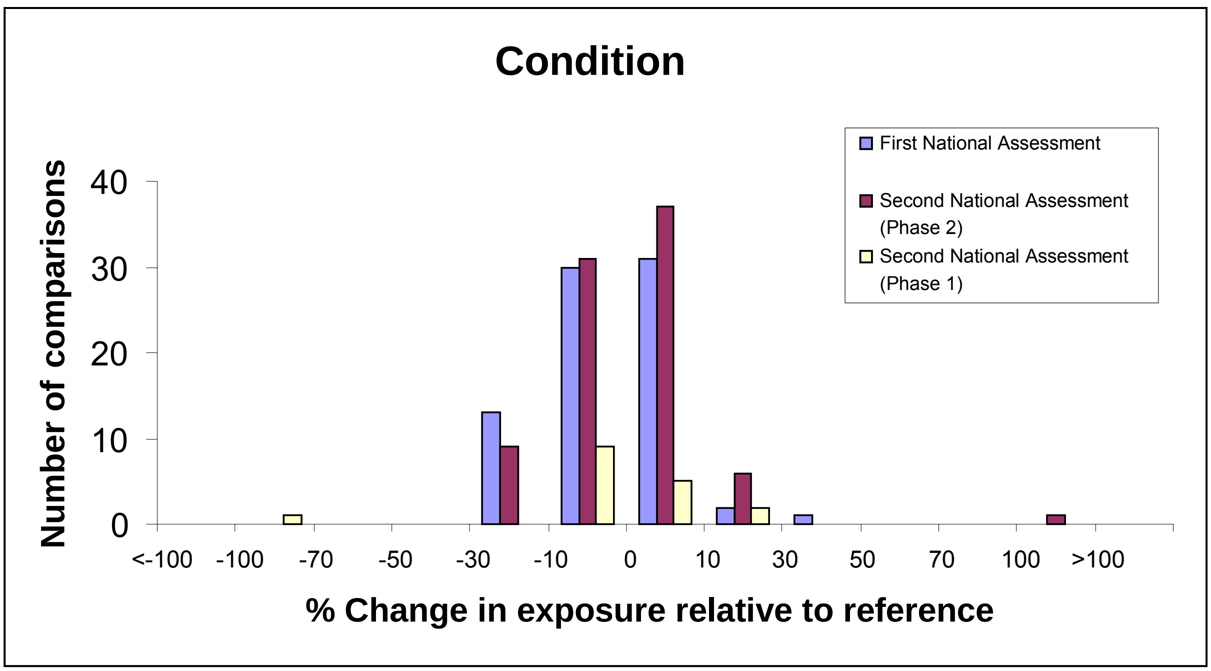

Figure 2a. Distribution of measured percent differences between exposure and reference area fish for condition.

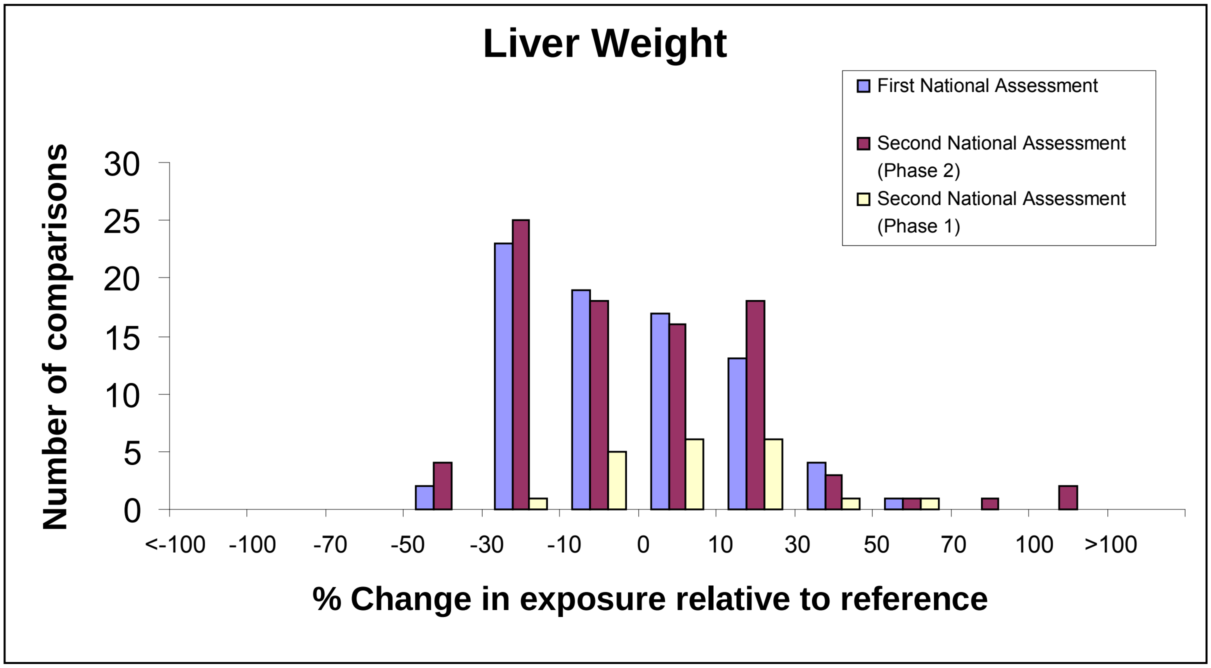

Figure 2b. Distribution of measured percent differences between exposure and reference area fish for relative liver weight.

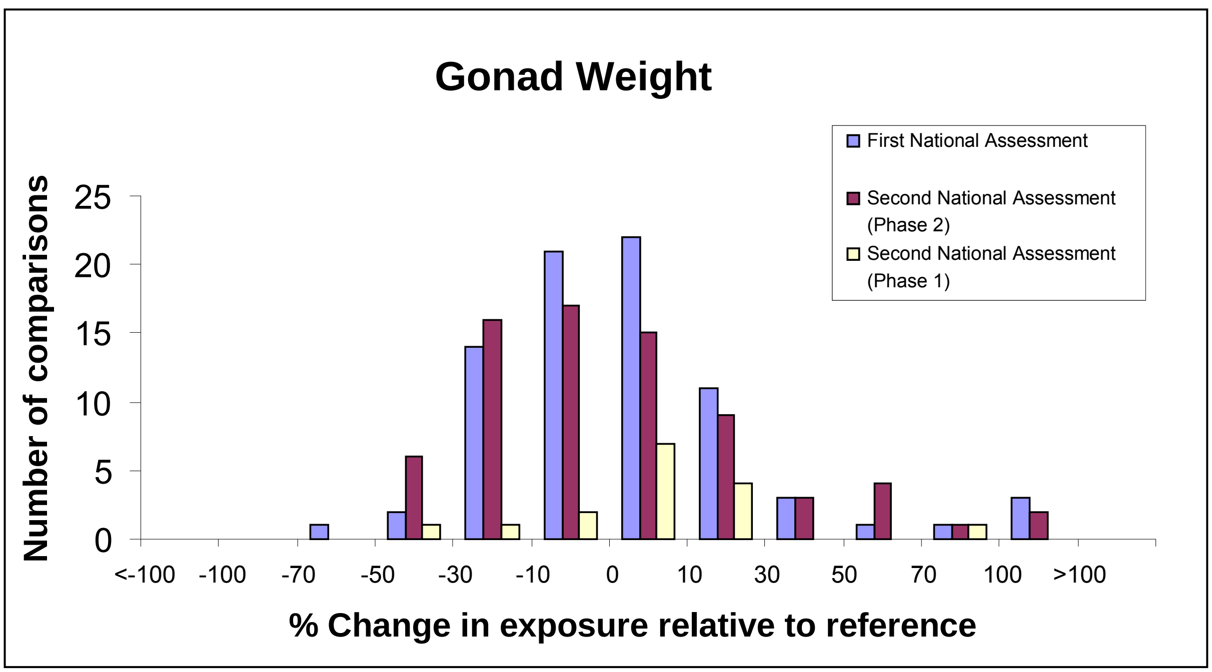

Figure 2c. Distribution of measured percent differences between exposure and reference area fish for relative gonad weight.

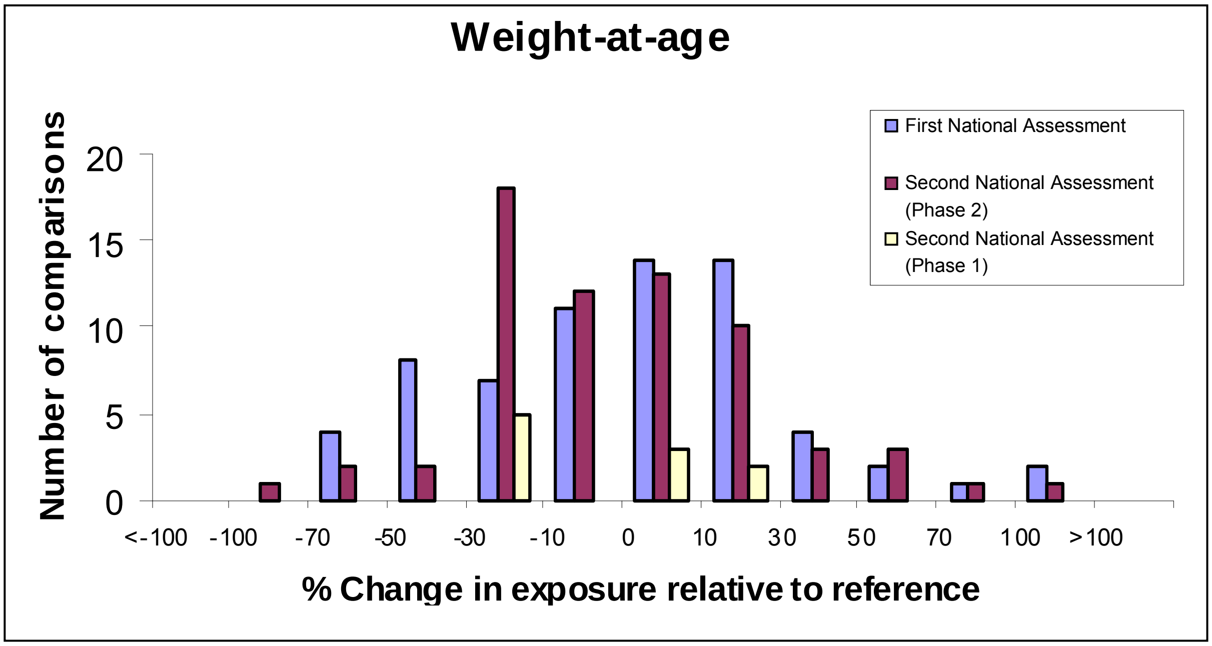

Figure 2d. Distribution of measured percent differences between exposure and reference area fish for weight-at-age.

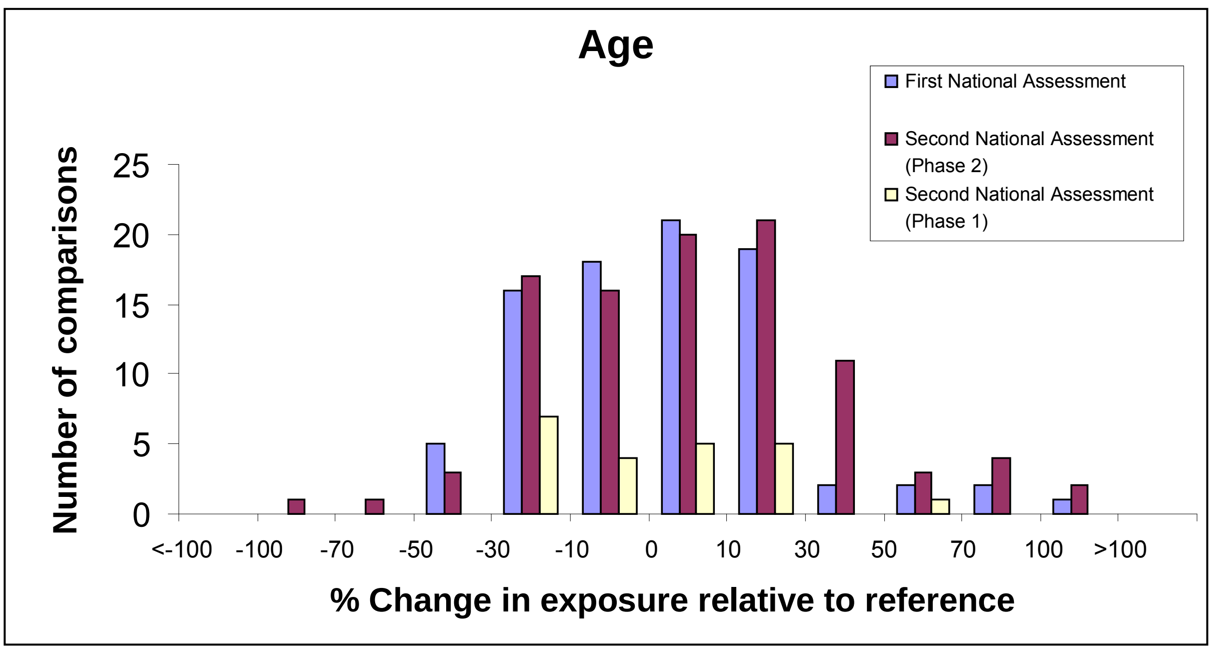

Figure 2e. Distribution of measured percent differences between exposure and reference area fish for age.

For all study periods and phases, condition showed the narrowest range of percent differences, with most measured effects ranging from -30% to 30%. 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; Tessier et al. 2009). Liver weight showed a broader distribution, with most measured effects ranging from -50% to 70%. Gonad weight, weight-at-age, and age showed the widest range, with many measured effects ranging from -70% to well over 100% (up to 350% for gonad weight).

Except as noted in section 4.3, similar distributions of effects were observed for all metal mine categories represented in Figure 2. For the liver and gonad endpoints for the mines conducting their Phase 1 EEM during the second national assessment period, the distributions were skewed farther to the right (i.e., toward more increases than decreases for these endpoints). Due to the smaller sample sizes, however, it is likely too early to ascertain whether these apparent variations are meaningful.

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. The significant differences in means are further broken down by those equal to or greater than and less than CES. 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 the significant interactions are tabulated using the methodology used during the first and second national assessment periods (i.e., not yet using the newer methodology described in Barrett et al. 2010). Note also that age data were analyzed with ANOVA and therefore did not produce interactions. See Environment Canada (2012) for further information on ANCOVA procedures and interpretation.

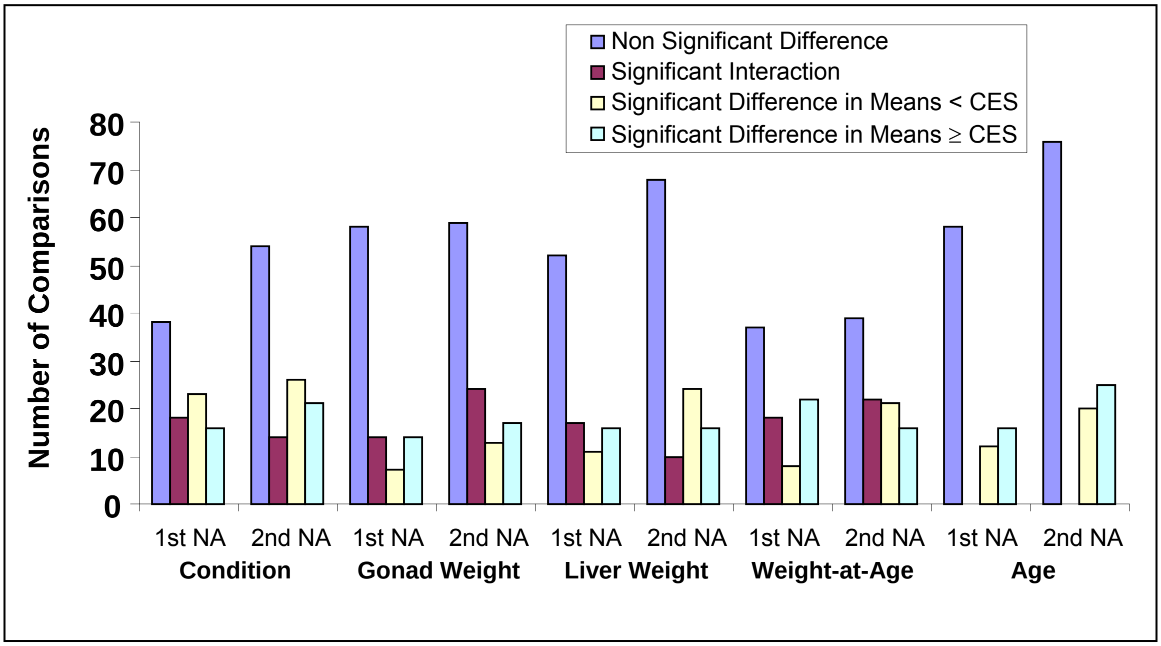

Figure 3. Number of exposure versus reference fish comparisons showing non-significant differences, significant differences in means (smaller or greater or equal to CES), or significant interactions during the first and second national assessment periods. NA – national assessment.

For the five endpoints, between 33% (age – first national assessment period) and 60% (condition – first national assessment period; weight-at-age – second national assessment period) of the comparisons were significant (including both significant differences in means and significant interactions; Figure 3). Within each endpoint, the proportion of significant versus non-significant comparisons was fairly similar between the first and second national assessment periods.

4.3 Response Patterns – National Averages

The national average response patterns for fish exposed in the field to metal 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 (Figure 4). During the first national assessment period (when all mines were doing Phase 1 studies), these analyses showed that, on average, exposure-area fish exhibited significantly lowered condition and relative liver size—that is, they were thinner and had smaller livers. During the first national assessment period, 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.

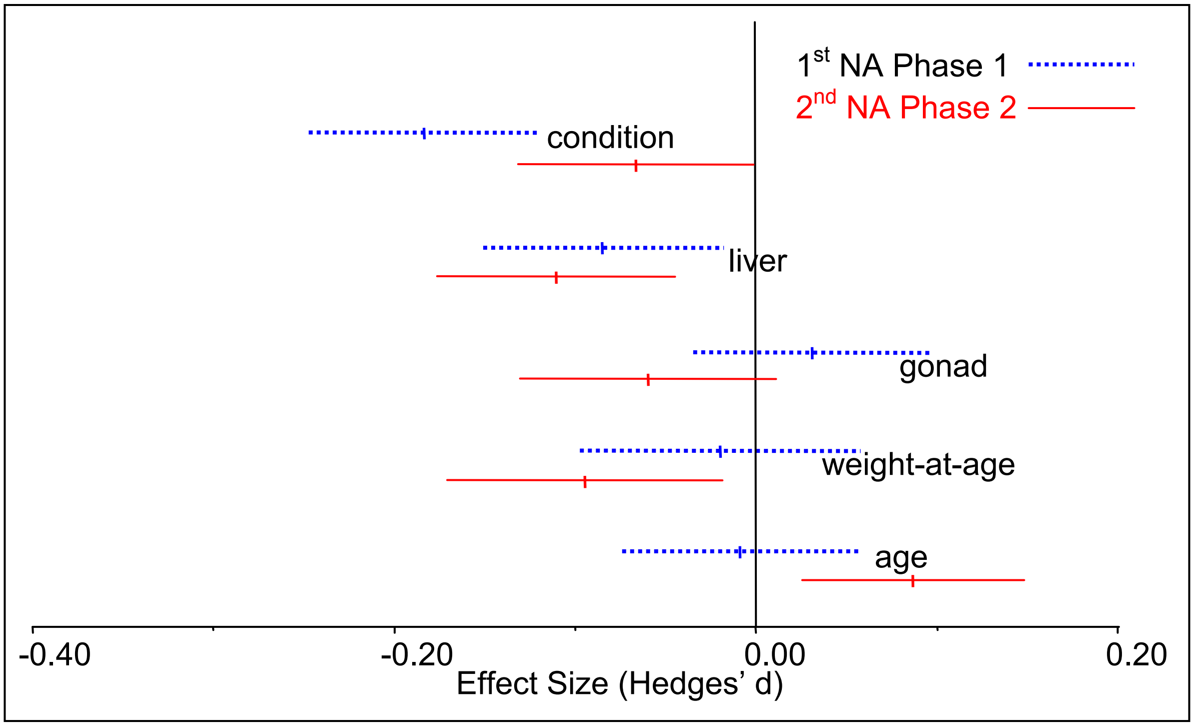

Figure 4. National average fish effects for metal mines in Phase 1 (P1) during the first national assessment period and Phase 2 (P2) during the second national assessment period. Error bars represent 95% confidence intervals. Number of comparisons: condition (P1 = 77, P2 = 80), liver (P1 = 79, P2 = 84), gonad (P1 = 79, P2 = 70), weight-at-age (P1 = 67, P2 =62), age (P1 = 86, P2 = 89). NA – national assessment.

During the second national assessment period, exposure-area fish again showed significantly reduced condition and relative liver size (Figure 4). In addition, they further exhibited (significant) lowered growth rate and (not significant) smaller gonad size. A significant change in age structure was also observed; on average, older fish were found in exposure areas. Note that the data shown in these and the following meta-analysis figures are restricted to the majority of metal mines that were in Phase 2 studies during the second national assessment period (and had been in Phase 1 studies during the first national assessment period). Thus, the meta-analysis figures reveal variations (or lack of) in response patterns for the same metal mines over both national assessment periods. In these and the following meta-analyses, the number of comparisons varies slightly between phases due to differences between phases in the number of usable data sets.

This national average response pattern for metal 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; Tessier et al. 2009). Fish exposed to pulp and paper mill effluents are frequently fatter and faster growing, with bigger livers, but smaller gonads. 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, 2008; McMaster et al. 2005; Parrott 2005; Kovacs et al. 2011). 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 metal mine effects shown in Figure 4 indicate an inhibition response pattern on fish, and this was seen in more endpoints during the second national assessment period than in the first national assessment period. During the second national assessment period, exposure-area fish were, on average, older, thinner and slower growing, with smaller livers and with a tendency toward reduced gonad size. Similar types of inhibitory responses 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 (for reviews, see Munkittrick and Dixon 1988; Munkittrick et al. 1991, 1994, 2000). 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.

4.4 Response Patterns – Additional Meta-analyses

The metal mining industry in Canada is diverse. Thus, it is instructive to break down the meta-analysis results by dividing the mines into smaller subgroups. The first national assessment (Lowell et al. 2008) provided detailed breakdowns by habitat and ore type. For the second national assessment, it is particularly interesting to take a closer look at three of the endpoints that showed variations in response pattern during the second assessment period: weight-at-age, relative gonad size and age.

When subdividing by major receiving water habitat types over both assessment periods, more detailed response patterns became apparent (Figure 5, which also includes the national average grand means for weight-at-age from Figure 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). During the first national assessment period, weight-at-age was significantly reduced in river habitats but was increased in lake habitats. During the second national assessment period, weight-at-age was significantly reduced in both river and lake habitats, which was the main factor for the national average reduction in weight-at-age during the second national assessment period.

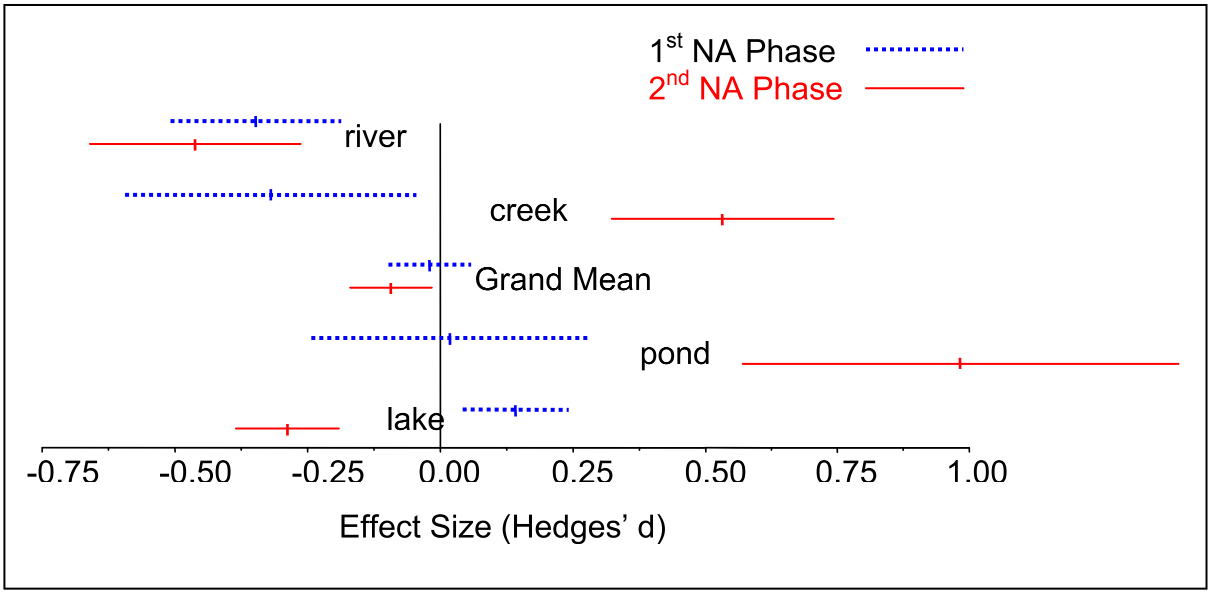

Figure 5. Fish weight-at-age by habitat in Phase 1 (P1) during the first national assessment period and Phase 2 (P2) during the second national assessment period. Error bars represent 95% confidence intervals. Number of comparisons: river (P1 = 15, P2 = 12), creek (P1 = 5, P2 = 8), pond (P1 = 4, P2 = 4), lake (P1 = 43, P2 = 38). NA – national assessment.

An increase in weight-at-age for exposure-area fish was seen in pond and creek habitats during the second national assessment period (Figure 5), but the low sample size for these two habitat types indicate that this could have occurred due to factors other than habitat type (e.g., differences in nutrient input). Therefore, more confidence can be assigned to conclusions based on the lake and river results.

During the first national assessment period, relative gonad weight was shifted more toward increases than the other endpoints (Figure 4). Many of these increases were attributable to fish sampled in river habitats (Figure 6). Although still farther to the right than most of the other endpoints during the second national assessment period, the national average distribution was much more shifted toward decreases than observed during the first national assessment period. This shift toward decreases was primarily attributable to decreases observed in river habitats. The gonad weight responses in the other habitat types were fairly constant across assessment periods.

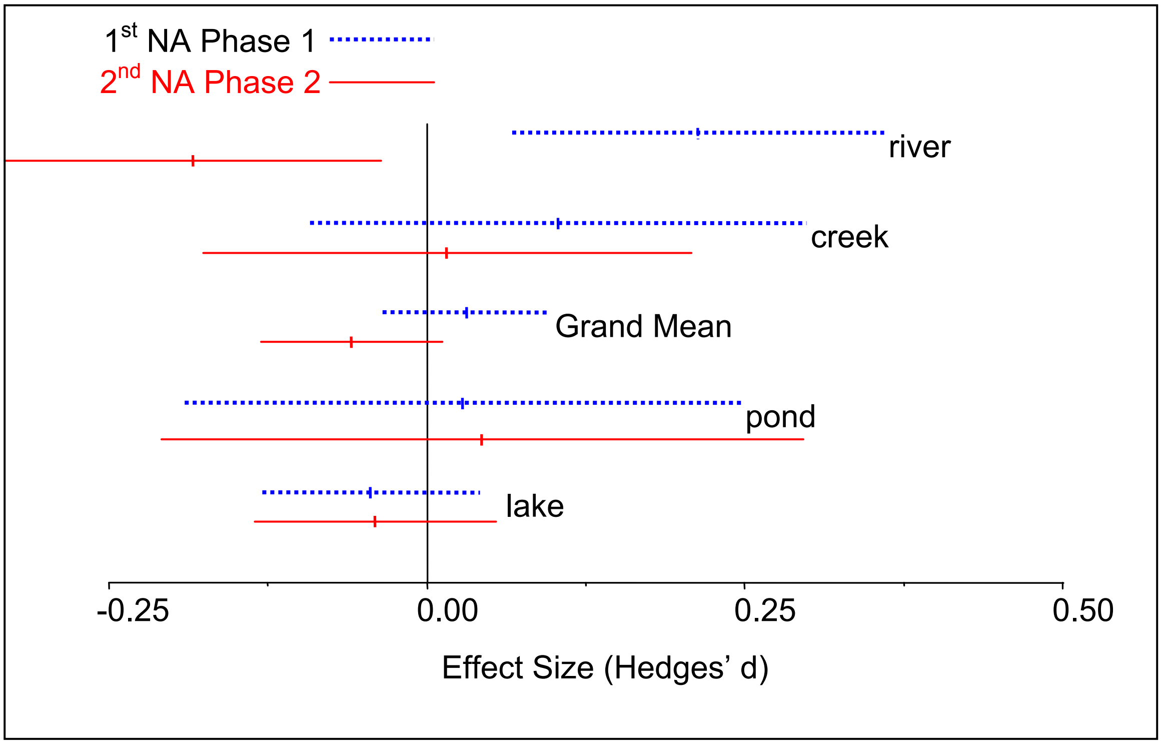

Figure 6. Fish relative gonad weight by habitat in Phase 1 (P1) during the first national assessment period and Phase 2 (P2) during the second national assessment period. Error bars represent 95% confidence intervals. Number of comparisons: river (P1 = 17, P2 = 18), creek (P1 = 7, P2 = 9), pond (P1 = 5, P2 = 5), lake (P1 = 50, P2 = 38). NA – national assessment.

During the first national assessment period, there was no national average tendency for exposure-area fish to be older or younger than reference fish, although significant differences were observed when subdividing by habitat type (Figure 7). A significant national average change in age structure was observed during the second national assessment period, with exposure-area fish being older. This was primarily attributable to observations of older age structures in exposure-area fish in the two most common habitat types: rivers and lakes.

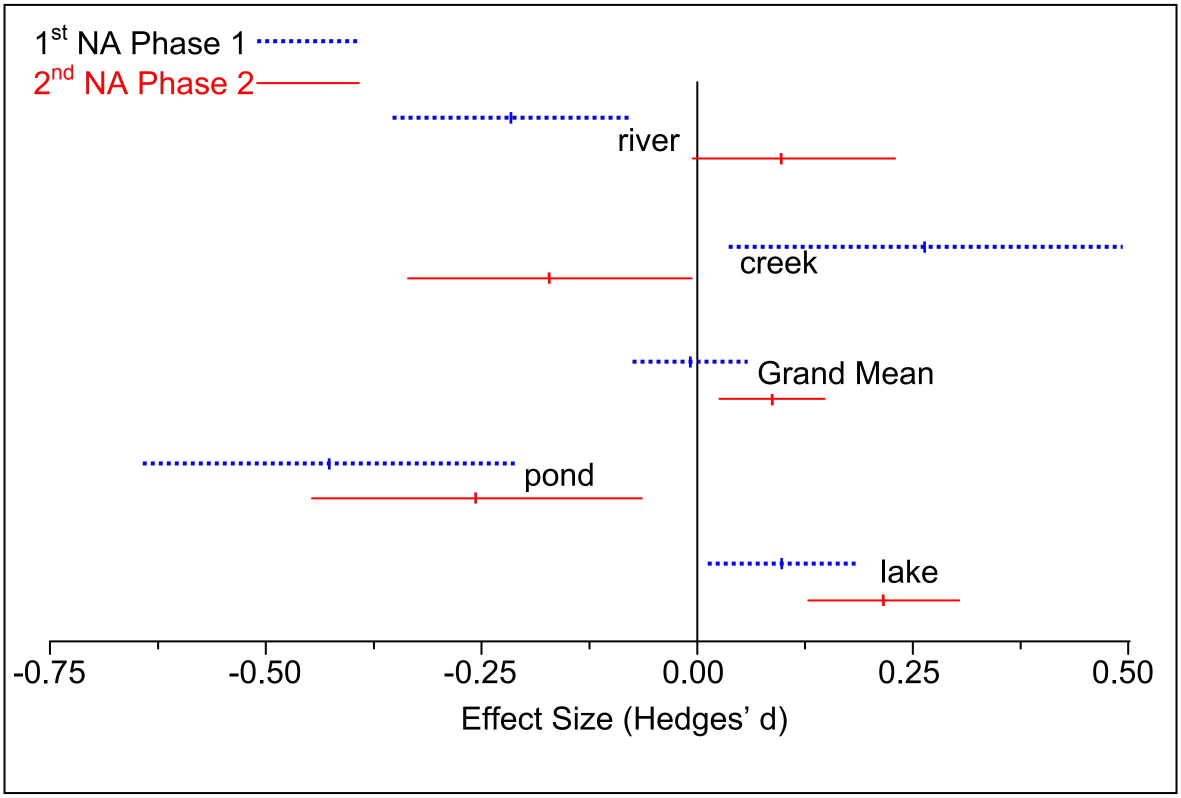

Figure 7. Fish age by habitat in Phase 1 (P1) during the first national assessment period and Phase 2 (P2) during the second national assessment period. Error bars represent 95% confidence intervals. Number of comparisons: river (P1 = 18, P2 = 25), creek (P1 = 6, P2 = 12), pond (P1 = 6, P2 = 8), lake (P1 = 56, P2 = 44). NA – national assessment.

Subdividing metal mines by ore type further revealed more detailed response patterns. Again, for the second national assessment it is particularly interesting to take a closer look at three of the endpoints that showed variations in their response pattern during the second assessment period: weight-at-age, relative gonad size and age. The two most common ore-type categories were precious metal and base metal. During the first national assessment period, weight-at-age for exposure-area fish was significantly reduced for the precious metal subgroup and significantly increased for the base metal subgroup (Figure 8). During the second national assessment period, the precious metal group showed a lessened decrease in weight-at-age, but the base metal group showed a more pronounced decrease in weight-at-age, which led to a displacement of the national average (grand mean) to the left. This, together with the observed decreases in weight-at-age for both the uranium and iron ore subgroups, resulted in the national average reduction in weight-at-age during the second national assessment period.

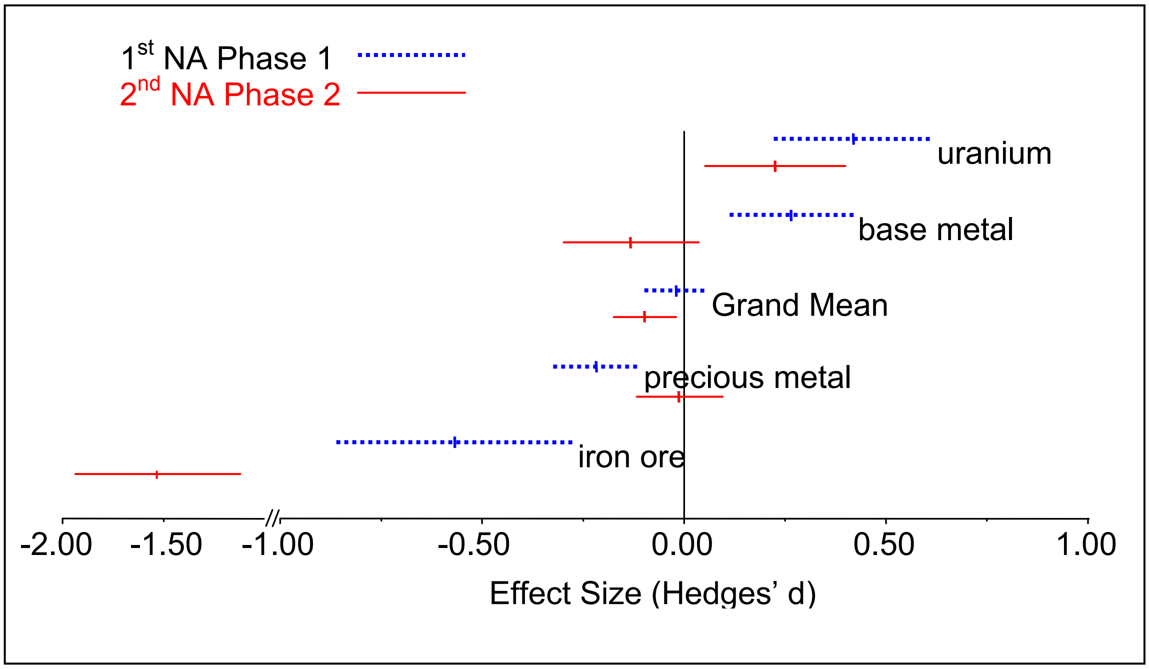

Figure 8. Fish weight-at-age by ore type in Phase 1 (P1) during the first national assessment period and Phase 2 (P2) during the second national assessment period. Error bars represent 95% confidence intervals. Number of comparisons: uranium (P1 = 10, P2 = 11), base metal (P1 = 18, P2 = 15), precious metal (P1 = 33, P2 = 31), iron ore (P1 = 6, P2 = 5). NA – national assessment.

During the first national assessment period, relative gonad weight was not statistically significant for any of the ore type subgroups (Figure 9). During the second national assessment period, however, significant decreases were observed for both the most common ore types (precious and base metals), resulting in a national average shift toward decreased gonad weights for exposure-area fish. For the age endpoint, a large change toward older fish was observed for fish exposed to precious metal effluents during the second national assessment period (Figure 10). This was a primary factor in the national average shift toward older exposed fish during the second national assessment period, along with older age structures for fish exposed to uranium and, to a lesser extent, base metal effluents.

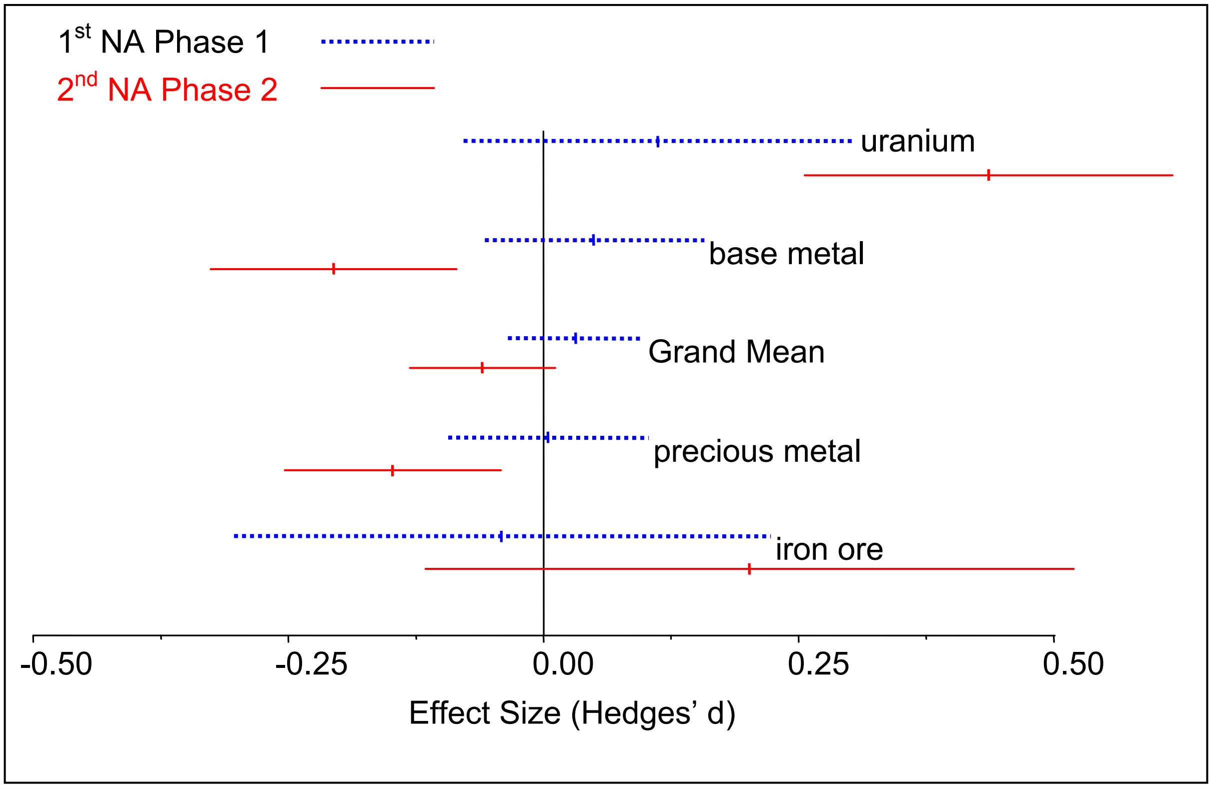

Figure 9. Fish relative gonad weight by ore type in Phase 1 (P1) during the first national assessment period and Phase 2 (P2) during the second national assessment period. Error bars represent 95% confidence intervals. Number of comparisons: uranium (P1 = 10, P2 = 11), base metal (P1 = 27, P2 = 24), precious metal (P1 = 36, P2 = 32), iron ore (P1 = 6, P2 = 3). NA – national assessment.

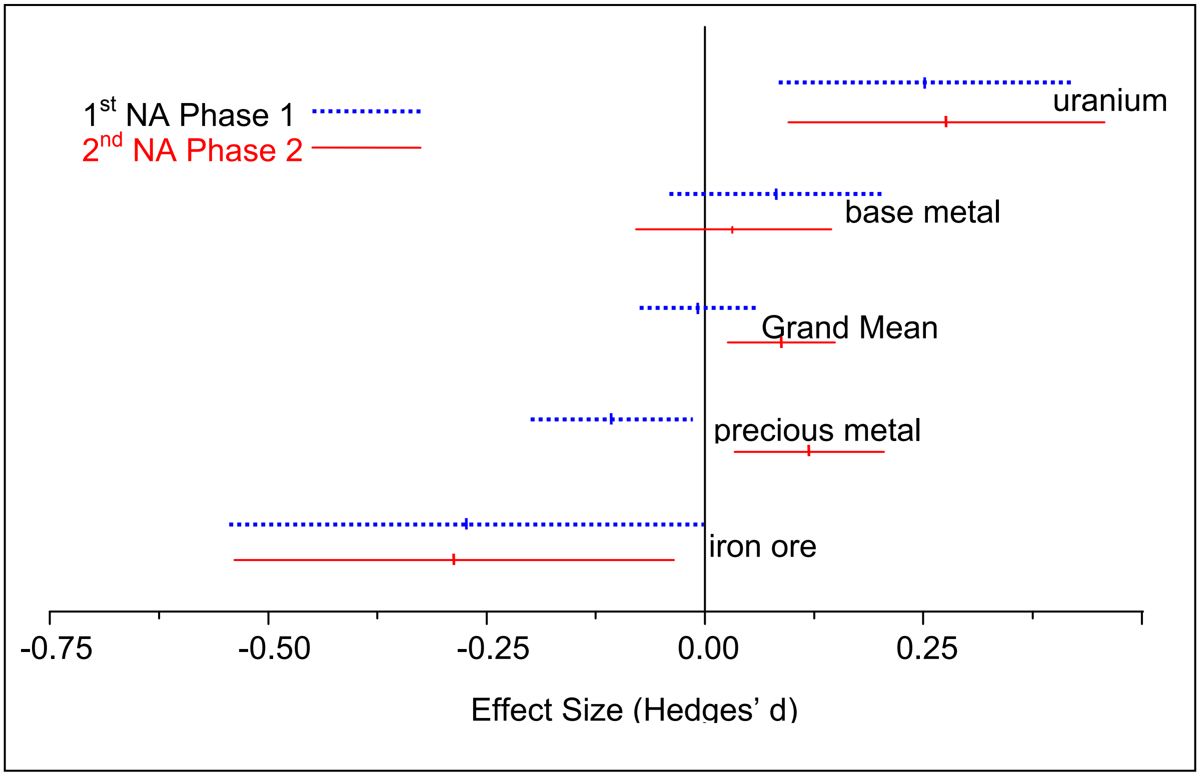

Figure 10. Fish age by ore type in Phase 1 (P1) during the first national assessment period and Phase 2 (P2) during the second national assessment period. Error bars represent 95% confidence intervals. Number of comparisons: uranium (P1 = 13, P2 = 11), base metal (P1 = 23, P2 = 26), precious metal (P1 = 44, P2 = 47), iron ore (P1 = 6, P2 = 5). NA – national assessment.

5.0 Usability of Fisheries Resources: Mercury Analyses in Fish Tissue

Under the MMER, effects on fish usability are evaluated by measuring concentrations of mercury in tissue from fish in the exposure and reference areas. Mines are required to conduct fish tissue analyses, if effluent levels of total mercury are greater than or equal to 0.1 µg/L. The Metal Mining Environmental Effects Monitoring (EEM) Technical Guidance Document (Environment Canada 2012) recommends that tissue analyses 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 (Schedule 5, section 1) 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 in a reference area."

A total of 18 metal mines completed a fish tissue analysis during the second phase of the monitoring, and 3 additional mines completed a fish tissue analysis in their first phase of monitoring.

A national summary of the results of the mercury in fish tissue analyses is presented in Figure 11. One study reported concentrations of mercury in fish tissue greater than 0.45 µg/g wet weight in the exposure area (0.55 µg/g). However, the same study reported a reference mercury concentration in fish tissue (1.45 µg/g) almost three times higher than the concentration found in the exposure area. This suggests that the mine's effluent may not be responsible for the fish tissue effect observed in this study. All other studies reported mercury concentrations in effluent-exposed fish tissue below 0.45 µg/g total mercury.

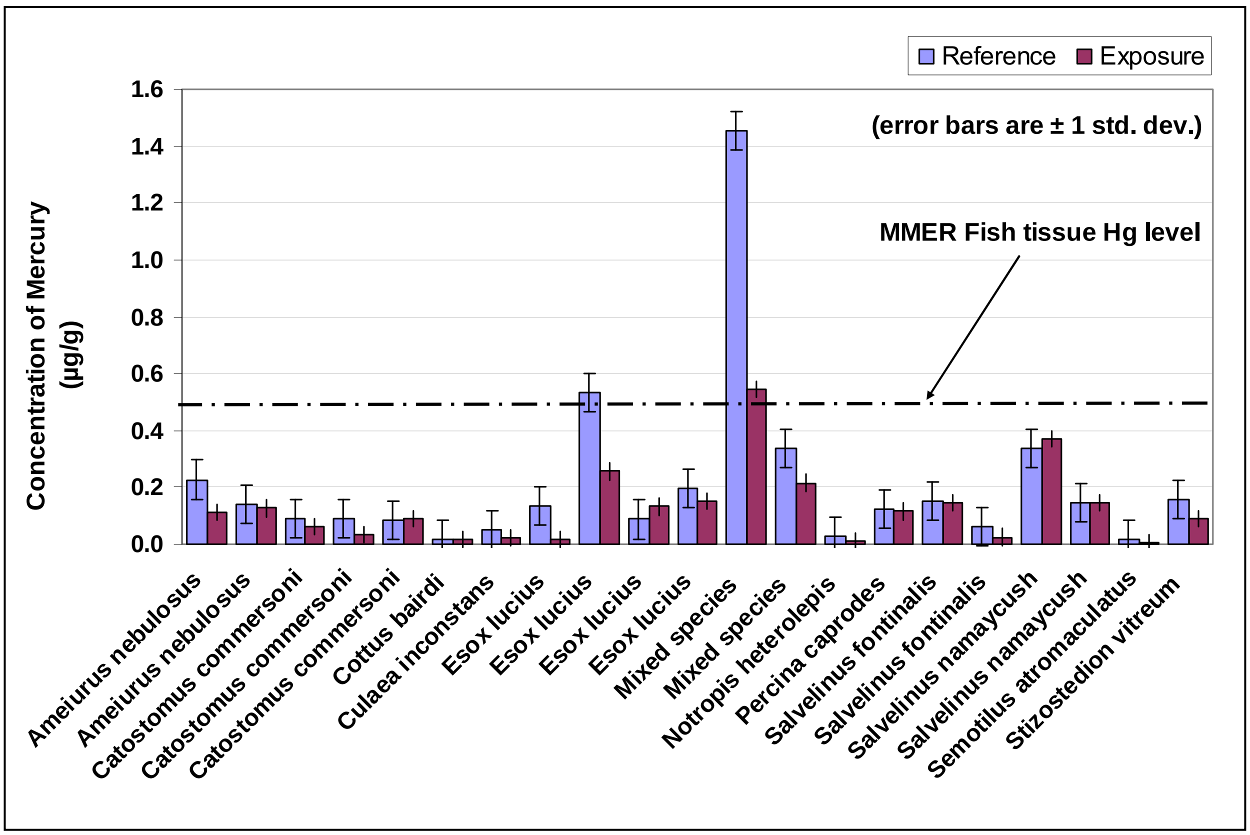

Figure 11. National summary of mercury analyses in fish tissue for the second national assessment period. Each pair of bars represents one study.

6.0 Benthic Invertebrate Community Survey

The third component of the EEM program is the benthic invertebrate community survey, which assesses the impacts of metal 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 Index 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

Seventy-eight mines conducted a benthic invertebrate community survey, with a total of 77 studies (1 joint study conducted by 2 mines) during the second national assessment period. Data from 66 studies were provided in an electronic format sufficient to be included in the national assessment (Table 4). Of these 66 surveys, 59 used a control-impact design, and 7 used a multiple control-impact design. As outlined in section 3.1, all 66 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), one mine used artificial substrates, and one mine conducted a gradient study; these were excluded from these summary analyses due to the different nature of their endpoints.

Table 4. Frequencies of benthic invertebrate community studies done by all metal mines and frequencies of studies included in the national assessment, by design type and by phase of study.

Study design type	Number of studies			Number of studies in national assessment		
	Phase 1	Phase 2	Total	Phase 1	Phase 2	Total
Control-impact	16	47	63	15	44	59[b]
Multiple control-impact	0	10[a]	10	0	7	7[b]
Gradient	1	0	1	0	0	0
Artificial substrata	0	1	1	0	0	0
Reference condition approach	0	3[c]	3	0	0	0
Total	78			66		

[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] These studies were conducted jointly by three mines, and separate data were reported for each mine.

6.2 Summary of Measured Effect Sizes

Figure 12 presents the distributions and ranges in measured exposure versus reference area percent differences for density, taxon richness, the Bray-Curtis Index of dissimilarity and Simpson's Evenness Index. The measured differences were calculated as the exposure area mean minus the reference area mean, expressed as a percentage of the reference area mean. As for fish, in order to facilitate comparisons of Phase 2 mines in the second national assessment period to Phase 1 mines in the first national assessment period (i.e., the same mines over both national assessment periods), results of Phase 1 and Phase 2 mines from the second national assessment period are displayed separately.

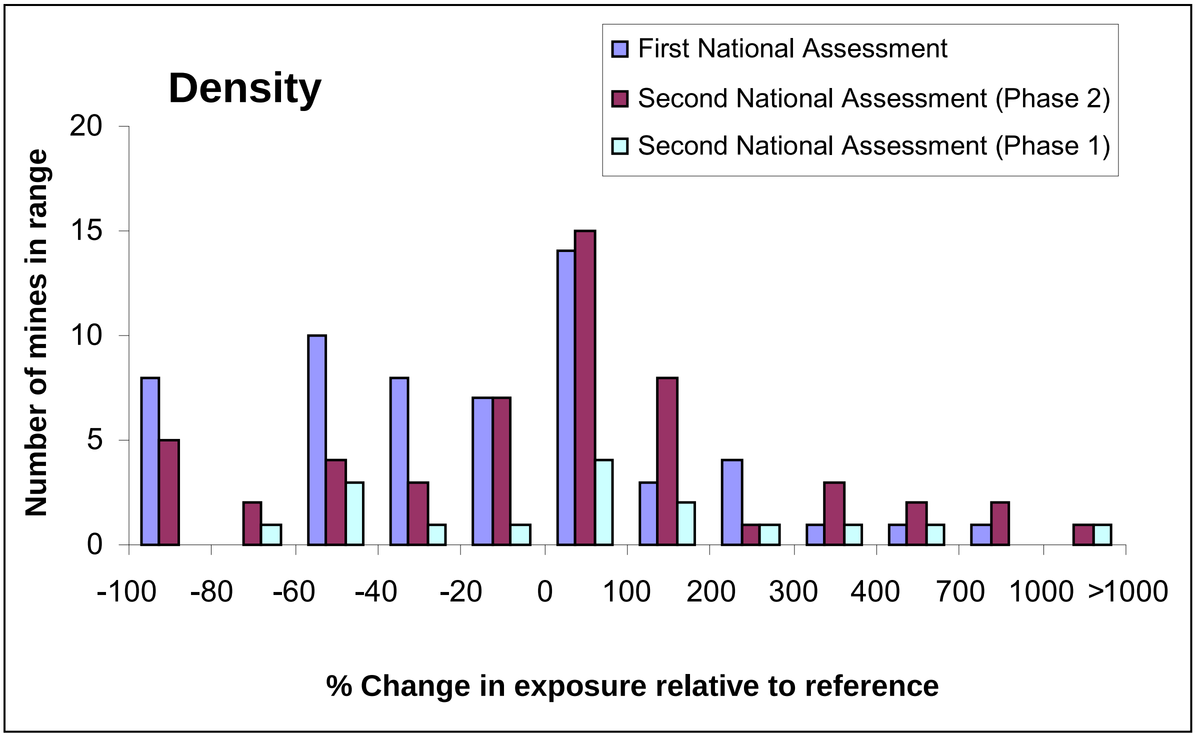

Figure 12a. Distribution of measured percent differences between exposure and reference areas for the benthic invertebrate survey for density.

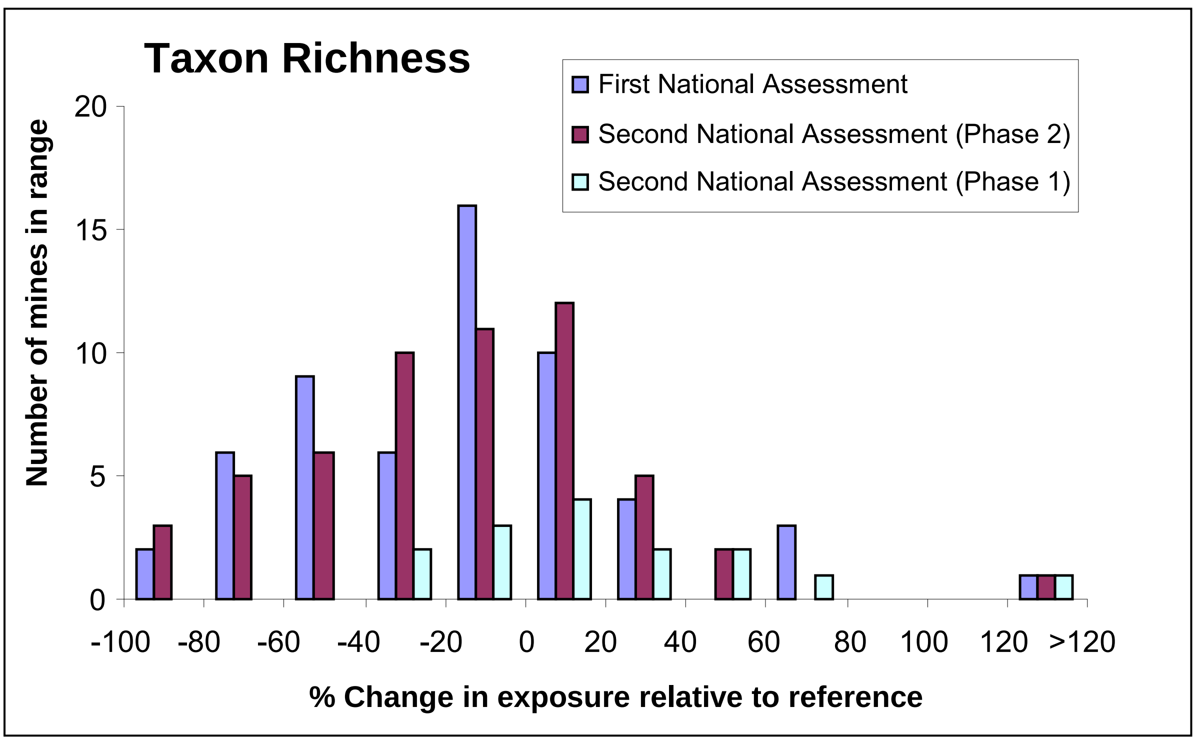

Figure 12b. Distribution of measured percent differences between exposure and reference areas for the benthic invertebrate survey for taxon richness.

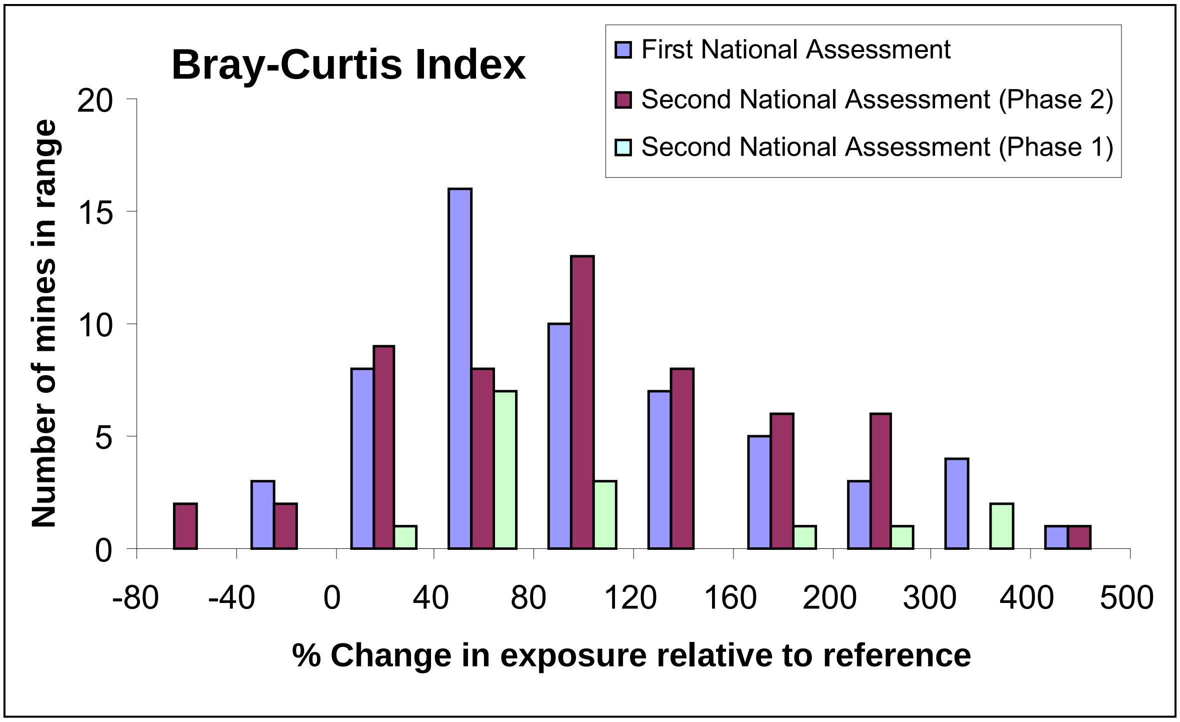

Figure 12c. Distribution of measured percent differences between exposure and reference areas for the benthic invertebrate survey for the Bray-Curtis Index.

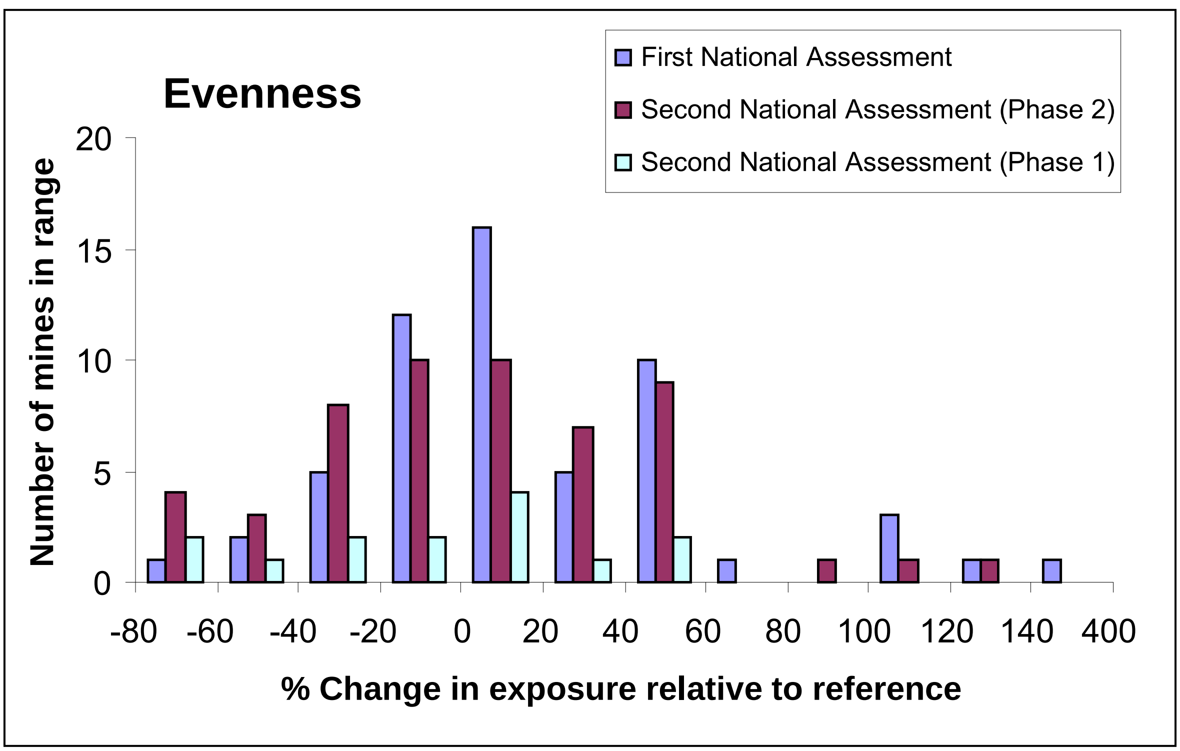

Figure 12d. Distribution of measured percent differences between exposure and reference areas for the benthic invertebrate survey for evenness.

For both national assessments, density is known to typically show the greatest range in measured effects (Lowell et al. 2003, 2005) and is the endpoint showing the most extreme range (ranged from -99% to 1070% in the first national assessment period and from -99% to 5484% for Phase 2 mines in the second national assessment period; Figure 12a). Taxon richness effects ranged from -85% to 125% in the first national assessment period and from -92 to 163% for Phase 2 mines in the second national assessment period. For the Bray-Curtis endpoint, the majority of mines fell within the 0% to 400% range. For Simpson's Evenness endpoint, the majority of mines fell within the -60 to 60% range. Note that, due to the method of calculation, Bray-Curtis measured effects are usually positive. The few negative values for this endpoint were due to unusual data distributions.

Except as noted in section 6.3, similar distributions of effects were observed for all three categories of mines in Figure 12. For the second national assessment Phase 1 mines (i.e., newly regulated mines), the taxon richness endpoint was skewed farther to the right. Due to the smaller sample size, however, it is likely too early to ascertain whether this apparent shift was meaningful.

Figure 13 shows the number of mines measuring statistically significant (i.e., an effect) or non-significant differences in means for each of the four endpoints. Mines that had an effect are divided into those where the effect was less than the CES of ± 2 standard deviations (± 2SD) from the reference mean and those where the effect equaled or exceeded the CES of ± 2SD. Similar patterns of results were observed between the first and second national assessments, where the Bray-Curtis Index was the most sensitive of the four benthic invertebrate endpoints (Lowell et al. 2003, 2005; Tessier et al. 2009), with effects equal to or above the CES as the most common result. In contrast, a higher number of mines showed no significant difference for the other three endpoints in both assessments. For density, taxon richness and evenness, there were similar percentages of mines showing non-significant differences. Specifically, 60% and 63% of first and second national assessment mines, respectively, showed non-significant differences for the density endpoint. The percentages of non-significant differences observed during each of the two national assessment periods for richness are, respectively, 58% and 63%, whereas for evenness these numbers are 77% and 74%.

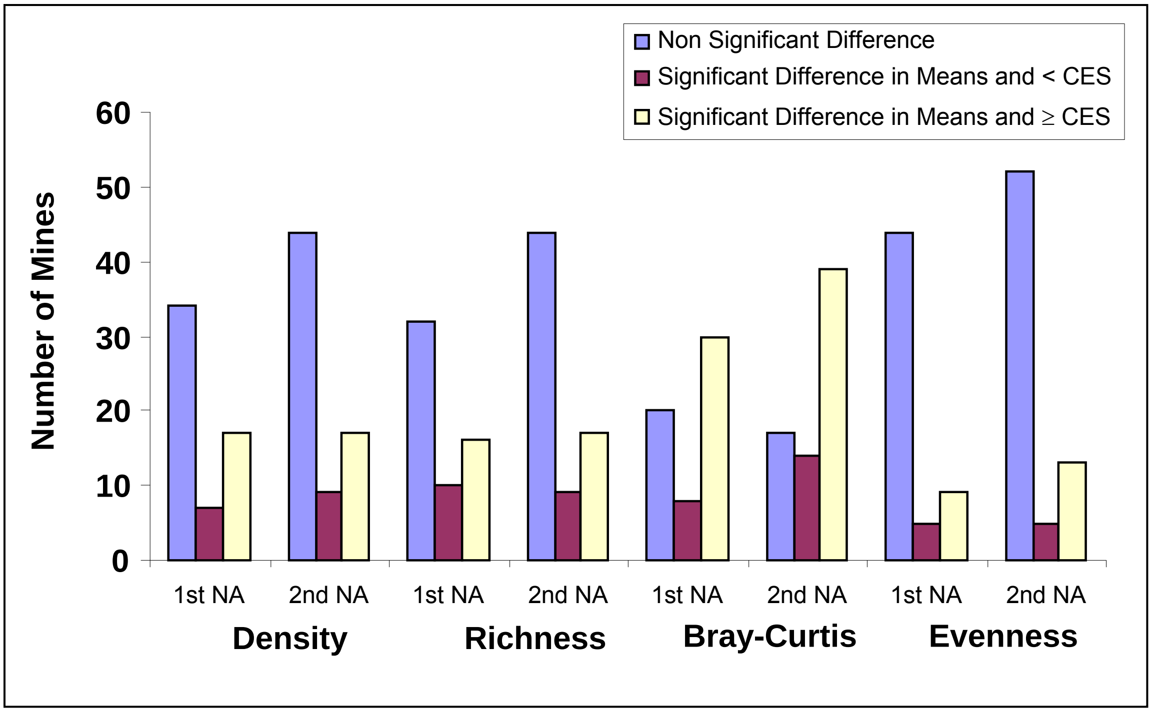

Figure 13. Number of metal mines showing non-significant and significant differences during the first and second (phases 1 and 2) national assessment periods for the benthic invertebrate community endpoints. NA – national assessment.

6.3 Response Patterns – National Averages

On a national basis, during the first national assessment period, benthic invertebrates showed significant changes in effluent exposure areas for all four of the core endpoints (Figure 14). On average, both density and taxon richness were reduced in exposure areas relative to reference areas, reflecting overall inhibitory effects on effluent-exposed benthic 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 Index endpoint measures effects in the positive direction. This index is known to be the most sensitive of the four endpoints (Lowell et al. 2003; also see Figure 13), and showed significant differences in community structure in effluent-exposed areas. The Simpson's Evenness Index endpoint also showed significant differences in community structure for effluent-exposed benthic invertebrates.

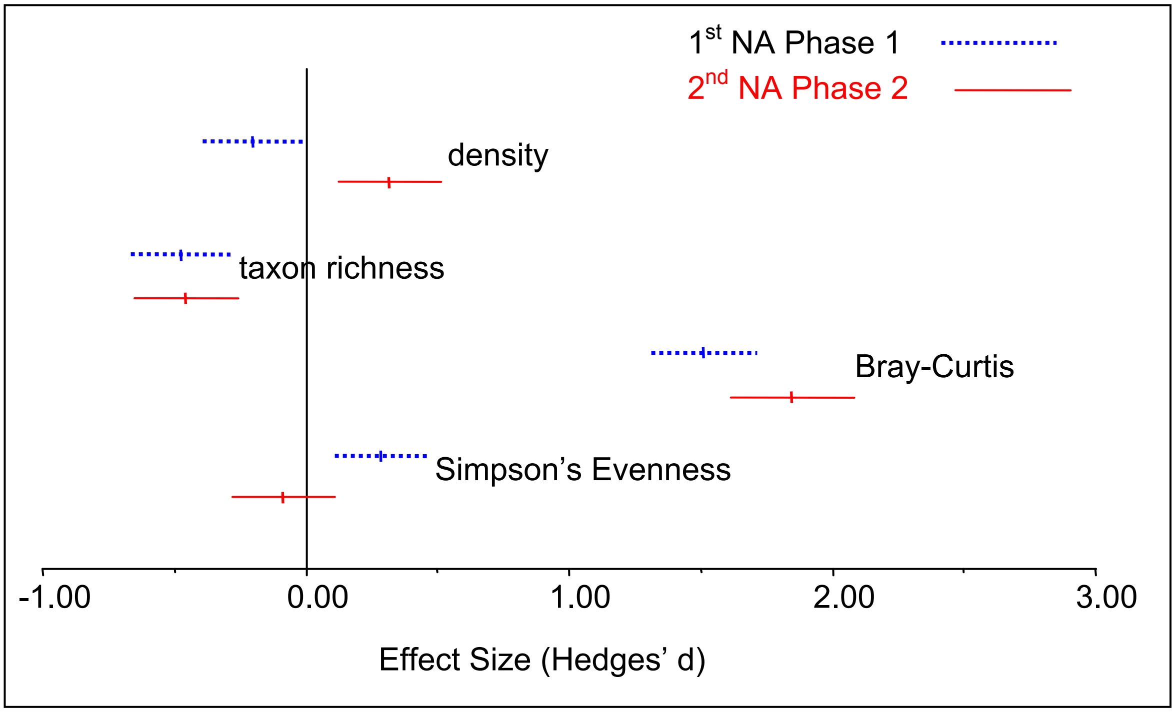

Figure 14. National average benthic invertebrate effects for metal mines in Phase 1 (P1) during the first national assessment period and Phase 2 (P2) during the second national assessment period. Error bars represent 95% confidence intervals. Number of mines: (P1 = 57, P2= 50). NA – national assessment.

When comparing results from both national assessment periods, a consistent significant reduction in taxon richness was observed in both periods, an increase in density and the Bray-Curtis Index occurred in the second national assessment period; evenness showed more mixed responses and overlapped the zero-effect line during the second national assessment period (Figure 14).

As was seen for fish (section 4.3), the national average response pattern for benthic invertebrates exposed to metal mine effluents differed noticeably from the response patterns that have been repeatedly observed for benthic 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, with an increase in exposure area taxon richness compared to what has been observed during either metal mining national assessment period. For pulp and paper mills, the higher exposure versus reference endpoint differences, which shape the observed national average response patterns, are due to the eutrophication effects of nutrient addition (Chambers et al. 2000; Culp et al. 2000; Lowell et al. 1995, 2000). These overall pulp mill stimulatory effects contrast with the more inhibitory effects of metal mine effluent exposure.

6.4 Response Patterns – Additional Meta-analyses

As was done for fish, the benthic invertebrate response patterns can be better understood by dividing the meta-analysis data set into smaller subgroupings. The first national assessment (Lowell et al. 2008) provided detailed breakdowns by habitat and ore type. It is particularly interesting to take a closer look at the benthic invertebrate endpoint density, which showed the most notable variation in response pattern during the second assessment period.

During the first national assessment period, breaking the analyses down by habitat type showed that density was significantly reduced in effluent-receiving lake and creek habitats (Figure 15), although sample size was low for creeks. During the second national assessment period, however, density increased for all habitat types except river erosional.

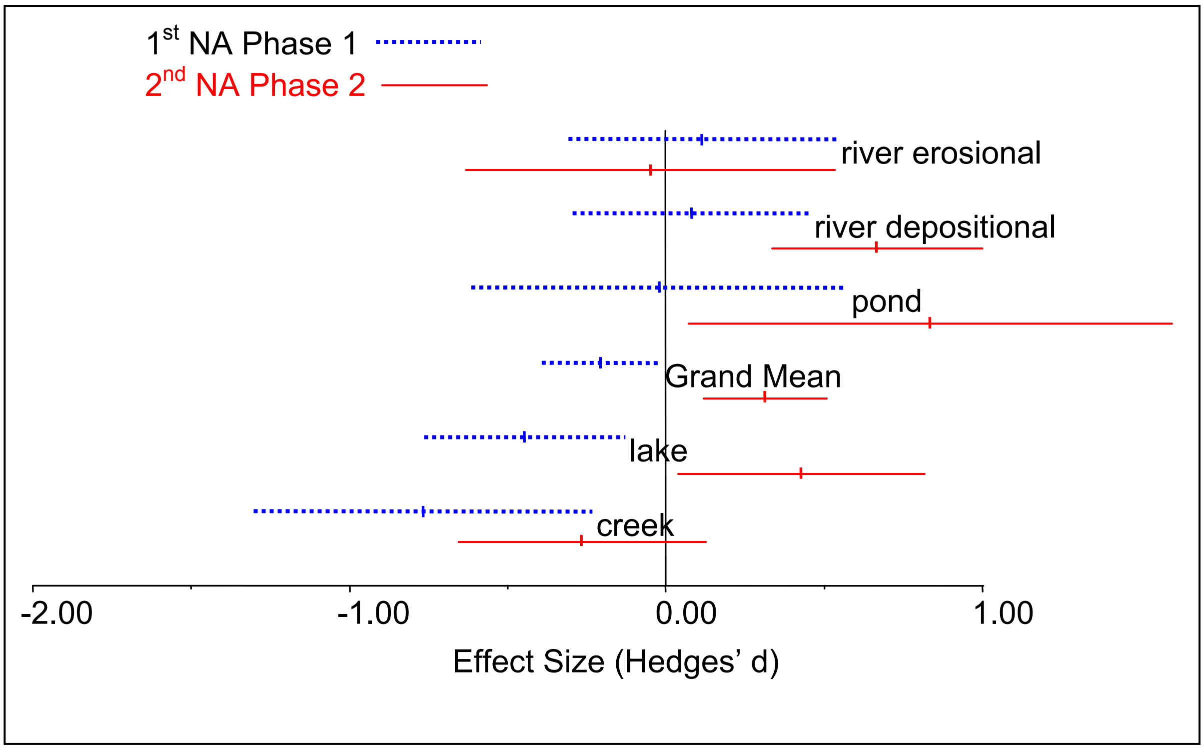

Figure 15. Benthic invertebrate density by habitat in Phase 1 (P1) during the first national assessment period and Phase 2 (P2) during the second national assessment period. Error bars represent 95% confidence intervals. Number of metal mines: river erosional (P1 = 12, P2 = 5), river depositional (P1 = 13, P2 = 16), pond (P1 = 5, P2 = 3), lake (P1 = 20, P2 = 16), creek (P1 = 7, P2 = 10). NA – national assessment.

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. During the first national assessment period density was significantly reduced for iron ore mines, although the sample size was low (Figure 16). 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. During the second national assessment period, density increased for all ore types.

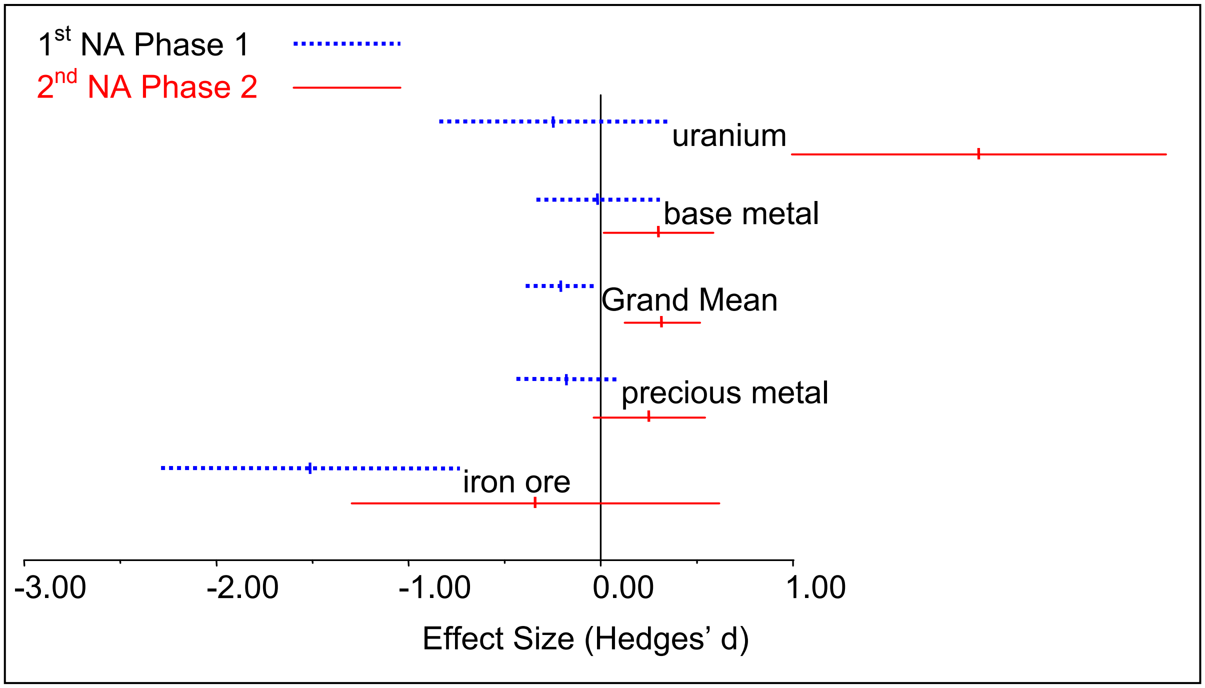

Figure 16. Benthic invertebrate density by ore type in Phase 1 (P1) during the first national assessment period and Phase 2 (P2) during the second national assessment period. Error bars represent 95% confidence intervals. Number of metal mines: uranium (P1 = 6, P2 = 3), base metal (P1 = 20, P2 = 22), precious metal (P1 = 27, P2 = 24), iron ore (P1 = 4, P2 = 2). NA – national assessment.

6.5 Relationship to Effluent Flow

One hypothesis that has been raised to explain the increases in density observed during the second national assessment period is that they may be a response to increasing mining activities over recent years, as reflected by increases in effluent flow at existing mines, leading to increasing nutrient enrichment effects. To investigate whether this may have been a cause for the variation in the response pattern, the influence of effluent flow on the magnitude of density and taxon richness effects was analyzed using a combination of regression and meta-analyses.

For each metal mine included in the above meta-analyses, total effluent flow for the year was extracted from the metal mining database for the year of benthic invertebrate sampling during the first and second national assessment periods. To correct for very large differences in effluent flow among mines, the change in effluent flow from the first to second national assessment period was calculated as a ratio (second national assessment period minus first national assessment period divided by first national assessment period). The parameter calculated in this manner reflects the change in effluent flow expressed as a proportion of first national assessment period flow. This was also done for the year prior to benthic invertebrate sampling (to check for possible lag effects, i.e., delayed effects), but the results were the same, therefore the same-year results are shown here.

In addition, for each mine included in the analyses, the increase or decrease in magnitude of density and taxon richness effect was calculated using standardized effect sizes (i.e., the Hedges' d values calculated during the meta-analyses). The change in magnitude of effect from the first to second national assessment period was calculated as a difference (second national assessment period minus first national assessment period).

Inspection of Figures 17 and 18 shows that there was no overall increase in effluent flow from the first to the second national assessment period. This can be seen by the fact that there are more points on the left side of the figures than on the right side, which reflects that more mines showed decreases than showed increases in effluent flow during the second national assessment period. Note that each point in these figures represents a different mine study.

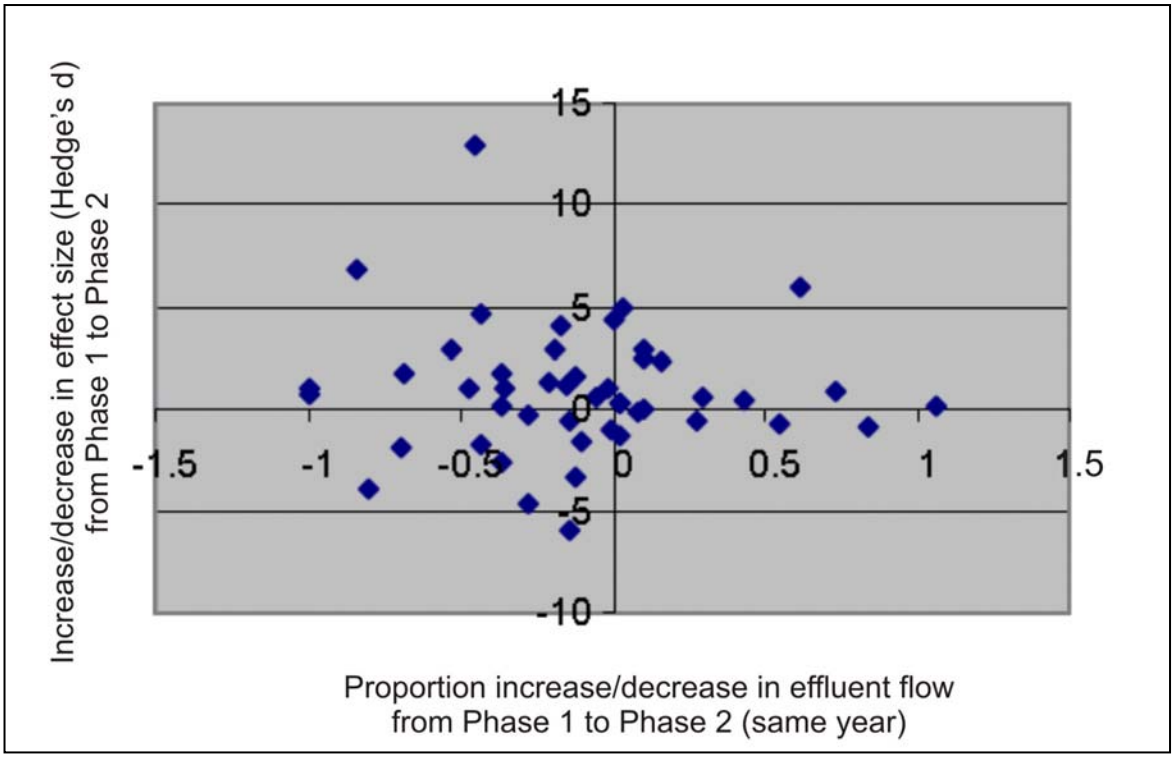

Figure 17. Change in density vs. effluent flow from Phase 1 during the first national assessment period to Phase 2 during the second national assessment period.

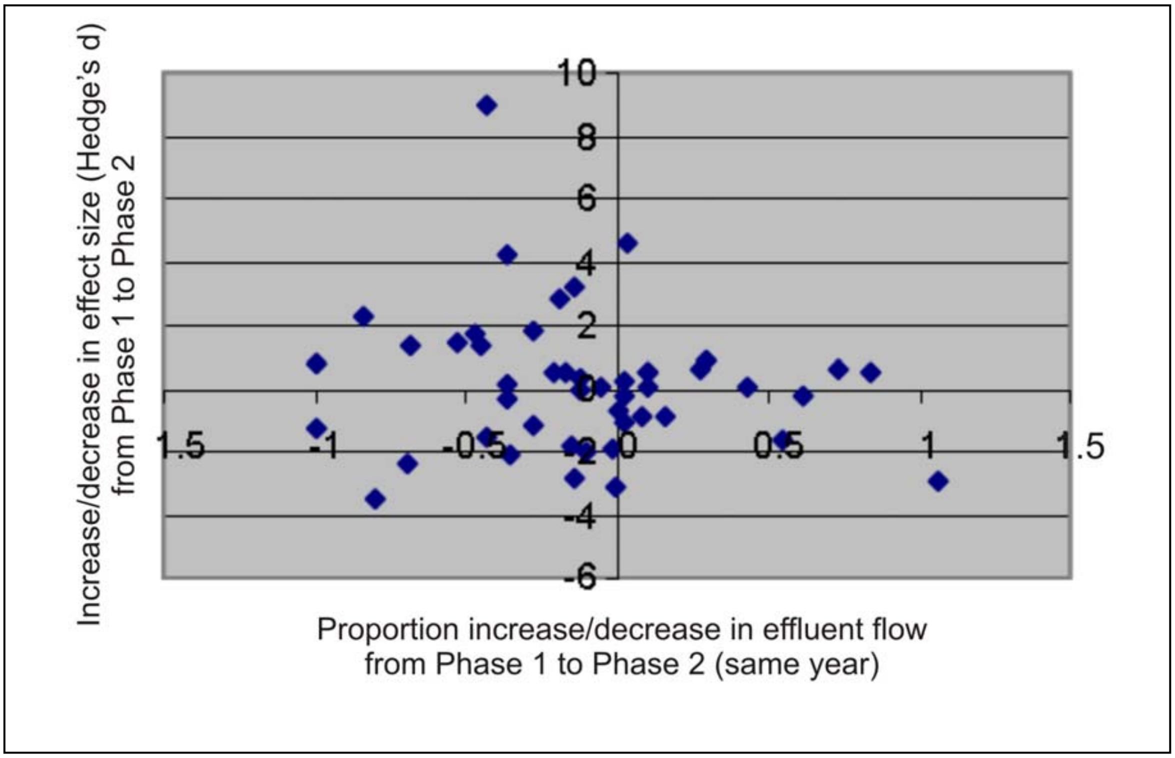

Figure 18. Change in taxon richness vs. effluent flow from Phase 1 during the first national assessment period to Phase 2 during the second national assessment period.

Furthermore, if the hypothesis was true that increases in density during the second national assessment period were due to increases in effluent flow, one would expect a positive slope in Figure 17. Instead, there was no positive slope and the correlation was very low ($r = -0.048$, $p > 0.5$). Thus, changes in effluent flow did not have a predominant overall influence on changes in magnitudes and patterns of effect for density. This is not to say that effluent flow had no influence. There were some individual mines where decreases in effluent flow were associated with either large decreases or large increases in density (greater range of effect changes on left side of Figure 17). Interestingly, only one large increase in effluent flow was associated with a large change in density (i.e., mostly a narrow range of effects changes on right side of Figure 17). Effluent flow is likely just one of several factors that can influence the magnitude and pattern of effects.

The results for taxon richness paralleled those for density (Figure 18). There was virtually no positive (or negative) slope and the correlation was very low ($r = -0.142$, $p > 0.2$). Therefore, changes in effluent flow also did not have a predominant overall influence on changes in magnitudes and patterns of effect for taxon richness. And similar to density, most of the larger changes in taxon richness were associated with decreases rather than increases in effluent flow.

7.0 Sublethal Toxicity Tests

7.1 Introduction

In addition to monitoring endpoints for fish, fish tissue and benthic invertebrates, metal mines are required to conduct SLT on effluent from their final discharge point that has potentially the most adverse environmental impact. Mines conduct a battery of SLT tests twice a year for three years and once each year after the third year, including a fish early-life-stage development test, an invertebrate reproduction test, and plant and algal growth inhibition tests. The SLT testing component of the metal mining EEM includes specific test methods prescribed in Schedule 5 of the MMER (see also Environment Canada 2012).

For the purposes of this national assessment, the results of SLT testing are used to measure any changes in effluent quality over time and compare effluent quality between mine types. On a site-specific basis, SLT data may also be used to help understand and estimate the relative contribution of the mine effluent, in multiple discharge situations, to observed effects in the receiving environment (see also Taylor et al. 2010).

The endpoint used to measure effluent quality in freshwater is typically the inhibiting concentration for 25% effect (IC_{25}), i.e., that concentration causing performance (e.g., growth, reproduction) 25% inferior to that of the control organisms. In a freshwater test, if full-strength effluent did not cause 25% inhibition/effect, then the endpoint was reported as > 100% concentration. For the rainbow trout embryo viability test, the endpoint is an EC_{25}, or effect concentration for 25% effect, i.e., the concentration of effluent estimated to cause an effect to 25% of the test organisms compared to control organisms.

7.2 Overview of Sublethal Toxicity Data Submitted in Phases 1 and 2

The assessment of SLT data was done by analyzing and comparing, on a national scale, data from two submission periods: 2003 to 2005 (first national assessment period) and 2006 to 2008 (second national assessment period). The first national assessment period includes data from a total of 1648 test results from 78 metal mines, and the second national assessment period includes data from 1657 test results from 99 metal mines. It should be noted that ~65% of the 99 metal mines included in the second national assessment period were conducting their Phase 2 studies and therefore had also submitted data in the 2003–2005 submission period representing the data set for the first national assessment.

In each of the two national assessment periods, only one marine metal mine submitted SLT data. These were excluded from the national analyses. Most metal mines conducted the fish early-life-stage development tests on fathead minnow; however, metal mines west of the Canadian Rockies used the rainbow trout. The invertebrate reproduction test was conducted on the waterflea *Ceriodaphnia dubia*, while growth inhibition tests were done on the algae *Pseudokirchneriella subcapitata* (based on cell yield) and the macrophyte *Lemna minor* (using frond number and frond dry weight).

7.3 Monitoring Changes in Effluent Quality among Phases

Sublethal toxicity endpoints can be used to compare the quality of effluent at different times. Comparisons among results of the first and second national assessment periods were made for the distributions of endpoints from Canadian metal mines discharging into freshwater environments (Figures 19 and 20). For each test, all IC_{25}s, or EC_{25}s when applicable, were compiled, and the percentage of tests falling into each defined category range of effluent concentration (i.e., ≥ 100%, < 100 to ≥ 80%, < 80% to ≥ 60%, < 60% to ≥ 36%) was calculated. For example, Figure 19a illustrates the results of SLT tests conducted on *C. dubia* during the first and second national assessment periods. The vertical bars indicate the percentage of tests conducted in each national assessment period in which *C. dubia* exhibited a 25% decrease in function at that threshold of effluent concentration. A higher percentage of tests carried out in the second national assessment period (42%) showed this effect at concentrations equal to or greater than 100% effluent than tests carried out in the first national assessment period (28%).

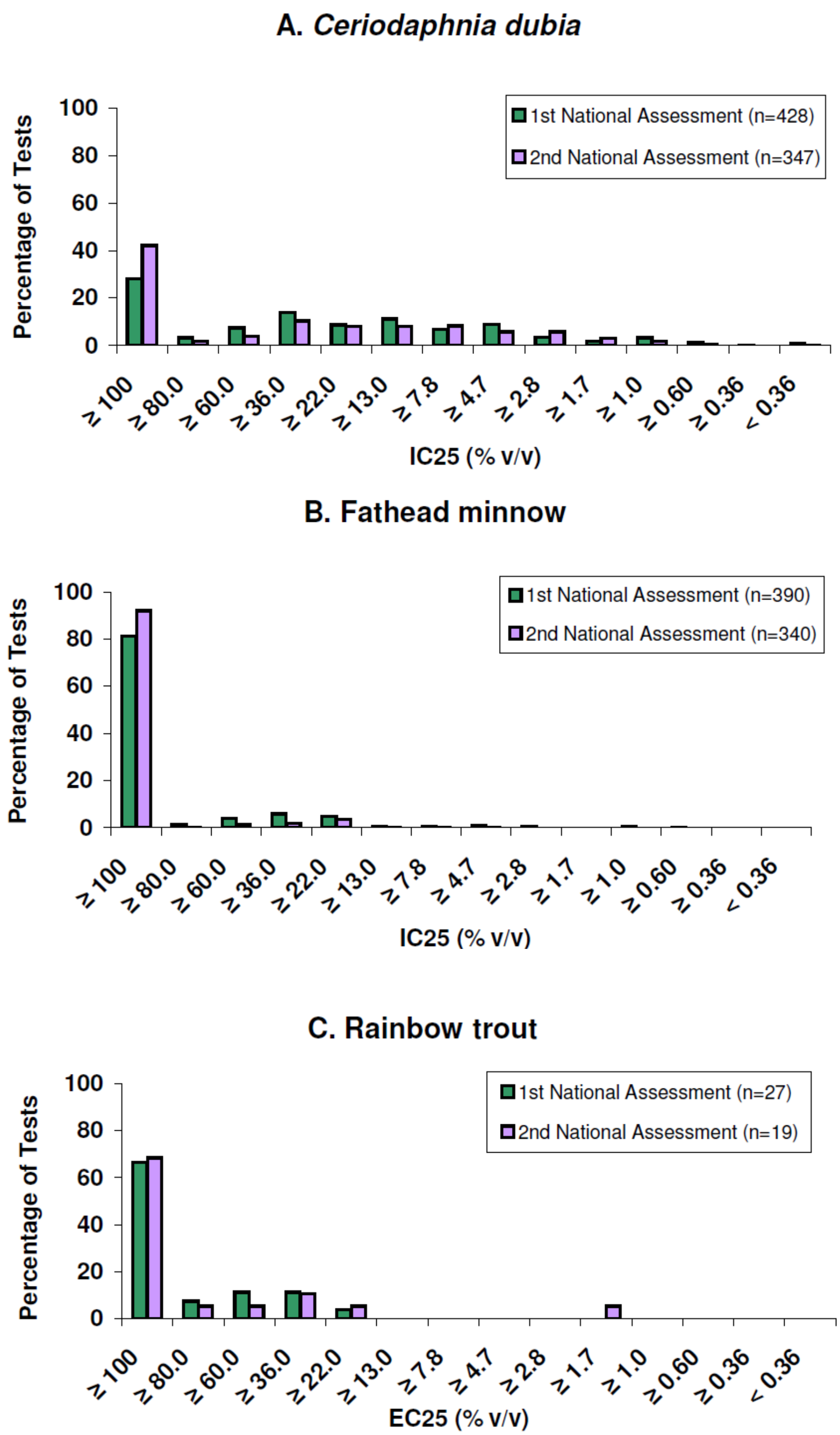

Figure 19. Comparison of sublethal toxicity of metal mining effluents to freshwater invertebrate *(C. dubia)* and fish (fathead minnow and rainbow trout) species.

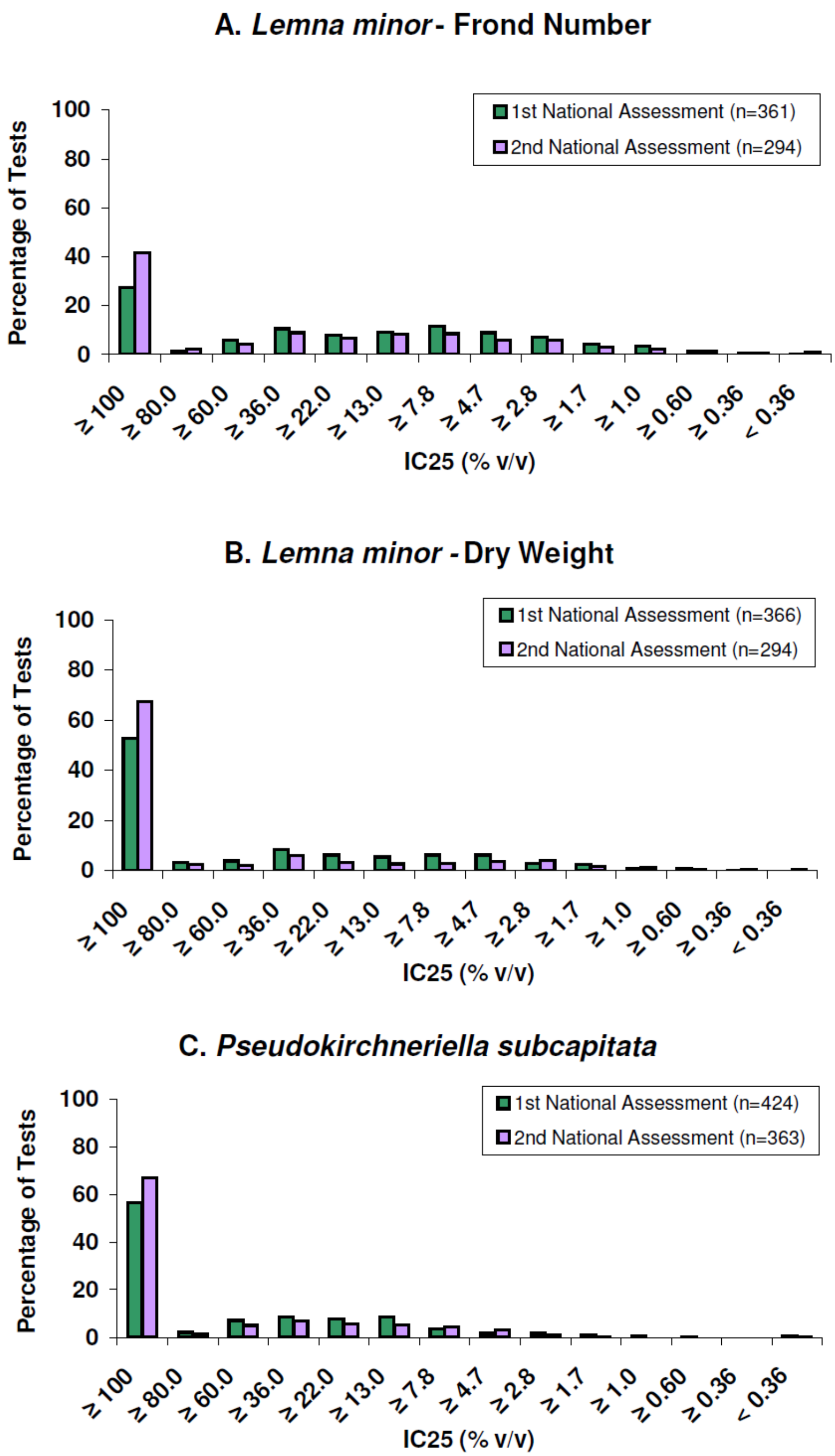

Figure 20. Comparison of sublethal toxicity of metal mining effluents to freshwater plant *(L. minor)* and algae *(P. subcapitata)* species.

On a national scale, the frequency distributions of the first national assessment datasets were similar to the second national assessment distributions for all test species analyzed. Percentages of tests falling in the ≥ 100% category were slightly higher for the second national assessment period (Figures 19 and 20), indicating a possible improvement in effluent quality over the two time periods. It is too early, however, to evaluate whether this increase was large enough to be biologically meaningful.

7.4 Responsiveness of Sublethal Toxicity Tests

These national frequency distributions can also reveal valuable information on the responsiveness of the different test species and endpoints to metal mining effluents. Test species/endpoints with lower percentages of tests falling in the ≥ 100% category are more responsive than those with high percentages in that same concentration category. When applying this technique to the frequency distributions presented in Figures 19 and 20, *L. minor* (frond number) and *C. dubia* are shown to be the most responsive tests, while the fish tests (fathead minnow and rainbow trout) are the least responsive.

In Figures 21a and 21b, the percentages of tests with IC_{25}s (or EC_{25}s) showing no sublethal response in the highest test concentration are compared across metal mine types. Metal mines were classified into four main categories according to the primary metal produced, namely, base metal (e.g., copper, zinc), uranium, iron ore, and precious metals (e.g., silver, gold). All remaining metal mine types subjected to the MMER were classified as “other” (e.g., tantalum, tungsten, titanium). For most metal mine types, and similar to the responsiveness of tests observed when grouping all metal mine types, *L. minor* (frond number) and *C. dubia* are shown to be the most responsive tests, while the fish tests are the least responsive. One exception is for iron ore mines, for which the fathead minnow test is the second-most responsive test after *C. dubia*.

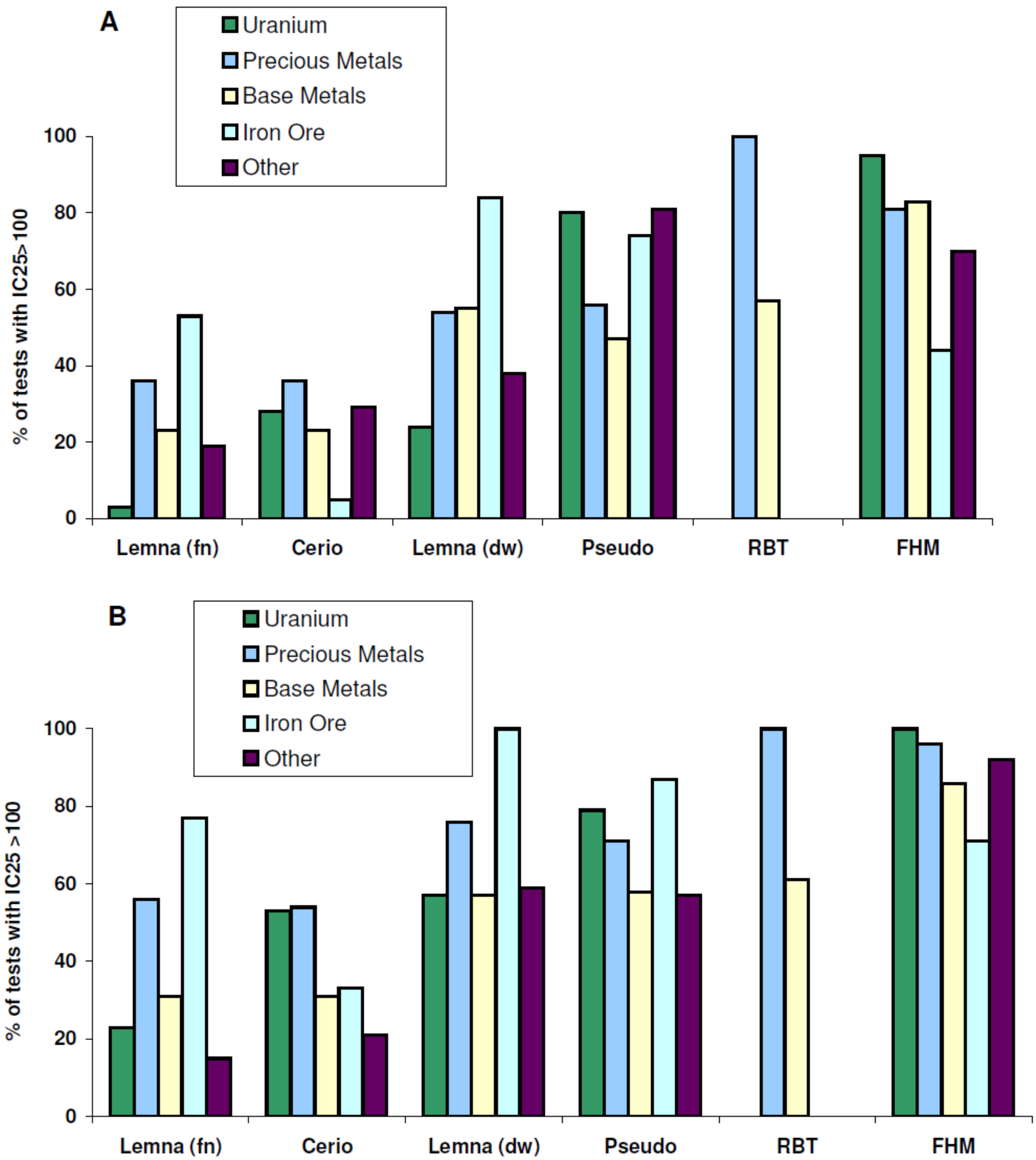

Figure 21. Percentages of sublethal toxicity tests showing IC_{25}s > 100% full-strength effluent in the first (A) and second (B) national assessment. Note: for the rainbow trout embryo viability test the endpoint is an EC_{25}, although for simplicity the y-axis label indicates only the percent of tests with IC_{25}s >100% effluent. Also, there is only one precious metal mine conducting the rainbow trout test, so test sensitivity cannot be determined for this mine type. Test names are abbreviated as follows:

Lemna (fn) = *Lemna minor* (frond number); Cerio = *Ceriodaphnia dubia* (reproduction); Lemna (dw) = *Lemna minor* (dry weight); Pseudo = *Pseudokirchneriella subcapitata* (growth); RBT = Rainbow Trout (embryo viability); FHM = Fathead Minnow (growth)

8.0 Summary and Conclusions

The second round of data collection for the metal mines conducting EEM has produced a geographically extensive database for evaluating the effects of mine effluents across the country. Nationally integrated analyses of the fish and benthic invertebrate data have revealed a number of response patterns in receiving water biota, as summarized in this report. Table 5 provides a summary of the fish and benthic invertebrates results from the first and second national assessment periods.

At a national scale, several lines of analysis showed that metal mine effluent effects tended to be more inhibitory than stimulatory. That is, effluent exposure was more often associated with reductions than increases in the indicators used to assess effects. For effluent-exposed fish during the first national assessment period, national-level meta-analyses revealed significant reductions in condition and relative liver size. For benthic invertebrates, the analyses showed significant reductions in the numbers (density) and kinds (taxon richness) of effluent-exposed benthic invertebrates, contributing to significant changes in community structure, measured by the Bray-Curtis and Simpson's Evenness Index endpoints. For both fish and benthic invertebrates, these conclusions were further reinforced by the study of the national distribution of measured effects shown in the histogram figures in sections 4.2 and 6.2, as well as by multivariate and bivariate statistical analyses published in the first metal mining national assessment report (Lowell et al. 2008).

During the second national assessment period, exposure-area fish again showed significantly reduced condition and relative liver size. In addition, they further exhibited significantly lowered growth rate and a change in age structure, and a shift toward smaller gonad size was also observed. That is, exposed fish during the second national assessment period were, on average, older, thinner and slower growing, with smaller livers and with more of a tendency toward reduced gonad size. These generally inhibitory effects may have a variety of causes, such as direct inhibitory effects of the effluent on fish and/or food limitation resulting from habitat alteration and inhibitory effects on prey organisms, such as benthic invertebrates.

During the second national assessment period, exposure-area benthic invertebrates again showed significantly reduced taxon richness, and the Bray-Curtis endpoint once more revealed notable changes in community structure in exposure areas. In contrast to the response pattern observed during the first national assessment period, a national average increase in density was observed during the second national assessment period. This underscores the potential for metal mines to have stimulatory effects for some endpoints.

Table 5. Summary of EEM findings for fish and benthic invertebrates in metal mine receiving waters during the first and second national assessment periods.

Endpoint	National average findings in exposure compared to reference areas during each national assessment period	
	During 1st national assessment period	During 2nd national assessment period
Fish		
Condition	thinner fish	thinner fish
Relative liver weight	smaller livers	smaller livers
Relative gonad weight	variable results	tendency toward smaller reproductive organs
Weight-at-age	variable results	slower growing
Age	variable results	changed population structure to older fish
Benthic invertebrates		
Total density	less individuals per unit area	more individuals per unit area
Taxon richness	fewer kinds of benthic invertebrates	fewer kinds of benthic invertebrates
Bray-Curtis Dissimilarity Index	change in community structure	change in community structure
Simpson's Evenness Index	change in community structure	variable results
General assessment to date: Inhibitory response patterns observed for exposure-area fish and benthic invertebrates, with possible stimulatory responses occurring at some mines.		

The greater overall tendency for inhibitory effects was particularly evident when comparing these results with those from the pulp and paper industry, the one other industry 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 fish gonadal growth (resulting in smaller gonads) 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. In contrast, the metal mining 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).

The general meta-analyses were broken down by dividing the metal mines into smaller subgroups, corresponding to metal mine ore and habitat types. Three of the fish endpoints that showed a change in response pattern during the second assessment period (weight-at-age, relative gonad size and age), and the benthic invertebrate density endpoint, were studied.

For the weight-at-age endpoint, the national pattern reflecting reduced growth rates in exposure fish was most strongly associated with metal mines that discharge to lake and river habitats, and base metal and iron ore mine types. For the fish gonad size endpoint, the national pattern of smaller exposed fish gonads was most strongly associated with metal mines that discharge to river habitats, as well as precious and base metal mine types. For the age endpoint, the national pattern showing older exposed fish was most strongly associated with metal mines that discharge to lake and river habitats, as well as precious metal and uranium mine types. The national pattern reflecting increased density in exposed benthic invertebrates was associated with all habitat and ore types, with the exception of river erosional habitat types.

Other factors have also been hypothesized to further influence metal mine effluent effects. The increases in density observed during the second national assessment period were thought to have been a response to increasing mining activities over recent years, as reflected by increases in effluent flow, leading to increasing nutrient enrichment effects. Analyses detailed in section 6.5, however, suggest otherwise. There was no overall increase in effluent flow from the first to the second national assessment period. Furthermore, there was no evidence that changes in effluent flow had a predominant overall influence on changes in magnitudes and patterns of effect for either density or taxon richness. Changes in effluent flow may have influenced effects at some individual metal mines, but this factor is likely just one of several that may influence the magnitude and pattern of effects.

Two other such potential factors are concentration of effluent and whether metal mines discharge intermittently versus continuously. Analyses in Lowell et al. (2008) showed that, 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. As also detailed in Lowell et al. (2008), none of the nine fish and benthic invertebrate core endpoints was significantly correlated with the number of months during which metal mines discharged during the year. Thus, effluent effects did not appear to be greatly influenced by whether mines discharge effluent intermittently or continuously.

Effluent effects on fish usability were 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, but the exposure area tissue mercury levels were almost threefold less 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.

Overall, the frequency distributions of sublethal toxicity data from the first national assessment period were similar to those from the second national assessment period. When looking only at data for test organisms exposed to 100% effluent, there was indication of a possible improvement in effluent quality over the two time periods, although it is too early to evaluate whether this possible improvement was large enough to be biologically meaningful. *C. dubia* and *L. minor* tests were usually the most responsive to mining effluents, regardless of mine type. For *L. minor*, frond number was more responsive than dry weight. Fish tests were relatively less responsive compared to the other tests, except for iron ore mine effluents where fathead minnows were fairly responsive.

Much effort has been expended by the metal mines conducting EEM 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 (multiple land uses, other industrial or municipal effluent sources, etc.). Even so, uncertainties remain at some mines. As metal mines progress through future rounds of EEM data collection, continuous improvements in study design and analysis, as well as ongoing research at selected mines, are expected to lead to a better understanding of how such factors may contribute to the effects that are measured.

Although a substantial amount of data for a large number of mines is summarized in this report, these data represent just two monitoring periods, and some of the variations between phases may have been partly due to factors other than effluent exposure. Further rounds of data collection will help to shed light on how constant or variable these response patterns are through time. Some metal mines with confirmed effects are now conducting their investigation of cause phase, and the information that will be gathered in the next round of data collection will be very important to help further elucidate the nature of metal mining effluent effects. Future analyses are expected to provide a more comprehensive picture of metal mining effluent effects in Canada.

9.0 Glossary

Benthic invertebrate community – The varied 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 lead to an understanding of 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 taxonomic 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 exposure area or a series of exposure areas, 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 EEM, 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 behavior, 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 represent different taxa. This measure helps to evaluate changes in the relative abundance of taxa.

Sublethal toxicity tests (SLT) – In the context of EEM, sublethal toxicity tests usually measure the effluent concentration for which a given effect (inhibition, usually) level is observed on the organisms exposed 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 (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.

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Appendix A

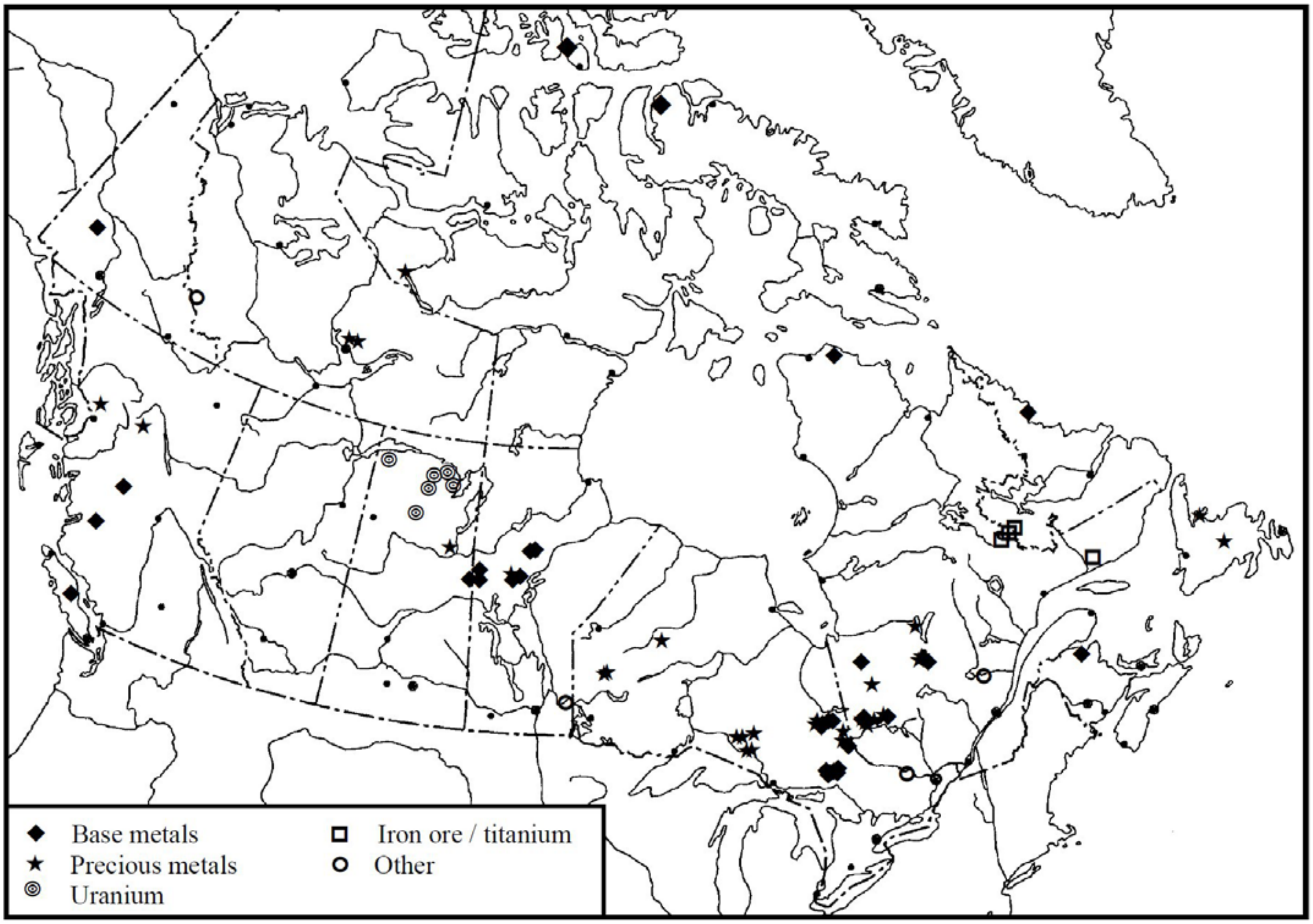

Figure A1. Metal mines subject to the *Metal Mining Effluent Regulations* in 2006 (Environment Canada 2008).

www.ec.gc.ca

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