Development of a Monitoring Design for Examining Effects in Wild Fish Associated with Discharges from Metal Mines

SANDRA C. RIBEY,1* KELLY R. MUNKITTRICK,2 MARK E. McMASTER,3 SIMON COURTENAY,4 CLAUDE LANGLOIS,5 STEVE MUNGER,6 ARDEN ROSAASEN7 AND GERRY WHITLEY8

1Environment Canada, 351 St. Joseph Boulevard, 8th Floor, Hull, Quebec K1A 0H3
2Current Address: University of New Brunswick, Canada Research Chair in Ecosystem Health Assessment, Department of Biology, Saint John, New Brunswick E2L 4L5
3Environment Canada, 867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario L7R 4A6
4Fisheries and Oceans Canada, Gulf Fisheries Centre, 343 Université Avenue, Box 5030, Moncton, New Brunswick E1C 9B6
5Environment Canada, 105 McGill Street, 4th Floor, Montreal, Quebec H2Y 2E7
6Canadian Nuclear Safety Commission, 280 Slater Street, Ottawa, Ontario K1P 5S9
7Cogema Resources Inc., 817–825 45th Street West, P.O. Box 9204, Saskatoon, Saskatchewan S7K 3X5
8Department of Indian Affairs and Northern Development, 345–300 Main Street, Whitehorse, Yukon Y1A 2B5

As part of the amended Metal Mining Liquid Effluent Regulations under the Fisheries Act, mines will be required to develop and conduct Environmental Effects Monitoring (EEM). EEM will be done to evaluate the effects of mine effluent on fish, fish habitat, and fish usability. Mines will be required to determine if there are changes in fish populations and/or in the usability of fish due to mine effluent. The EEM program has been designed with a tiered monitoring approach, with the first phase determining if an effect is present. Subsequent phases of EEM will focus on continued monitoring and determining the magnitude, geographic extent, and cause of effects (if any). Fish collected from the area exposed to mine effluent will be compared to fish from a reference area in order to determine if there is an effect, if the effect is mine related and the cause of the effect within the effluent. The fish population survey will examine the growth, reproduction, condition, and survival of one or more resident sentinel fish species. Fish usability will be determined based on the appearance of fish, their use, and the contaminant levels in fish tissue. It is recognized that some mines may not be able to implement a fish monitoring program as outlined, so it has been recommended that alternative methods, such as caged bivalves or on-site bioassays, may also be used. Frequency of monitoring will be dependent on the previous results of the fish and benthic invertebrate monitoring phases.

Key words: metal mining, EEM, fish survey, Fisheries Act, MMER, fish populations, environmental monitoring

*Corresponding author; sandra.ribey@ec.gc.ca
Introduction

In Canada, Environmental Effects Monitoring (EEM) programs began to develop as a regulatory tool in the early 1990s (Environment Canada 1997). Similar programs have also been developed for pulp and paper discharges in Sweden (Thoresson 1993; Swedish EPA 1997) and Australia (Keough and Mapstone 1995, 1997; Mapstone 1995; Terrens et al. 1998). For metal mines in Canada, a multistakeholder working group undertook an Assessment of Aquatic Effects of Mining in Canada and in the final report (AQUAMIN 1996) recommended that EEM be included as part of the amended Metal Mining Effluent Regulations (MMER), pursuant to the Canadian Fisheries Act (R.S., c. F-14, s. 1). Under this Act, the Federal government limits the deposit of deleterious substances to control impacts on fish, fish habitat and the use by man of fish (fish usability).

The design parameters for EEM in Canada are science based and evolving. Hodson et al. (1996) recommended a tiered design for EEM studies. The development of the requirements for fish monitoring included a large number of meetings of scientists and stakeholders to develop recommendations to design EEM in situations that may present a variety of problems for monitoring. The focus of many of the discussions was on increasing site specificity and flexibility in program design.

The original pulp and paper EEM program was developed without multistakeholder discussions, and the fish program was based on studies that had been conducted in large freshwater rivers and lakes. The study design for metal mines had to take into account that there are large differences between effluent receiving waters of most pulp and paper mills and metal mines. The differences include: mines are often located in headwater areas where water flows are low and fish populations are small, whereas pulp and paper mills are commonly located on larger water bodies; mine sites are often more limited geographically, both in terms of the area affected as well as the effluent volume released; mines may only discharge seasonally, typically when flows are high; mine effluent contains water, process and water treatment chemicals, such as sulphate, chloride, ammonia and cyanide, and trace metals, whereas pulp mill effluents typically contain more organic substances; and, mines can process ore from a host ore body, and may also have ore from other sites brought to the mill. As a result of these characteristics, mine effluent quality can illustrate a high level of variability. During rainfall events, discharges from mines can become concentrated, while those from pulp mills will typically be diluted. These differences were considered in the design of a fish survey for EEM for metal mines.

With these characteristics in mind, the multistakeholder group focused on the consideration of new monitoring approaches, baseline data, analytical approaches, data quality assurance parameters and interpretation tools that were either not available, or lacked consensus on implementation. Most of these discussions have not been widely represented in the scientific literature. The study design for the fish survey of the EEM program also involved discussions on power analysis, the defi-
nition of critical effects sizes, and interpretation of results. The purpose of this paper is to describe the fish program, outline the challenges and highlight the science and consensus-based solutions that were implemented in the program. More details on additional EEM components can be found in accompanying papers (Dumaresq et al. 2002; Glozier et al. 2002; Parker and Dumaresq 2002; Scroggins et al. 2002) as well as in the metal mining requirements and technical guidance documents (Environment Canada 2001; web site reference www.ec.gc.ca/eem).

EEM Program Design

The objective of EEM is to evaluate effects of mine effluent on fish, fish habitat and fish usability. As such, monitoring fish in the aquatic receiving environment (freshwater and marine) is of primary importance to the EEM program. There are receiving environments where monitoring programs will be difficult to design and implement, and there are some receiving environments where physical and logistical constraints will not allow a monitoring program to be conducted. For example, for metal mining EEM, the decision was made that a mine would not be required to conduct a fish survey if the effluent concentration in the receiving environment is less than 1% at 250 m from each final point of discharge (Environment Canada 2001). For the pulp and paper EEM program, a decision tree had been developed to assist in the decision of whether fish monitoring was required (Environment Canada 1997).

The metal mining program built on a multistakeholder review of existing monitoring data (AQUAMIN 1996) and a multistakeholder-funded research program to review monitoring tools and their applicability to evaluating the potential impacts of mining activities (AETE 1997, 1998, 1999). The final development of the monitoring requirements involved working groups comprised of experienced monitoring representatives from all relevant stakeholders. The process differed significantly from the development of the pulp and paper EEM program, which produced a strictly regimented program that has subsequently been altered to increase flexibility and site specificity (Environment Canada 1997, 1998).

The main focus of the EEM program for metal mining is to answer the following questions:

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

In the fish program for EEM, a fundamental change from the pulp and paper approach was the decision to base the fish program on addressing the required information needs, while maintaining flexibility in program design. Although core standardized components are provided, in the absence of consensus on acceptable study designs, priority is given to relevant, available information and flexibility in study design to answer the key questions.
To increase flexibility, key questions were developed that the fish component of EEM must address, which are:

- Has the fish community been modified by the effluent?
- Have the fish populations been modified by the effluent?
- Has there been a change in fish usability due to effluent?

A key component in the monitoring program design is the decision as to what changes represent impacts, and how the monitoring program will be designed to detect those changes (Munkittrick et al. 2000). For the EEM metal mining program, an effect was defined as a statistically significant difference in fish population endpoints and the benthic invertebrate community endpoints measured between an area exposed to effluent and a reference area. An effect in fish tissue was defined as a statistical difference as well as the levels of contaminants in fish tissue being higher than human health consumption guidelines. Under certain study designs, a statistically significant gradient in these endpoints will also be considered an effect. The definition of effect adopted by the EEM program does not consider ecological relevance of the change; other factors and issues associated with interpreting the relevance of an effect are discussed in Environment Canada (2000).

The metal mining EEM program has a general framework that includes phases for Initial Monitoring, Periodic Monitoring, Focused Monitoring and Investigation of Cause. The descriptions of these phases can be found in more detail in Dumaresq et al. (2002) and Environment Canada (2001). While the pulp and paper EEM program has utilized a standard three- or four-year cycle, the timing of the phases for the metal mining program will be dependent upon the results of previous monitoring. When effects are present, the subsequent monitoring is to be conducted in the next available field season. The description of the phases later in this paper will be restricted to the requirements for the fish survey.

The mining EEM program is tiered, with the monitoring frequency dependent on previous monitoring results for both the fish survey and the benthic invertebrate community survey. There were decisions made by the multistakeholder group that affected the fish survey study design, monitoring phases, and data assessment and interpretation. These factors are discussed in the following sections.

**Fish Survey Study Design**

There has been considerable controversy and discussion over the basic design components for monitoring, including whether monitoring be conducted at a biochemical level, individual, population or fish community level. A more complete description of the options can be found in Munkittrick et al. (2000).

The decision was made to focus the program on defining potential effects at the levels of the individual and/or population. The fish community survey was restricted to identify potential sentinel species and their relative abundance. There are difficulties in defining a more specific fish community approach on a national basis for a country as large and
diverse as Canada, including concerns about site specificity of designs, and sampling methodologies. Furthermore, due to the need to eventually link any fish changes that are evident to the effluent, there is a subsequent need in a community-based program to proceed to monitoring at the population and individual levels.

The emphasis of the fish component of the metal mining EEM program is to define whether discharges affect the growth, reproduction, condition and survival of resident fish. The EEM fish survey may include monitoring the fish to determine if there are differences in survival (age structure), energy use (growth and reproduction) and energy storage (condition) of fish exposed to effluent and fish caught in a reference area. “Fish,” as defined by the *Fisheries Act*, includes fish, shellfish, and crustaceans.

The fish program includes a number of elements in its tiered approach, including defining whether effects are present; can those effects be confirmed by a subsequent set of collections; what is the magnitude and geographic extent of effects; and what are the potential underlying causes? Due to the potential for sampling bias and year-to-year variability, the confirmation step was a key step in the fish survey design: effects should be confirmed before studies proceed to more detailed, and potentially more expensive, sampling.

Although the preference for population monitoring is to examine two sexually mature fish species, there may be situations where immature fish are the primary residents, or where fish population sizes are small and sampling mortality may constitute an impact on the resident. Several options were developed in these cases, including the option to examine mortality rates and growth rates in immature fish, and the option of non-lethal sampling where there are concerns about impacts related to sampling mortality. Additional guidance for the development of non-lethal sampling methods is presented elsewhere (Gray et al. 2002).

The fish survey may also include conducting a fish tissue analysis to determine if there are changes in fish usability because of increased levels of metals in fish tissue. If provincial, territorial or federal human health consumption guidelines have been established within the jurisdiction of the mine, and the metals for which there are guidelines are present in the effluent, a mine will be required to conduct a fish tissue analysis. The presence of trace metals is determined during Effluent Characterization, which is also required as part of the EEM program.

Specific triggers and monitoring requirements are highlighted in Table 1. Specific issues related to interpretation of the data and follow-up actions can be found in Environment Canada (2000) and Munkittrick et al. (2000). Some challenging issues arose in the discussions of the location of sampling areas, the selection of sentinel species and the sample size requirements.

**Location of Sampling Area**

The challenging issues related to sampling areas included the definition of reference areas, design issues related to the presence of potentially confounding discharges, and design issues in the absence of barriers to fish movement.
<table>
<thead>
<tr>
<th>Concern</th>
<th>Indicators (historical data)</th>
<th>Initial monitoring</th>
<th>Periodic monitoring</th>
<th>Focused monitoring</th>
<th>Investigation of cause</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Confirm</td>
<td>Surveillance</td>
<td>Minimal</td>
</tr>
<tr>
<td>Trigger for monitoring</td>
<td>No suitable historical data</td>
<td>Effect seen but not confirmed</td>
<td>Effect seen and confirmed and cause is known but ongoing surveillance necessary</td>
<td>No effects seen, and that has been confirmed (using historical data or periodic monitoring), and no significant change in conditions since last monitoring period</td>
<td>Mine-related effect seen</td>
</tr>
<tr>
<td>Community composition</td>
<td>Presence/ absence Abundance of species Rare/ endangered species</td>
<td>Relative abundance CPUE of species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population</td>
<td>Abundance Growth Reproduction</td>
<td>Size vs. Age Average gonad size Average age Relative year class strength</td>
<td>Size vs. age Average gonad size Average age Relative year class strength</td>
<td>Size vs. age Average gonad size Average age Relative year class strength</td>
<td>Detailed, focused studies on effects of concern</td>
</tr>
</tbody>
</table>

(continued)
### Table 1. (continued)

<table>
<thead>
<tr>
<th>Concern</th>
<th>Endpoints (historical data)</th>
<th>Indicators</th>
<th>Initial monitoring</th>
<th>Periodic monitoring</th>
<th>Focused monitoring</th>
<th>Investigation of cause</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Health</td>
<td>Condition factor</td>
<td>Condition factor</td>
<td>Condition factor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Physical</td>
<td>Physical abnormalities</td>
<td>Physical abnormalities</td>
<td>Physical abnormalities</td>
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<tr>
<td></td>
<td></td>
<td>abnormalities</td>
<td>abnormalities</td>
<td>abnormalities</td>
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<tr>
<td></td>
<td></td>
<td>Exposures</td>
<td>Metal levels in tissue</td>
<td>Metal levels in tissue</td>
<td>Metal levels in tissue</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Health</td>
<td>No baseline data; contaminants identified as concern during Site Characterization</td>
<td>Most recent levels are elevated</td>
<td>Levels elevated and extent known</td>
<td>Effect seen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Physical</td>
<td>most recent levels are elevated</td>
<td>Levels not elevated and no mine process changes since last monitoring period</td>
<td>Effect is mine-related and geographical extent and magnitude known</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>abnormalities</td>
<td>Tissue concentrations of Contaminant of concern</td>
<td>Tissue concentrations of Contaminant of concern</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fish usability</td>
<td>Tissue concentrations of Contaminant of concern</td>
<td>Tissue concentrations of Contaminant of concern</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metals levels in tissues</td>
<td>Tissue concentrations of Contaminant of concern</td>
<td>Tissue concentrations of Contaminant of concern</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>External appearance</td>
<td>Tissue concentrations of Contaminant of concern</td>
<td>Tissue concentrations of Contaminant of concern</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Registered complaints</td>
<td>Tissue concentrations of Contaminant of concern</td>
<td>Tissue concentrations of Contaminant of concern</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intervals</td>
<td>Program initiation if inadequate information for Site Characterization</td>
<td>Next available sampling period</td>
<td>3 years</td>
<td>6 years at most</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Next sampling period after confirmation of changes</td>
</tr>
</tbody>
</table>
The study design must include relevant information to characterize the area that surrounds the mine, including effluent mixture areas, geological, hydrological, chemical and biological features, information about the mine operations, and any confounding influences (such as other point or non-point sources of effluent, dams etc.). All of this information plays a role in selection of appropriate reference and exposure areas for the fish survey. The reference and exposure areas should be similar, with the exception of exposure to the mine effluent.

The exposure area is defined as all waters that are exposed to mine effluent. Initially, fish sampling should be done in an area most proximate to effluent discharge, where fish are found and it could reasonably be expected to find effects (Initial and Periodic Monitoring). Subsequent monitoring may require sampling further from the effluent discharge, until no effects are found, in order to determine the geographic extent of the effects (Focused Monitoring).

The reference area should be located adjacent to or upstream of the exposure area, but far enough away that fish populations are not likely to mix. Historic data or the use of multiple reference areas may assist in data interpretation. The appropriateness of the reference area should be re-evaluated during each study design, particularly if the geographic extent of monitoring is being expanded.

When choosing the sampling areas, it is important to consider fish species that are present in the exposure and reference areas, which is determined during the Initial Monitoring phase. This includes determining the potential mobility and the relative abundance of the fish species captured.

Selection of Fish Species

When selecting the fish species for the fish survey, the most important considerations are abundance, relevance to the study area, that endpoints of interest are easily measured (Munkittrick and McMaster 2000) and relative sensitivity to the effluent (Table 2).

The EEM program has been designed to monitor two fish species and the priority has been to monitor resident species as adults (e.g., sexually mature fish) when they are available (Environment Canada 1997). It is recognized that this will not be possible at all mine sites, and the discussions centred around recommending options for monitoring sexually immature species in areas where the most exposed species are only present as juveniles.

Species chosen for the fish survey should have a high abundance in the study area so that a long-term monitoring program using the same species is possible. Fish species that are used for commercial, sport and subsistence fishing are generally not recommended for the fish survey due to confounding effects of exploitation. For the fish species selected, one must be able to measure endpoints to determine the survival, energy use and energy storage for that species (Table 3). As well, the diet of the sentinel species should be known or evaluated, as those species that depend mostly on terrestrial insects would be of little relevance in assess-
Table 2. Monitoring species characteristics for optimizing the assessment of aquatic environmental health versus human health issues using fish populations

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fisheries health</th>
<th>Human health</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Point source</td>
<td>Non-point source</td>
<td></td>
</tr>
<tr>
<td>Residency (in absence of barrier)</td>
<td>Local</td>
<td>Wide ranging</td>
<td>Issue specific</td>
</tr>
<tr>
<td>Abundance</td>
<td>High</td>
<td>High</td>
<td>Issue specific</td>
</tr>
<tr>
<td>Longevity</td>
<td>Short-medium</td>
<td>Short-medium</td>
<td>Long</td>
</tr>
<tr>
<td>Food preference</td>
<td>Benthic</td>
<td>Issue specific</td>
<td>Piscivores</td>
</tr>
<tr>
<td>Fecundity and growth rate</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Age to maturation</td>
<td>Short</td>
<td>Short</td>
<td>Long</td>
</tr>
<tr>
<td>Spawning time</td>
<td>Site specific</td>
<td>Site specific</td>
<td>Site specific</td>
</tr>
<tr>
<td>Food chain involvement</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Source: Munkittrick and McMaster (2000).*
ing aquatic food-chain impacts, while those that are long-lived and predatory on fish would be most relevant for potential food chain bioaccumulation. Benthivorous fish can be a good choice for fish population surveys because they are usually less mobile than pelagic species, and they feed at the water-sediment interface where metals can accumulate. If bioaccumulation is a concern, piscivores, such as walleye (*Stizostedion vitreum*) or whitefish (*Coregonus clupeaformis*), may be used. A possible issue with using species such as these is that of exposure and potential mixing of fish populations between the exposure and reference area, especially in situations where there are no physical barriers separating the areas. For this reason, the use of small-bodied fish species (species that have a maximum size \(< 150 \text{ mm}\)), such as darters, minnows and sculpins, is also recommended and their use has been increasing in the pulp and paper EEM program (Munkittrick et al. 2000).

Small-bodied fish species are usually quite abundant, easy to capture and more sedentary than larger fish. It has been found that home range size is positively correlated with body size (Minns 1995). All measurements that should be taken (Table 4) can be taken easily using small-bodied species. There are some special considerations that should be taken into account when using small-bodied species. However, in some cases, less research has been done on the species, so less is known about their basic biology. Some species are multiple spawners (e.g., Heins and Rabito 1986; Burt et al. 1988; Paine 1990). Reproductive effort in these species is difficult to estimate from a single sample, because the reproductive tissue can be turned over almost completely between clutches (i.e., most of the

<table>
<thead>
<tr>
<th>Table 3. Fish measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length (fork or total or standard)</strong> (^a)</td>
</tr>
<tr>
<td><strong>Total body weight (fresh)</strong></td>
</tr>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td><strong>Gonad weight</strong></td>
</tr>
<tr>
<td><strong>Egg size</strong></td>
</tr>
<tr>
<td><strong>Fecundity(^b)</strong></td>
</tr>
<tr>
<td><strong>Weight of liver</strong></td>
</tr>
<tr>
<td><strong>External condition</strong></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
</tr>
</tbody>
</table>

\(^a\) If caudal fin forked, use fork length, otherwise, use total length. In cases where fin erosion is prevalent, standard length should be used.

\(^b\) Fecundity can be calculated by dividing total ovary weight by weight of individual eggs (individual egg weight can be estimated by counting the number of eggs in a sub-sample. The water displacement method is not recommended. The sub-sample should contain at least 100 eggs). The eggs should be weighed prior to preservation as preservation will change the weight of the eggs.
mass of ova in the ovary will be spawned, and then a new clutch of mature ova will be developed). The number of clutches produced during the spawning season becomes the important reproductive variable and is difficult to estimate for an individual female in the field, even with frequent sampling. It will be difficult to evaluate the significance of changes in egg production in multiple spawners if they show normal reproduction in the first clutches. Impacts will be difficult to detect and will be affected by a variety of site-specific factors such as survival, immigration, emigration, etc. Until more information is known, the program will consider that multiple spawners showing normal fecundity, normal egg size, normal size and normal age at maturity prior to the first spawn of the season are not experiencing a decrease in reproduction.

Guidance on non-lethal sampling of these species for the purposes of EEM programs can be found in Gray et al. (2002). Where no finfish are present, the mine may choose to sample molluscs such as oysters or mussels. Bivalves have been successfully used for population assessments of metal contamination (Elder and Collins 1991; MacMahon 1991).

For the fish tissue analysis, the species selected may not be the same species being used for the population survey. The species should be a sport, subsistence or commercial species that is locally consumed if bioaccumulation of metals is a local concern.

Sample Size

The mine must include in the study design a targeted number of fish that will be captured during the fish population survey. In the absence of previous monitoring data which would allow the use of a power analysis to select a sample size, it is recommended that 20 mature males and 20 mature females are captured in each sampling area, for each fish species used in the population survey. It has been found in studies using white

<table>
<thead>
<tr>
<th>Type of response</th>
<th>Effect endpoint</th>
<th>Statistical procedure (dependent on endpoint)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>Age</td>
<td>ANOVA</td>
</tr>
<tr>
<td>Energy use</td>
<td>Size-at-age (body weight against age)</td>
<td>ANCOVA</td>
</tr>
<tr>
<td></td>
<td>Relative gonad size (gonad weight against body weight)</td>
<td>ANCOVA</td>
</tr>
<tr>
<td>Energy storage</td>
<td>Condition (body weight against length)</td>
<td>ANCOVA</td>
</tr>
<tr>
<td></td>
<td>Relative liver size (liver weight against body weight)</td>
<td>ANCOVA</td>
</tr>
</tbody>
</table>

*a For the ANCOVA analyses, the first term in parentheses is the endpoint (dependent variable, Y) that is analyzed for an effluent effect. The second term in parentheses is the covariate, X (age, weight, or length).
suckers that there is little change in the 95% confidence limits with increasing sampling sizes beyond 20 when adult fish are sampled randomly (Munkittrick 1992). If a small-bodied fish species is selected, it is also recommended that an additional 20 juvenile fish be collected.

It has also been recommended that for the fish population survey, non-destructive sampling of fish be used as an alternative sampling design. If this option is chosen, a minimum of 100 individuals of the fish species should be collected in each sampling area (Gray et al. 2002). The fish collected should represent a range of sizes within the population.

If there are previous monitoring data, the sample size can be calculated once the effect size and power levels are known. Sample sizes can be calculated using the methods found in Green (1989) and Environment Canada (1998). In Cycle 1 of the pulp and paper EEM program, differences as high as 20% were seen in parameters between reference areas (Environment Canada 1997). The variability from the previous monitoring phase is used to set the estimated sample size required. Since variability will change between monitoring phases, the target effect size should not be a fixed number, but a range of changes, such as a 20 to 30% difference (Environment Canada 1997).

Fish characteristics that are monitored in the EEM program are not equally variable. Reproductive measurements have been found to be more variable on a relative scale than measurements such as length, weight and liver weight (Environment Canada 1997). If effect sizes are also expressed on a relative scale (e.g., as % difference), then any study that will generally be able to detect a 25% difference in relative gonad size, can detect similar or smaller differences in other measurements.

It has been recommended that the EEM population survey be designed to detect a 20 to 30% difference in gonad size, using a power level of 0.9 (1-β), where α and β are set equal at 0.10. There was considerable discussion given to the consequences of power levels by the benthic working group (Environment Canada 2001), and a decision was made to recommend that α and β levels be made equal in the EEM program (i.e., they could be set at 0.05 or 0.10, as long as they are equal; a more complete description of the issues can be found in Glozier et al. 2002). Priority should be given to reducing variability, rather than increasing sample sizes. When the variability is so high that sample sizes are not justifiable or cost effective, the mine should consider selecting a different fish species or an alternative monitoring method.

For the fish tissue analysis, it has been recommended that analyses be conducted on no less than eight fish of the same sex and species, in both the exposure and reference areas. The analysis is conducted on a composite sample of the fish, with five composites made up of equal wet weight.

### Fish Monitoring Methods Not Considered Appropriate for Mining EEM

The Aquatic Effects Technology Evaluation (AETE) program, which was a cooperative program between the Canadian mining industry and
various federal and provincial government departments, was established to review techniques for assessing impacts of mine effluents. AETE included an evaluation of fish monitoring methods (AETE 1998, 1999). Throughout the development of the EEM program, the results of AETE were referred to determine the suitability of various methods for the mining environment. Among the various techniques, the following were considered, but not recommended, for the mining EEM program.

Index of Biotic Integrity

The Index of Biotic Integrity (IBI) has been used successfully in determining fish community level differences in some North American streams, mostly in south and central U.S.A. (Karr et al. 1986). The studies are usually designed to be conducted in small streams or the littoral zones of lakes, in order to reduce the effort required to sample fish. Many types of sampling gear are used to obtain a representative collection of fish. Fish are generally only identified, counted and returned to the water.

At this time in Canada, there are no national, provincial or regional requirements for any community level surveys being conducted during mine operation. Community surveys are often included in baseline studies prior to operation of a new mine. In the Québec region, IBI type monitoring is being developed for broad-based environmental quality assessment.

It is unknown whether the IBI would be successful in northern regions because there are generally few species. As EEM is a national program, this was one consideration in rejecting IBI as a monitoring technique. As well, community surveys often require greater sampling effort than a fish population survey, therefore may be less cost effective despite low processing costs (AETE 1998).

Metallothionein as a Biomarker

In the pulp and paper EEM program, one of the issues of concern that remains is fish residency, including whether or not fish have been exposed to effluent, as well as the length of time fish have been in the exposure area. To address this in the mining EEM program, the use of metallothionein as a biomarker of exposure was considered.

Based on the AETE findings (AETE 1997), it was found that metallothionein can be a useful biomarker of exposure to some metals, including Cd, Zn, Cu and Ag. But, metallothionein was not found to be an effective biomarker in highly mobile species, which would be of concern when determining fish residency. At the time of the report, metallothionein analyses were not available commercially, and there were no standard protocols for sample preparation, extraction and quantification. Because of these reasons, this tool was not recommended at this time.

Histopathology

Determining alterations in organs and tissues at the microscopic level using histopathological techniques was also considered for the EEM
program. The AETE (1998) studies found that there are few histological effects that have shown a high specificity for metals and in the 1996 field program (Beak 1996) there was a lack of consistent responses in fish. Furthermore, there are few private sector companies with the technical expertise to conduct the analyses. Therefore, this technique was not included for routine monitoring in the EEM program, although this tool may be useful during investigation of cause.

Monitoring Phases

There were decisions made by the working groups that affected the design of the monitoring phases. Discussion here will be restricted to the specifics of the fish monitoring program. The important components of the monitoring design include the selected fish species, the sampling areas, sample size, and sampling time.

Initial Monitoring

The first mining EEM study, Initial Monitoring, is conducted in order to determine if the effluent has modified fish populations and if there has been a change in fish usability due to the effluent. In Initial Monitoring, fish population measurements examining fish growth, reproduction, condition and survival, from the exposure and reference area, are compared statistically to determine if there are differences between the areas. Tissue analyses on metals of concern may be conducted as well, and examined to see if there are statistical differences between the exposure area and reference area, as well as whether the levels found in the exposure area are higher than human health consumption guidelines.

Periodic Monitoring

Subsequent to Initial Monitoring, the mine will continue in Periodic Monitoring to either:

- Ensure effects are replicable, consistent and mine related (confirmation monitoring);
- Ensure that there are still no effects (after one survey), or that confirmed effects for which the magnitude, extent and cause are known, are not changing (surveillance monitoring); and
- Ensure after two consecutive fish surveys and benthic invertebrate community surveys have found no effects, that there are still no effects (minimal monitoring).

The differences between the three Periodic Monitoring phases (Confirmation, Surveillance and Minimal) are the sampling intervals and the triggers to conduct the sampling (Table 1). Periodic Monitoring should be sampled in the same month/season as the previous monitoring phase. At any time, if an effect is found, the mine may choose to proceed directly to Focused Monitoring.
Confirmation monitoring will be conducted when a mine finds an
effect in fish after one survey. Mines must conduct confirmation monitor-
ing within 24 months of the submission of the previous interpretative
report (the report submitted to Environment Canada by the mine, outlin-
ing the results of the EEM study). Mines may wish to modify their study
design prior to confirmation monitoring if it was questionable that the
effect found was mine related (e.g., minimize monitoring effects from any
confounding influences), since if the effect is found in two consecutive
surveys, it will be considered mine related.

Surveillance monitoring is conducted when no effects in fish have
been found or to monitor effects where the magnitude, geographic extent
and cause of the effect is known. The surveillance phase of periodic mon-
itoring must be completed with 36 months of the submission of the pre-
vious interpretative report.

If, after two consecutive EEM surveys, no effects have been found in
fish or benthic invertebrates, the mine may conduct minimal monitoring.
The minimal monitoring phase must be completed within 72 months of
the submission of the previous interpretative report. If there are perma-
nent changes in mine operations (e.g., a change in the location of the final
points of discharge, new ore bodies being processed) or a permanent
physical change to the study area (e.g., a dam) it is recommended that
mines conduct confirmation monitoring as soon as possible, to ensure
there are still no effects on fish or benthic invertebrates.

**Focused Monitoring**

Once effects have been confirmed, the mine is required to determine
the magnitude and geographic extent of the effects. Focused monitoring
involves sampling exposure areas further from the final discharge points
until no effects are found. The mine may have to select new reference
areas if the habitat type in the exposure area differs. As well, different fish
species, possibly from a different trophic level, could be sampled to find
out if the same effects are being found throughout the exposure area.
Focused monitoring must be completed within 24 months of the effect
being confirmed. If an effect is found at any time, mines may choose to
proceed directly to Focused Monitoring.

**Investigation of Cause**

If, after determining the magnitude and geographic extent, the cause
of the effects in fish are still not known, the mine must conduct an
Investigation of Cause within 24 months of the previous monitoring
phase. Investigation of Cause may not include a fish survey as was con-
ducted in Periodic or Focused Monitoring. The recommended tools for
determining cause of effects in fish include using ecoepidemiological tech-
niques, the identification of specific stressors that are primarily responsi-
ble for changes, or the identification of specific chemicals associated with
changes (Fox 1991; McMaster et al. 1996; Culp et al. 2000). The type of
study appropriate for each mine is determined site specifically, based on the effects being found.

**Data Assessment and Interpretation**

To determine if there are effects on the fish population, statistical analyses of the data are necessary. The endpoints that will be used to determine effects are outlined in Table 4. Supporting analyses of the additional data can also be conducted (Table 5), but any statistically significant differences in these endpoints may not necessarily be considered an “effect.” Because there are sex differences in overall energetic requirements, sexes should be treated separately for the statistical analysis. Also, results from sexually immature fish should not be mixed with those from mature fish for statistical analysis.

The recommended order of data analysis can be divided into five stages: a) preparing the data for analyses, b) initial summary statistics, c) ANOVA analyses, d) ANCOVA analyses, and e) power analyses.

Preparing the data for analyses: this step includes data entry and QA/QC, including a check for data entry errors, and confirmation that all fish which are assumed to be adults are undergoing or have undergone gonadal development for the next spawning season. Data that will be necessary for interpretation should also be summarized, including significant confounding factors. Other significant information will include the gear used and catch per unit effort calculations. This step should also include an examination of the exposure data, and determination of whether there was a possibility of fish moving between areas, including whether the potential existed for reference area fish to be exposed.

Summary statistics: summary statistics should be calculated by species and sex, and should include means, standard deviations, standard error, minimum and maximum values and sample sizes. The sample size collected should be compared to the objectives for the sampling program, and the variability obtained should be compared to previous studies and the values used during study design. The data should also be examined for normality and equality of variances (basic statistical assumptions) (Environment Canada 2001).

ANOVA analyses should examine whether there was a statistically significant difference in length, weight, age, egg size or fecundity for either species or sex; p level; % difference; and an indication of which area was larger when differences existed.

ANCOVA analyses should be performed for size-at-age, condition factor (length-weight regressions), gonadal weight (gonad weight-carcass weight or gonadal weight-length regressions), and liver weight (liver weight-body weight and length regressions). The analyses should examine whether there were significant regressions, and if there was a significant interaction between areas. If slopes were equal, the data should be examined for a difference in elevation between areas. Information to report include: area in which condition index was higher, percent difference in CI and significance (p) of difference in slopes or elevations. If there
is an interaction between the variables tested the analysis continues by examining the plotted data, to see if the differences are interpretable. The statistical power of the comparisons should be examined, and sample sizes defined for subsequent monitoring phases.

If an alternative to the fish survey is conducted, the mine should do a scientifically defensible data analysis applicable to that alternative in order to determine whether or not there is an effect on fish (Environment Canada 2001).

### Alternatives to the Fish Survey

It is recognized that there may be situations where a fish survey, as outlined above, may provide minimal useful information (e.g., presence of many confounding influences), may not be cost effective, or may subject persons doing the monitoring to hazardous conditions. In these situations, the mine may choose an alternative option for the fish survey.

One recommended alternative to the population survey and fish tissue analysis is the use of caged bivalves. Caged bivalves can be effectively used to circumvent problems with confounding discharges. The methodology for caged bivalves has recently become well known and well-established endpoints (growth and bioaccumulation) have been

**Table 5. Endpoints to be used for supporting analyses—these analyses are for informational purposes and significant differences between exposure and reference areas are not necessarily used to designate an effect**

<table>
<thead>
<tr>
<th>Type of Response</th>
<th>Supporting response variable</th>
<th>Statistical procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy use</td>
<td>Body weight (whole)</td>
<td>ANOVA</td>
</tr>
<tr>
<td></td>
<td>Length</td>
<td>ANOVA</td>
</tr>
<tr>
<td></td>
<td>Size-at-age (length against age)</td>
<td>ANCOVA</td>
</tr>
<tr>
<td></td>
<td>Relative gonad size (gonad weight against length)</td>
<td>ANCOVA</td>
</tr>
<tr>
<td></td>
<td>Relative fecundity (# of eggs/female against body weight)</td>
<td>ANCOVA</td>
</tr>
<tr>
<td></td>
<td>Relative fecundity (# of eggs/female against length)</td>
<td>ANCOVA</td>
</tr>
<tr>
<td></td>
<td>Relative fecundity (# of eggs/female against age)</td>
<td>ANCOVA</td>
</tr>
<tr>
<td>Energy storage</td>
<td>Relative egg size (mean egg weight against body weight)</td>
<td>ANCOVA</td>
</tr>
<tr>
<td></td>
<td>Relative egg size (mean egg weight against age)</td>
<td>ANCOVA</td>
</tr>
</tbody>
</table>

*a For the ANCOVA analyses, the first term in parentheses is the endpoint (dependent variable, Y) that is analyzed for an effluent effect. The second term in parentheses is the covariate, X (age, weight, or length).*
used in Canada and the United States in a cost-effective manner (Courtenay et al. 1998; Environment Canada 2001).

Mesocosms/on-site bioassays are another recommended alternative method to design a study around confounding influences. A mesocosm is a field-based, artificial stream system that simulates the natural environment, while allowing for control of variables, such as sex ratios and effluent exposure (Culp and Podemski 1995; Culp et al. 1996; Dubé et al. 1998). Studies have shown that standard EEM population survey endpoints, as well as physiological endpoints, can be measured in fish exposures of 30 to 60 days, with little more cost than the “standard” population survey (Dubé et al. In press).

When it is not possible to sample fish directly, it may be possible to estimate the potential of the effluent to affect fish, using fish sublethal toxicity studies at environmentally relevant concentrations. The validity of extrapolating toxicity test results to field results is to be assessed as more information from the EEM program becomes available. Therefore, the utility of this alternative remains to be determined.

Conclusions

Based on multistakeholder consultation, consensus on a number of key elements of the fish monitoring component of the mining EEM program was achieved.

In order to determine the potential effects of mine effluent on fish, the most cost-effective method that was able to produce reproducible and scientifically defensible results was a fish population survey, where measurements of energy use, energy storage and survival would be examined. The fish population survey would ideally look at two fish species that were sexually mature at the time of sampling, although the program includes the flexibility to allow for the use of juvenile fish. Non-lethal sampling of some fish species is also recommended, as are other alternative monitoring methods, such as the use of caged bivalves and on-site bioassays/mesocosms when wild fish studies are not appropriate.

To determine fish usability, fish caught during the population survey are examined for external lesions, tumors or other abnormalities. As well, a fish tissue analysis may be required if the jurisdiction has human health consumption guidelines for certain metals, such as mercury, and that metal is present in the mine’s effluent. Fish usability would be considered impaired if the level of the metal was significantly different from the level found in the reference area and above the human health consumption guideline.

Acknowledgment

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References


Environment Canada. 2000. Guidance for determining follow-up actions when effects have been identified in environmental effects monitoring (EEM). Environment Canada, Hull, Quebec. October, 2000 (draft).


