



Environment  
Canada

Environnement  
Canada



# Environmental Effects Monitoring Investigation of Cause Workshop for

---

## **METAL MINING**

ISBN: 978-1-100-19968-9

Cat. No.: En14-59/2012E-PDF

Information contained in this publication or product may be reproduced, in part or in whole, and by any means, for personal or public non-commercial purposes, without charge or further permission, unless otherwise specified.

You are asked to:

- Exercise due diligence in ensuring the accuracy of the materials reproduced;
- Indicate both the complete title of the materials reproduced, as well as the author organization; and
- Indicate that the reproduction is a copy of an official work that is published by the Government of Canada and that the reproduction has not been produced in affiliation with or with the endorsement of the Government of Canada.

Commercial reproduction and distribution is prohibited except with written permission from the Government of Canada's copyright administrator, Public Works and Government Services of Canada (PWGSC). For more information, please contact PWGSC at 613-996-6886 or at [droitdauteur.copyright@tpsgc-pwgsc.gc.ca](mailto:droitdauteur.copyright@tpsgc-pwgsc.gc.ca).

© Her Majesty the Queen in Right of Canada, represented by the Minister of the Environment, 2012

Environmental Effects Monitoring  
Investigation of Cause Workshop for Metal Mining

Proceedings

Prepared by  
The National Environmental Effects Monitoring Office  
Environment Canada

*Holiday Inn Chaudière in Gatineau, QC*

*December 8<sup>th</sup> and 9<sup>th</sup>, 2009.*

## ACKNOWLEDGEMENTS

Special thanks are extended to the organizing committee for coordinating the workshop, to all the presenters for their valuable contributions, and to the Mining Association of Canada for co-hosting this workshop with Environment Canada. The Mining Association of Canada and Environment Canada wish to thank the participants (Appendix 2) for their involvement and participation. The discussion surrounding policy, process, and scientific aspects of Investigation of Cause provided valuable input (particularly methodologies and tools) to all stakeholders who need to design Investigation of Cause Studies for metal mining, and to the Environmental Effects Monitoring Program in preparation for the Investigation of Cause stage within the *Metal Mining Effluent Regulations*.

### Organizing Committee

Robert Prairie (co-chair)  
Rick Lowell (co-chair)  
Elizabeth Gardiner  
Jean-François Doyon  
Kent England  
Bernard Vigneault

Charles Dumaesq  
Sue Ellen Maher  
Jim McGeer  
Mark McMaster  
Sherry Walker  
Bonna Ring

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS .....	II
INTRODUCTION AND OBJECTIVE OF WORKSHOP .....	1
Introduction .....	1
Objectives .....	1
OVERVIEW OF THE WORKSHOP .....	1
BACKGROUND OF THE METAL MINING EEM PROGRAM .....	2
EXTENDED ABSTRACTS* .....	3
Session 1: Overview of Environmental Studies .....	3
National Assessment of Effluent Effects on Fish and Invertebrates over the First Two Phases of the Metal Mining Environmental Effects Monitoring Program .....	3
Update on National Investigation of Cause Project on Reduced Gonad Size in Fish Exposed to Pulp and Paper Effluent.....	6
Overview of Existing Guidance for Investigation of Cause in the Metal Mining Environmental Effects Monitoring Program .....	8
Review of Regulatory Requirements and Policy for Metal Mines in the Investigation of Cause Phase of the Environmental Effects Monitoring Program .....	11
Overview of Proposed Amendments to Environmental Effects Monitoring Provisions of the Metal Mining Effluent Regulations .....	15
Session 1 Discussion Period .....	15
Session 2: Potential Causes of Effects.....	18
Ecotoxicology of trace metals in the aquatic environment .....	18
Investigating Selenium Toxicity Using an Environmental Effects Monitoring Approach .....	20
Cyanide speciation and fate in gold mine tailings: A case study .....	23
Mining Reagents and By-Products (e.g. Thiosalts) As Potential Toxicants in Mine Effluents. ....	26
Session 3: Regional Overview and Case Studies .....	33
Metal Mining Environmental Effects Monitoring in the Atlantic Region .....	33
Metal Mining Environmental Effects Monitoring in the Quebec Region .....	35
Brief Overview of Metal Mining Environmental Effects Monitoring in the Ontario Region.....	35
The Metal Mining Environmental Effects Monitoring Program in the Prairie and Northern Region. ....	39
Metal Mining Environmental Effects Monitoring in Pacific and Yukon Region .....	40
Investigation of Cause of Effects on Benthic Invertebrates at the Kidd Metallurgical Site.....	43
Con Mine: Investigation of Cause Study on fish livers – Challenges to designing a new Investigation of Cause study .....	44
Session 4: Tools and Approaches for Investigation of Cause Studies.....	49
An update on the use of caged fish for Environmental Effects Monitoring.....	49
<i>In situ</i> measurements of toxicity and contaminant bioaccumulation with caged amphipods exposed to water and sediment for Investigation of Cause in metal mining Environmental Effects Monitoring studies.....	52

Experimental Designs Using Artificial Streams (Mesocosms) to Address Cause for Metal Mine Effluents .....	58
Toxicity Reduction Evaluations as a Tool for Investigation of Cause - Mining Case Studies .....	62
Fathead Minnow Lifecycle Assays for Assessment of Complex Effluents .....	63
Using bioaccumulation models for predicting dissolved metal toxicity .....	65
Quantifying the Cumulative Effects of Multiple Stressors: Using Redundancy Analysis.....	71
Separating Current Effluent Quality from Historical Contamination Using a Laboratory-Based Monitoring Tool for Investigation of Cause.....	75
Use and Application of Benthic-Transplant-Devices BTDs <sup>®</sup> in Environmental Effects Monitoring Investigations.....	82
Should Biological Recovery Trigger an Investigation of Cause Study, in the context of Historical Impacts and Upcoming Mine Closure? .....	85
DISCUSSION TO IDENTIFY CHALLENGES, AND RESEARCH NEEDS .....	87
What Did the Workshop Accomplish? .....	87
Challenges and Research Needs Identified.....	87
Investigation of Cause Related Challenges .....	87
Program Challenges Not Specific to Investigation of Cause .....	89
Program Review Update.....	90
PATH FORWARD .....	90
APPENDICES .....	91
Appendix 1: Agenda .....	91
Appendix 2: Participant List.....	95
Appendix 3: Acronyms List .....	98

## **INTRODUCTION AND OBJECTIVE OF WORKSHOP**

### **Introduction**

During the summer of 2009 a committee, which included representatives from industry, non-governmental organizations, academia, Environment Canada and Natural Resources Canada (see Acknowledgements Section) for the organizing committee list) was developed to organize a workshop on Environmental Effects Monitoring (EEM) Investigation of Cause (IOC). The workshop was hosted jointly by the Mining Association of Canada and Environment Canada on December 8<sup>th</sup> and 9<sup>th</sup>, 2009 in Gatineau, QC. Presenters and session chairs were invited by the organizing committee and overall approximately 100 invited guests attended the workshop.

### **Objectives**

The objective of the workshop was to explore various aspects and challenges related to the IOC component of the Metal Mining EEM program. The intent of the workshop was to bring together stakeholders to discuss policy, process and scientific aspects of the Investigation of Cause stage, (including environmental studies, potential causes of effects, regional perspectives and case studies, and tools and approaches for IOC). Participating stakeholders at the workshop included the National EEM Team, Scientific Committee, industry, consultants, representatives from other government departments and agencies, environmental non-governmental organizations, and representatives from aboriginal communities. The proceedings of the workshop will be used to develop the future IOC guidance for the Metal Mining EEM program. The workshop provided a forum for discussion and development of IOC for metal mines, and allowed research needs to be identified.

## **OVERVIEW OF THE WORKSHOP**

The workshop opened with welcoming remarks from the Director of the Environmental Effects and Monitoring Program. Following an introduction of the workshop and its objectives by the Chairs, the workshop was underway. Presentations were made by Industry representatives, consultants, academic scientists, and Environment Canada officials, and were organized into the following four topics (see Appendix 1 for the Workshop Agenda):

- Session 1: Introduction, Background and Overview of Environmental Studies.
- Session 2: Potential Causes of Effects.
- Session 3: Regional Overview and Case Studies.
- Session 4: Tools and Approaches for IOC Studies.

There was an opportunity for questions and answers after each presentation, and there was a discussion period following Session 1. At the end of Session 4 there was a discussion to identify gaps and research needs and following this discussion there was a short session to wrap up and develop a path forward.

Each presenter provided a short abstract which was compiled into a book of abstracts which was distributed to the workshop participants. Where possible, the extended abstracts are included in these proceedings. When an extended abstract was not provided, the short abstract was included.

## **BACKGROUND OF THE METAL MINING EEM PROGRAM**

\*Compiled by Environment Canada using material presented by Robert Prairie, Xstrata Zinc Canada.

The development of the Environmental Effects Monitoring (EEM) program for the metal mining sector began in 1993 with the Assessment of the Aquatic Effects of Mining in Canada (AQUAMIN), which evaluated the effectiveness of the 1977 *Metal Mining Liquid Effluent Regulations* (MMLER). The *Assessment of the Aquatic Effects of Mining in Canada Final Report*, released in 1996, summarized the results of the assessment, and presented recommendations for amendments to the MMLER and the design of an EEM program for metal mining. A multi-stakeholder consultation process (led by Environment Canada) then took place between 1997 and 1999 to develop the metal mining EEM program requirements and detailed guidance. In parallel (1995-1999), the Aquatic Effects Technology Evaluation (AETE), led by Natural Resources Canada, with funding provided from the Government of Canada and the Mining Association of Canada, evaluated the environmental monitoring technologies and methodologies available to cost-effectively assess and characterise environmental impacts of mine effluents on the receiving aquatic environment. The consultation on the EEM program culminated in 1999 with a consensus agreement on proposed EEM requirements, and the completion of a guidance document. The *Metal Mining Effluent Regulations* (MMER) were promulgated in June, 2002 under the authority of the *Fisheries Act*.

Under the MMER, metal mines are required to undertake an EEM study to determine effects, if any, on fish, fish habitat and fisheries resources. EEM studies consist of biological monitoring as well as effluent characterization and water quality monitoring studies. The progression of the program follows a tiered monitoring approach, where the monitoring requirements of each phase (i.e. each round of monitoring within a defined regulatory time period) is dependent upon results of the previous monitoring phase. Once effects are confirmed in two studies (i.e. periodic monitoring), and the results of the focussed biological monitoring study indicate the magnitude and geographic extent of an effect on the fish population, on fish tissue, or on the benthic invertebrate community, mines are required to investigate the cause of the effects as per Subsection 19(2) of Schedule 5 of the MMER. Investigation of Cause monitoring studies attempt to determine the specific causes of the effects under investigation, including what components of the effluent are responsible for the effects. Once the cause of the effect is known, the mine will conduct periodic monitoring (i.e. surveillance) as per Subsection 22 (1) of Schedule 5 of the MMER. Although not required by the regulation, mines are also encouraged to study and implement possible corrective actions for the cause of effects.

There are currently (winter 2009) several mines in the IOC phase and additional mines are expected to conduct IOC studies in the near future. A Metal Mining EEM Technical Guidance Document was developed to assist industry and consultants in conducting EEM studies. Experience gained through program implementation (i.e. completing the EEM field studies and analyzing data) and multi-stakeholder consultation results in continuous improvements to the program. As well, external research initiatives conducted to respond to monitoring issues contribute to the development of new EEM methods. The outcomes of the Metal Mining IOC Workshop will be used to update guidance on Investigation of Cause in the Metal Mining EEM Technical Guidance Document. For additional information on the EEM Program or on the Technical Guidance Document refer to the following website: <http://www.ec.gc.ca/esee-eem/>.

Several industry challenges were introduced for later discussion.

## EXTENDED ABSTRACTS\*

\*Or short abstracts, if no extended abstract was submitted by request of the author.

### **Session 1: Overview of Environmental Studies**

#### **National Assessment of Effluent Effects on Fish and Invertebrates over the First Two Phases of the Metal Mining Environmental Effects Monitoring Program**

Lowell<sup>1</sup>, R.B. Walker<sup>1</sup>, S.L., Ring<sup>1</sup>, B., Buckland<sup>1</sup>, B. and Tessier<sup>1</sup>, C.

<sup>1</sup>National EEM Office, Environment Canada

The National Environmental Effects Monitoring (EEM) Program has recently completed the second three-year round of periodic metal mining biological monitoring, which tracks the effects of mining effluents across the country. Findings from the first three-year round of monitoring were summarized in the first Metal Mining EEM National Assessment (Lowell *et al.* 2007). This first assessment showed overall inhibitory effects on fish and invertebrates on a national scale, although this varied depending upon site-specific factors. For some mines and receiving environments, stimulatory effects were also measured, and at other mines, effects were minimal.

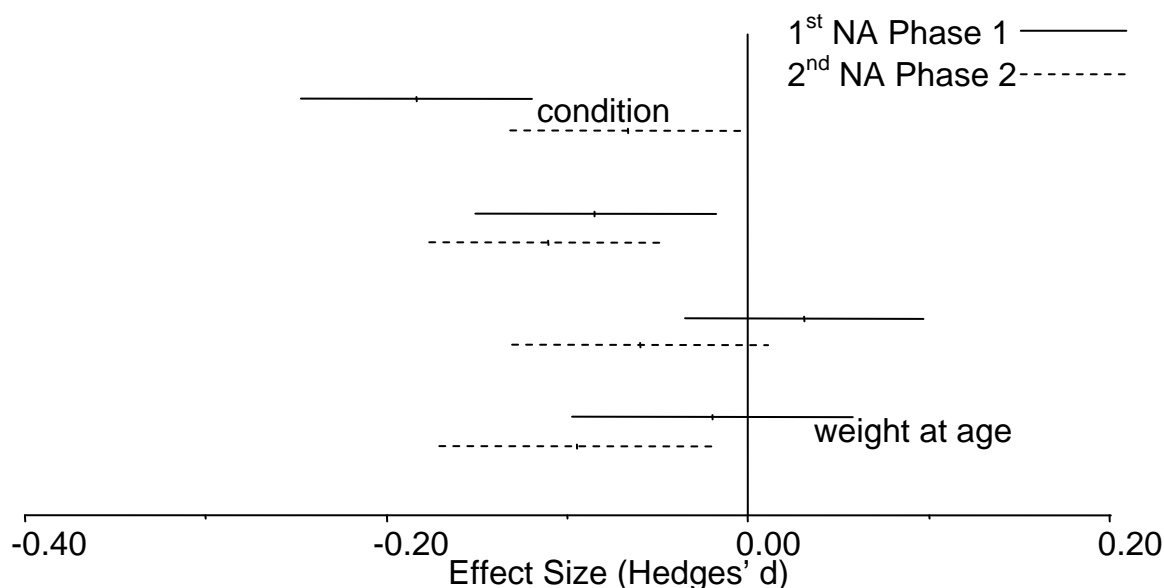
The ongoing second Metal Mining EEM National Assessment compares findings from the second round of monitoring to those from the first round of monitoring. All mines in the first round of monitoring (covered by the 1<sup>st</sup> National Assessment) were conducting their first EEM studies and, thus, were all doing their Phase 1 studies (Table 1). In the second round of monitoring (covered by the 2<sup>nd</sup> National Assessment), the majority of mines had progressed to their Phase 2 studies. A smaller number of mines, however, had just entered the EEM program during the second round of monitoring and were, therefore, doing their first set of studies (i.e., Phase 1) during the period of time covered by the 2<sup>nd</sup> National Assessment (Table 1).

		Atlantic	Quebec	Ontario	Prairie Northern	Pacific Yukon	Total
Mines conducting EEM studies							
1st Assessment (1st three-year period)	Phase 1	4	19	20	22	5	70
	Phase 2	0	0	0	0	0	0
	Total	4	19	20	22	5	70
2nd Assessment (2nd three-year period)	Phase 1	2	6	6	1	1	16
	Phase 2	4	16	20	18	4	62
	Total	6	22	26	19	5	78

Table 1. Number of mines conducting Phase 1 versus Phase 2 studies during the 1<sup>st</sup> and 2<sup>nd</sup> Metal Mining EEM National Assessments.

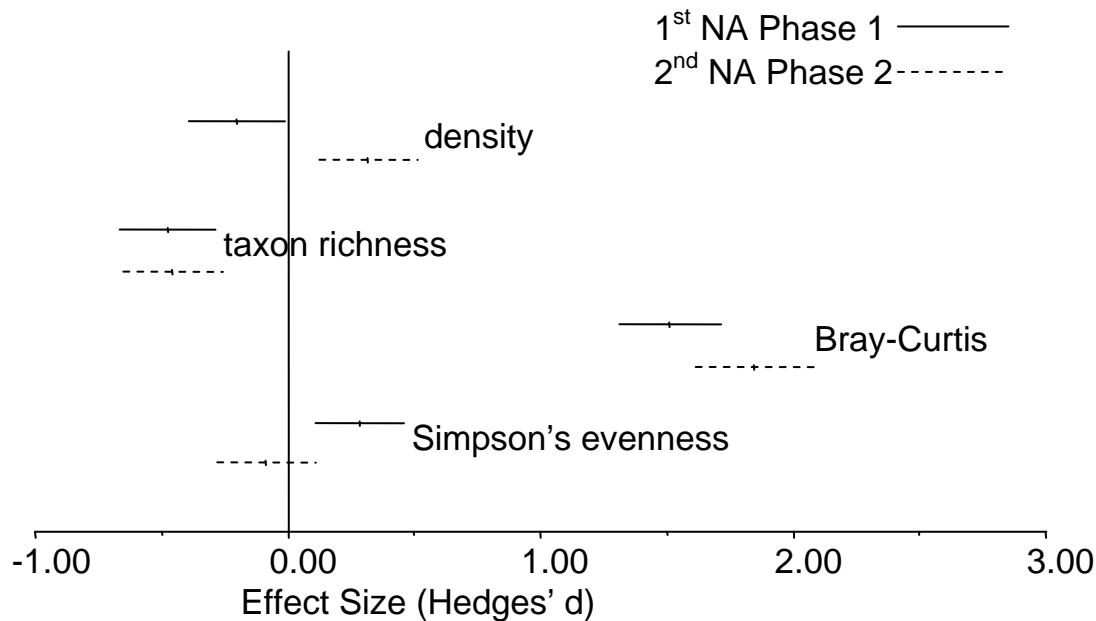
National patterns and site-specific heterogeneity in effects were investigated for mines that completed their Phase 2 studies, and these findings were compared to the responses measured for those same mines during their Phase 1 studies. Meta-analyses and effect size summaries were used to examine effects on a broad geographic scale. The key response variables were the magnitudes of measured effects on core fish (condition, relative gonad and liver size, age, size-at-age) and invertebrate (density, taxon richness, evenness, Bray-Curtis Index) endpoints.

Preliminary findings from the Phase 2 studies were consistent with those for the Phase 1 studies, with overall inhibitory effects again being observed at a national scale. As was observed in Phase 1, fish condition (relationship of body weight to length) and relative liver size in effluent exposure areas continued to be significantly decreased in Phase 2 (Fig. 1). Weight at age and relative gonad size both decreased, with the decrease in weight at age being statistically significant.



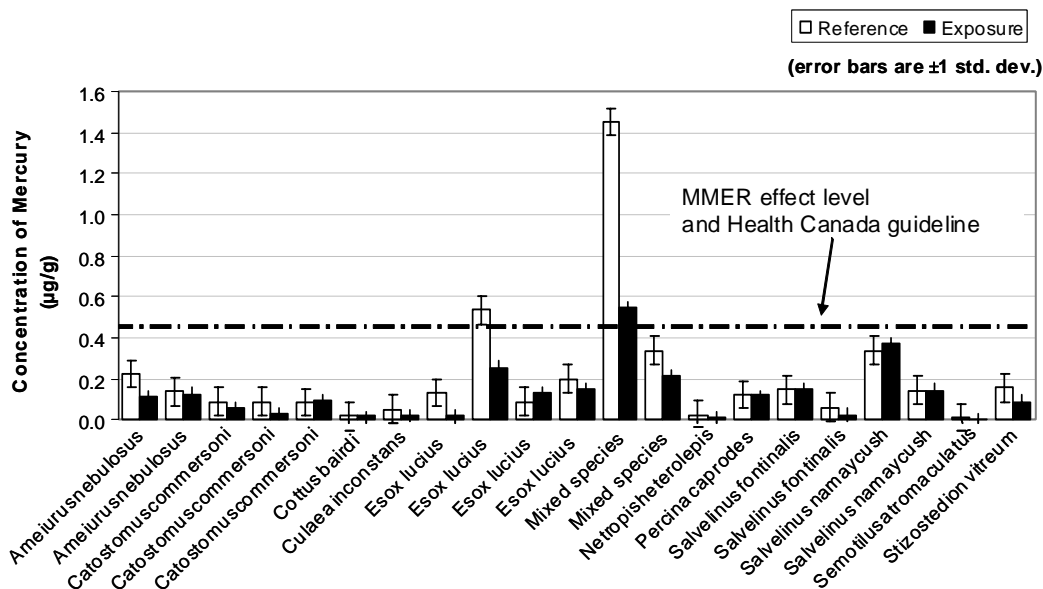
**Figure 1:** National average fish effects for metal mines. Standardized effect sizes (Hedges' d) show decreases (negative values) or increases (positive values) in effluent exposure areas. Horizontal bars show 95% confidence intervals for all mines nationally. Bars that do not overlap the vertical zero effect size line indicate that the decrease (or increase) was statistically significant. NA – National Assessment.

For benthic invertebrates, total density and taxon richness were significantly reduced in Phase 1, with this trend being continued for taxon richness in Phase 2 (Fig. 2). The Bray-Curtis and Simpson's evenness endpoints revealed significant changes in invertebrate community structure in Phase 2. For total density, however, there was a significant shift toward increased invertebrate density, underlining the potential for stimulatory effects on some endpoints. This shift may have been influenced by changes in effluent effects and/or study design. The MM EEM Program is still in its early stages, and additional rounds of monitoring will help to further interpret these effects.



**Figure 2:** National average benthic invertebrate effects for metal mines. See Figure 1 legend for further details.

With regard to the utilization of fisheries resources, no evidence was found of effluent-associated increases in fish tissue mercury levels over guideline levels. There were no instances where effluent exposed fish exceeded guideline levels, while also exceeding mercury levels in reference fish (Figure 3).



**Figure 3:** National summary of mercury levels in fish tissue for phase 2 of the Metal Mining EEM Program.

Each pair of bars represents one study for a given fish species and mine.

On a national scale Phase 2 findings were consistent with those from Phase 1, although some shifts were also seen. Overall inhibitory effects were observed for fish condition, liver size, and growth rate, as well as for invertebrate taxon richness. But some stimulatory effects were also observed, as well as minimal effects for some endpoints and mines, underscoring that effects can be heterogeneous among mines. As ongoing EEM data collection progresses, these national-scale analyses are helping to provide a more comprehensive picture of metal mining response patterns in Canada.

#### Reference

Lowell, R.B., C. Tessier, S.L. Walker, A. Willsie, M. Bowerman, and D. Gautron. 2007. National Assessment of Phase 1 Data from the Metal Mining Environmental Effects Monitoring Program. National Environmental Effects Monitoring Office, Environment Canada, Gatineau, QC, 45pp.

### **Update on National Investigation of Cause Project on Reduced Gonad Size in Fish Exposed to Pulp and Paper Effluent**

Mark Hewitt<sup>1</sup>, Pierre Martel<sup>2</sup>, Tibor Kovacs<sup>2</sup>, Deborah MacLachy<sup>3</sup>, Mark McMaster<sup>1</sup>, Joanne Parrott<sup>1</sup>, Michael van den Heuvel<sup>4</sup> and Glen Van Der Kraak<sup>4</sup>

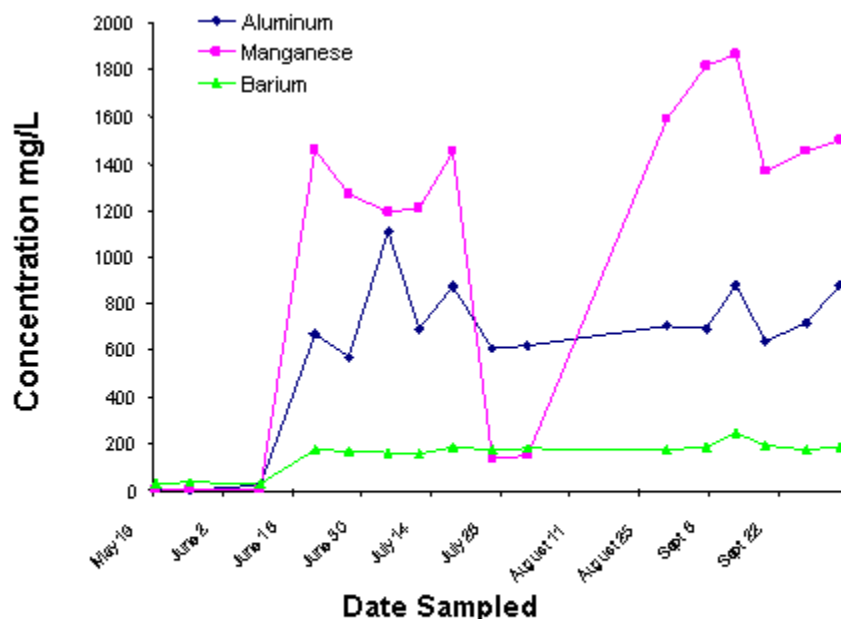
<sup>1</sup>Aquatic Ecosystem Protection Research Division, Environment Canada, Burlington, ON Canada; <sup>2</sup>FPIInnovations-Paprican Division, Pointe-Claire, QC Canada; <sup>3</sup>Canadian Rivers Institute and Department of Biology, Wilfrid Laurier University, Waterloo ON Canada; <sup>4</sup> Canadian Rivers Institute and Department of Biology, University of Prince Edward Island, PEI Canada; <sup>5</sup>Department of Integrative Biology, University of Guelph, Guelph, ON Canada

Through the Environmental Effects Monitoring (EEM) Program in Canada, two national response patterns in fish residing below mill effluent discharges have emerged: responses related to nutrient enrichment, and responses described as metabolic disruption, typified by larger liver size and smaller gonad size. A team comprised of key researchers from industry, government and academia from across Canada has been assembled to tackle this long-standing issue comprehensively. A multi-year plan to evaluate in-mill and end-of-pipe treatment options for removing substances affecting fish reproduction from pulp and paper mill effluents has been developed and work is underway. The purpose of the initial phase of the work is to select appropriate laboratory tests of fish reproduction to apply to the program. Currently, the utility of different laboratory bioassays to characterize the reproductive effects seen in wild fish as a consequence of exposure to pulp mill effluent are being evaluated. In addition to wild fish collections and lifecycle tests at mill study sites, laboratory tests in five fish species are being conducted concurrently. The results of the first mill effluent studied showed wild fish results can be replicated with laboratory lifecycle experiments and that egg production in shorter term tests show promise for investigating causes and solutions. Measured parameters of effluent quality required for regulatory purposes (BOD, TSS) remained stable throughout the study, however unexpected variations in other parameters (metals, dissolved organics, ligands for fish androgen receptors) over six months of monitoring were greater than expected. More recent results at a second study site have shown that mill operation upsets may be the major contributors to these variations and the resultant effects on fish reproduction.

#### Results

One of the most interesting results learned thus far that can be applied to the metal mining EEM

Program is the temporal variability in metals in mill effluents. We monitored one effluent weekly for six months and determined that several metals fluctuated significantly during that time.



**Figure 1:** Metals levels in La Tuque effluent during Cycle 4 IOC studies.

As can be seen in Figure 1, temporal fluctuations of metals and other effluent components, present a significant challenge in conducting IOC and IOS studies.

### Lessons Learned

- IOC is a process
  - Usually requires multi-EEM cycles to conduct
  - It is not necessary to find the actual causative chemicals, knowing their source and finding a solution are the significant drivers
  - An EEM cycle will be required as the ultimate validation of the solution(s) implemented
- In the centralized Pulp and Paper IOC project, a critical mass of knowledge, resources and experience was necessary to make real progress
- It was critical to maintain good communication with the National EEM Office and the respective regions involved in the multi-mill joint project

### **Questions/Discussion:**

**Q:** Could you give a sense of the components of structure?

**A:** Government and industry are working together, with a goal of centralizing IOC. Cycle 4 will be the most difficult.

[Editor's note: The author clarified that "centralized" means doing the research in a multi-agency

consortium that has the critical mass to make progress towards solution.]

## **Overview of Existing Guidance for Investigation of Cause in the Metal Mining Environmental Effects Monitoring Program**

Ring, B.L.<sup>1</sup>, Walker, S.<sup>1</sup>, Lowell, R.<sup>1</sup>

<sup>1</sup> National EEM Office, Environment Canada

### Background

The "Metal Mining Technical Guidance Document for Aquatic Environmental Effects Monitoring" (Environment Canada, 2002) was developed when the *Metal Mining Effluent Regulations* were promulgated. It is an essential document that guides and aids industry and their consultants by providing information on the recommended methodologies, based on generally accepted standards of good scientific practice, and options on how to carry out the Environmental Effects Monitoring (EEM) studies. The guidance document was originally prepared based on recommendations from a series of multi-stakeholder Technical Subgroups (Fish, Benthic Invertebrate Community, Toxicity, Water and Sediment, and Stakeholder Involvement). Experience gained through program implementation (i.e. completing the EEM field studies and analyzing data) and multi-stakeholder consultation results in continuous improvements to the EEM program. As well, external research initiatives conducted to respond to monitoring issues contribute to the development of new EEM methods. Therefore the Guidance Document is viewed as a document which is updated regularly as new information and research becomes available. The Guidance Document is available to industry and consultants on the National EEM Website (<http://www.ec.gc.ca/esee-eem>).

In the EEM program, when the cause of an effect has not been determined, the mine conducts an Investigation of Cause (IOC) study. Although a separate, specific chapter on IOC was not included in the 2002 version, IOC concepts, considerations and issues were incorporated throughout the original version. The Metal Mining Guidance Document is currently being updated to ensure consistency with the 2006 amendments to the MMER, to address improvements in the program, incorporate outcomes from the Metal Mining EEM Review Team, and to add new science developments. During this update, all the IOC related information throughout the 2002 version will be incorporated into one tentative chapter. This chapter will be updated based on the presentations, case studies and outcomes from this workshop.

Listed below is an overview of the existing IOC guidance provided in the 2002 version of the Guidance Document, as presented at the IOC workshop:

### General Overview of IOC provided

Throughout the 2002 version of the Guidance Document the specific IOC regulatory requirements (MMER) are provided, which includes references to specific MMER sections and timelines. This version of the Guidance Document also notes that a study may progress to Investigation of Cause prior to confirmation of the effect or determining the magnitude and extent. In the revised Guidance Document (in preparation), the requirements and timelines will be tentatively included in the Introductory chapter and readers will be referred to the MMER.

It is emphasized in several instances that the methodologies provided in the Guidance Document,

although considered the most applicable generic designs available, are not an exhaustive list of all the possible means and ways of conducting EEM. The tools chosen should be cost-effective, recognized with primary literature documentation, readily available from consulting, academic or government laboratories, and applicable to the EEM program. Also individual techniques or combinations of techniques may be used to address site-specific questions, as long as the regulatory requirements (MMER) are met.

The document titled: “Guidance for Determining Follow-up Actions when Effects Have been Identified in EEM” is referred to in several instances. This document was created by Environment Canada, in consultation with Fisheries and Oceans Canada, in 2000 and its purpose is to provide guidance to regulatory agencies, regulated facilities and EEM practitioners for determining follow-up actions when effects have been identified. This document is outdated and will not be referenced in the revised version of the Guidance Document.

#### Possible tools for an IOC Fish Survey Study

There were a number of IOC tools mentioned in the 2002 version of the Fish Population Survey Chapter, including:

- Ecoepidemiological Criteria for Attributing Cause: to determine lines of evidence for defining cause and effect;
- The use of various study designs (e.g., gradient designs, caged fish / non mobile fish or invertebrates, on site bioassays / mesocosms and the Sediment Quality Triad) to eliminate or prioritize stressors; and
- The identification of specific chemicals associated with changes using methods such as selective operation of the discharge or in-plant processes or the use of toxicity identification and evaluation procedures (TIEs).

#### Possible tools for an IOC Benthic Invertebrate Study

The following is a summary of the possible IOC tools discussed in the 2002 version of the Benthic Invertebrate Survey Chapter:

- use of weight-of-evidence approaches to establish cause of effect(s);
- methods for determining Biomarkers and Metal Body Burdens;
- lethal and sublethal toxicity tests (e.g., whole sediment survival and growth or reproduction tests with *Hyalella azteca*, *Chironomus* sp., *Tubifex tubifex*, and *Hexagenia* sp);
- analysis of sediment cores for historical trends;
- additional benthic invertebrates measures (e.g., biomass, lower level identification, secondary production, and population fitness parameters can provide additional useful information; and
- the use of other organisms (e.g., phytoplankton, macrophytes, and periphyton).

For each of the above recommendations numerous references are listed. Screening evaluations for tests on alternative organisms (phytoplankton, macrophytes and periphyton) are also provided.

#### Possible tools for an IOC related to Effluent Characterization and Water Quality Monitoring

There are a number of effluent characterization and water quality monitoring techniques available

which may help in understanding the nature and cause of effects. These include:

- effluent characterization on additional mine related contaminants from other sources, (particularly non-point sources);
- measurement of dissolved metals (when total metal measurements in the exposure area are elevated);
- measurement of reagents and reagent by-products used in processing, particularly where observed effects cannot be attributed to metals and other parameters monitored regularly as part of the EEM;
- metal speciation for metals of concern (the bioavailability and toxicity of that metal to aquatic organisms) (e.g., chemical analysis of metal speciation and chemical speciation models);
- measurement of chlorophyll A and/or phaeo-pigment, especially in cases where upstream discharges of nutrients represent confounding influences; and
- monitoring of flow and loadings in the exposure areas which may help with the data interpretation and understanding of the dilution ratio, mixing, mass balance, fate and effects of contaminants and causal relationships (mass balance calculation).

The Guidance Document provides references for each of the tools mentioned above, particularly the chemical analysis of metal speciation and chemical speciation models, and also provides an example of a mass balance calculation.

#### Possible tools for an IOC related to Sediment Monitoring

At sites where there are effects in the benthic invertebrate community, there are a number of sediment monitoring techniques which may help in understanding the nature and cause of effects. There was extensive guidance on these techniques in the 2002 version, which are summarized below:

- sediment mass transport / sediment depositional rate;
- sediment coring (provides references, listed recommendations for core sampling, and advantages and disadvantages of different sediment corers);
- Sediment chemistry (e.g., partial metal concentrations in sediment (partial extraction), sequentially extracted metals, SEM/AVS ratios, C:N ratio for marine sediment);
- whole sediment toxicity testing: Information is provided on the following tests:
  - survival and growth of *Hyalella azteca*,
  - survival and growth of the freshwater midges *Chironomus tentans* or *Chironomus riparius*, survival and reproduction of the oligochaete *Tubifex tubifex*,
  - survival and growth of the mayfly *Hexagenia limbata*,
  - using a battery of whole sediment toxicity tests: *Hyalella azteca*, *Chironomus tentans* or *Chironomus riparius*, *Tubifex tubifex* and *Hexagenia limbata*,
  - metals in Overlying Water in Whole Sediment Toxicity Tests
  - Bioaccumulation: Measurement of Metals in Test Organisms from Sediment Toxicity Testing;
- sediment quality triad; including background and a description of a sediment quality triad approach;
- sediment pore water monitoring with information on background and methodologies including the following tests: sea urchin fertilization bioassay, Microtox acute test; acute lethality with

*Daphnia magna*, reproduction and survival using the cladoceran *Ceriodaphnia dubia*, growth inhibition of the alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*); and,

- Toxicity Identification Evaluation and Toxicity Reduction Evaluation (TIE/TRE) to determine and eliminate the cause(s) of toxicity. A description of the protocols and objectives are provided and the three phases of TRE (i.e. characterization of toxicity, identification and confirmation of suspected toxicants) are discussed.

As seen from the lists above there are already many existing tools for IOC studies described in the original version of the Guidance Document. References, background information and in some cases detailed lists and screening tests are provided. All these tools and related information will be incorporated into the new IOC chapter which will be updated based on the presentations, case studies and outcomes from the IOC workshop. The updated version of the Guidance Document will be available on the National EEM Website. (<http://www.ec.gc.ca/esee-eem>)

#### Reference

Environment Canada. 2002. Metal Mining Guidance Document for Aquatic Environmental Effects Monitoring.

#### **Questions/Discussion:**

Q: There are a number of mine sites whose data could not be incorporated into the National Assessment, even starting at phase 1. Will there be changes in the technical guidance to address this?

A: The program will consider this issue further.

[Editor's note: See *The National Assessment of Phase 1 Data from the for Metal Mining Monitoring Effects Program*, R.B. Lowell, C. Tessier, S.L. Walker, A. Willsie, M. Bowerman, and D. Gautron, National EEM Office, Environment Canada, 2007 for further details regarding why certain data could not be used.]

#### **Review of Regulatory Requirements and Policy for Metal Mines in the Investigation of Cause Phase of the Environmental Effects Monitoring Program**

S.L. Walker  
National EEM Office  
Forestry, Agriculture & Aquaculture Division

Under the *Metal Mining Effluent Regulations* (MMER), metal mines subject to the regulations are required to conduct an environmental effects monitoring (EEM) program as specified in Section 7 of the MMER as follows:

- ss. 7(1) "... mine shall conduct environmental effects monitoring studies of potential effects on the fish population, on fish tissue and on the benthic invertebrate community in accordance with the requirements and within the periods set out in Schedule 5"
- ss. 7(2) Results...submitted to authorization officer
- ss. 7(3) Studies shall be performed ....in accordance with generally accepted standards of good scientific practice...

The requirements for EEM are detailed in Schedule 5 of the MMER, which is divided into two parts. Part 1 specifies the requirements for effluent characterization, sublethal toxicity testing and water quality monitoring. Mines are required to submit a report as well as their electronic data annually by March 31. For effluent characterization, hardness, alkalinity, aluminum, cadmium, iron, mercury, molybdenum, ammonia, and nitrate are measured four times per calendar year while the mine is depositing effluent. Sublethal toxicity tests are required on four test species including a fish, invertebrate, plant and algal species twice per calendar year for first phase of EEM. Following the first phase, the MMER permits mines to reduce the frequency of sublethal toxicity tests to once per year. Similar to effluent characterization, water quality monitoring is conducted four times per calendar year as well as at the time of biological sampling. Water quality monitoring is conducted on the parameters specified for effluent characterization, the Schedule 4 substances, as well as temperature, dissolved oxygen (DO). In addition, pH, hardness, alkalinity are measured for mines depositing to freshwater environments, and in addition, salinity is measured for estuarine environments, whereas only salinity is measured for mines depositing to marine environments.

The biological monitoring requirements of EEM are specified in Part 2 of Schedule 5. The requirements include a fish population survey, a survey of mercury in fish tissue and a benthic invertebrate community survey. An interpretive report is submitted at the end of each monitoring phase (two, three or six year) depending on the results of the previous studies.

Mines are required to conduct a fish population study “if the concentration of effluent in the exposure area is greater than 1% in the area located within 250 m of a final discharge point” (Sch 5, paragraph 9(b)). For this survey, data are collected on indicators of growth, reproduction, condition and survival and the following endpoints are reported: age, size at age, relative liver size, relative gonad size, and condition.

A study of mercury in fish tissue is required, “if during effluent characterization ...a concentration of total mercury in the effluent is identified that is equal to or greater than 0.1 µg/L” (Sch 5, paragraph 9(c)).

Under Sch. 5, paragraph 9(d) of the regulation, the mine is also required to undertake a benthic invertebrate community study and under subparagraph 16(a)(iii) collect data to calculate the total benthic invertebrate density, the evenness index, the taxa richness and the similarity index. These data are used to assess effects on structure and function of the benthic invertebrate community.

Mines undertake initial EEM studies to assess effects on fish populations, the use of fisheries resources, and effects on benthic invertebrate communities as an indicator of effects on fish habitat. Once effects are found, a subsequent EEM study is undertaken to confirm effects. Specifically, mines are required to confirm effects or the absence of effects in two consecutive phases as indicated in Sch. 5, paragraph 22(2)(c) which states “if the results of the two previous biological monitoring studies indicate **an effect** on the fish population, on fish tissue or on the benthic invertebrate community...”, the mine is then required to submit a study on the extent and magnitude in 24 months. However, “if the results of the previous two consecutive biological studies indicate **no effect** on fish populations, on fish tissue and on the benthic invertebrate community” (Sch. 5, Paragraph. 22(2)(b)), then the next interpretive report is submitted in 72 months.

The Science Committee has provided the following advice regarding fish data for metal mines:

An effect should be considered confirmed in 2 consecutive phases if:

- A similar type of effect is found in two consecutive phases in the **same or different species / gender** is considered a confirmed effect
- A significant interaction is considered a significant effect – methods to determine the magnitude of the effect are under development

[Editor's note: the above guidance concerning confirmed effect will be updated in the revised *Metal Mining Technical Guidance Document*.]

When an effect has been confirmed in two phases, the next study is submitted in two years with “a description of one or more additional sampling areas within the exposure area that shall be used to assess the magnitude and extent of the effect” (Sch. 5, Paragraph. 19(1)(d)). Paragraph 19(1)(d) of Schedule 5 of the Regulations state that “if the results of the two previous monitoring studies indicate a similar type of effect on the fish population, on fish tissue or on the benthic invertebrate community, [the biological study shall include] a description of one or more additional sampling areas within the exposure area...to assess the magnitude and extent of the effect.”

An “effect” is defined in Schedule 5 of the MMER as follows:

- For indices for the benthic invertebrate community survey and the fish population survey, an “effect... **means a statistical difference** between ...a) an exposure area and a reference area or b) sampling areas within an exposure area where there are gradually decreasing effluent concentrations.”
- “effect on fish tissue means 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 taken in a reference area.”

Interim critical effect sizes have been adopted by Environment Canada as a tool to help determine the level of effort that should be put into future studies based on the results of past studies (Table 1).

Endpoints	Critical effect size
<b>Fish population survey</b>	
Gonad weight relative to body weight	± 25%
Liver weight relative to body weight	± 25%
Condition	± 10%
Age	± 25%
Body weight relative to age	± 25%
<b>Benthic invertebrate survey</b>	
Total density	± 2 SD
Taxon richness	± 2 SD
Simpson's evenness	± 2 SD
Bray-Curtis index	+ 2 SD

Table 1: Interim  
Critical Effect Sizes

If an effect has been confirmed but is below critical effect size (CES), the mine would not be expected to find a larger effect further away or at a higher level. Therefore, we can then consider that “the results of previous biological monitoring study indicate the magnitude and extent of an effect” and that the mine may go directly to Investigation of Cause (IOC) (Sch. 5, ss. 19(2))(required in two years).

If, after having performed an extent and magnitude study, the cause of the effect is still not known, the mine will conduct a study (Sch.5, ss.19(2)) to determine the cause of the effect (Investigation of Cause). The study design is required to be submitted at least six months before the Investigation of Cause study is conducted, and shall include a summary of previous biological monitoring studies “and a detailed description of what field and laboratory studies will be used to determine the cause of the effect” (Sch. 5, ss. 19(2)). Two years are provided in the MMER (from the date that the interpretive report from the previous study was required to be submitted) to initiate the Investigation of Cause study and submit an interpretive report. If the mine is in the Investigation of Cause phase of EEM, “the interpretative report shall contain only the cause of the effect...and **if the cause was not determined, an explanation of why and a description of any steps that must be taken in the next study to determine that cause**” Sch. 5, ss. 21(2).

After having performed an Investigation of Cause study, if the cause was not determined, a report will be submitted with “an explanation of why and a description of any steps that must be taken in the next study to determine the cause” (Sch. 5, ss. 21(2)). The subsequent study will then be a continuation of the Investigation of Cause. Otherwise, if the cause is known, the next biological monitoring study will be in three years (Sch. 5, ss. 22(1)).

Regarding Environment Canada guidance concerning EEM studies for extent and magnitude and Investigation of Cause studies, if a study finds fish results with large effects (i.e. greater than critical effect size), the complete biological study for Extent and Magnitude (in two years) would be composed of additional sampling on fish only (sampling at sites further downstream and/or another fish species). After having performed Extent and Magnitude, the Investigation of Cause study (Sch. 5, Paragraph. 22(2)(c)), should include a summary of previous studies “and a detailed description of what field and laboratory studies will be used to determine the cause of the effect” (Sch. 5, ss.19(2)) to investigate the effect on fish. An Interpretive Report is submitted in two years which will either identify the cause of the effect or indicate why the cause was not identified and describe the next steps that will be undertaken to determine the cause.

### **Questions/Discussion:**

Q: For effects, the terms statistically different and interim CES are both used. Is this why a lot of mines are in IOC?

A: No. The *Metal Mining Effluent Regulations* dictate the regulatory decision making framework for IOC.

Q: Can you combine the results from both the benthic and fish surveys to show that an effect is present at a mine site (e.g. if an effect is observed in both surveys)?

A: This situation should be discussed with the regional coordinator.

Q: How do water quality and biological quality align?

A: Water quality monitoring is used as supporting information to the Biological Monitoring, and aims to identify concerns at their respective sites.

Q: If a significant difference noted in Phase 1 goes one way, then the opposite way in Phase 2, is this considered an effect according to the regulations?

A: This is not a similar effect. However, this situation should be discussed with the regional coordinator to determine if this is of a concern to the mine. Perhaps it is a significant interaction?

Q: Why are mines doing sublethal toxicity testing in addition to EEM?

A: It was pointed out that sublethal toxicity testing can inform IOC.

## **Overview of Proposed Amendments to Environmental Effects Monitoring Provisions of the Metal Mining Effluent Regulations**

Dumaresq<sup>1</sup>, C.G.

<sup>1</sup>Head, Regulatory Development, Mining and Processing Division Environment Canada

Environment Canada is proposing five amendments to the EEM provisions of the MMER and three amendments to address minor inconsistencies in language between the English and French versions of the Regulations. The objective of the presentation is to inform stakeholders of the proposed amendments and provide an opportunity for comment.

The proposed amendments to the EEM provisions would: revise the definition of an effect on fish tissue; add selenium and electrical conductivity to the list of parameters that must be periodically monitored in effluent and receiving waters; exempt monitoring of radium 226 in receiving waters if conditions of subsection 13(2) of the MMER are met; remove a requirement to compare sublethal toxicity results to biological monitoring results; and, make the wording of paragraph 22(2)(c) of Schedule 5 consistent with paragraph 19(1)(d). The proposed amendments would also address recommendations of the Standing Joint Committee for the Scrutiny of Regulations regarding minor inconsistencies in wording between the English and French versions of the Regulations.

There has been consultation on the first four proposed EEM amendments, since these reflect recommendations of the multi-stakeholder Metal Mining EEM Review Team. There has been no consultation on fifth proposed amendment, but for the previous amendment to paragraph 19(1)(d) there was significant consultation. There has been no consultation on the proposed amendments in response to recommendations of the Standing Joint Committee for the Scrutiny of Regulations since these are administrative in nature. Comments on the proposed amendments may be submitted to the author by January 8, 2010.

## **Session 1 Discussion Period**

Q: Will Industry be informed of case materials or other information to change the timing from 24 months to 36 months for IOC studies?

A: Environment Canada will attempt to do so.

Q: How was the critical effect size (CES) of 25% chosen for fish endpoints?

A: There is a long history of this value used in scientific studies, and this value was recommended in a recently published review: Munkittrick, K.R., C.J. Arens, R.B. Lowell and G.P. Kaminski. 2009. A review of potential methods for determining critical effect size for designing environmental monitoring programs. *Environ. Toxicol. Chem.* 28:1361-1371. This value is also based on the magnitude of effect in different scenarios. There has always been a grey area (~20-30%), but it is the most agreed-upon value. This will be into the new guidance, and will be incorporated into the guidance update.

[Editor's Note: This endpoint was published in the *Pulp and Paper Technical Guidance for Environmental Effects Monitoring* (2010). For Metal Mining, this endpoint was recommended, and will be used in the interim until validated.]

Q: Are the effect sizes going to be finalized? Some mines have study designs that detect smaller effect sizes, and they feel that they are being penalized for it.

A: The EEM program agreed that this would need to be further addressed.

Q: Currently, the cut-off for statistical significance is at  $p = 0.1$ . The probability of finding an effect increases with more parameters.

A: This is the reason that current guidance has shortened the list of endpoints to 9 parameters. The goal is to reduce the variation from all the different parameters, in order to eliminate or reduce the number of false positives (i.e. when effects are detected through statistical analysis, but when they do not actually exist). The occurrence of false positives is further reduced by the requirement to find the same effect for two phases in a row.

Q: What does the consultant do when a consistent effort has been expended but there just aren't enough fish in the area to be caught? There are then not enough fish to run the statistical analyses. Can the lack of fish be considered an effect?

A: Yes, the lack of fish can be considered an effect, but the uncertainty associated with that must be taken into account. (There could be other factors influencing the availability of fish in the area, ones that may or may not be mine-related). One possible course of action is to move downstream to see if fish can be caught there, and determine if the effect noted is a mine-related effect. Moving progressively downstream also gives data (i.e. distance from outfall) that could be useful in determining the effect the mine has on the fish.

Comment: Sometimes fish are hard to find; this may or may not be mine-related (i.e. related to the effluent discharge). It could also be due to new confounding factors (e.g. new beaver dams built in the area). The conclusion that the effect noted is mine-related may not be correct.

[Editor's note: *The Metal Mining Technical Guidance Document for Aquatic Environmental Effects Monitoring* addresses confounding factors and the use of the weight of evidence approach.]

Q: For mines undergoing a decommission phase, is there room for change in the IOC protocol/process?

A: This will be addressed in a later presentation.

[Editor's note: See Session 4: LePage; Should Biological Recovery Trigger and IOC Study in the context of historical impacts and upcoming mine closure?]

Q: There does not seem to be consistency between the approaches for each region in how they handle similar situations.

A: This will be discussed further at a later time (in further discussion either during the workshop, or afterwards). The lines of communication, however, are open.

[Editor's note: Environment Canada personnel across the country work together to ensure national consistency (National EEM Team).]

## Session 2: Potential Causes of Effects

### Ecotoxicology of trace metals in the aquatic environment

Campbell, Peter G.C.

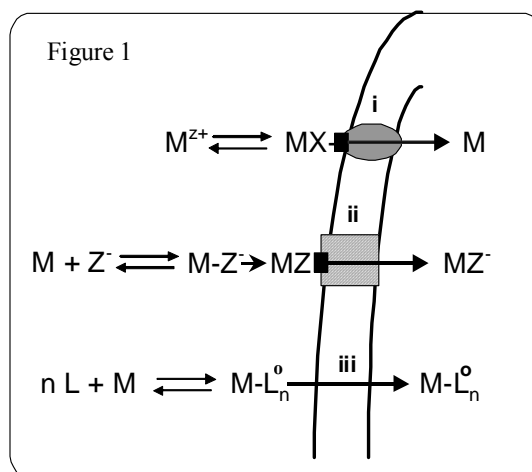
INRS, Centre Eau, Terre et Environnement, 490 de la Couronne, Québec, QC, Canada G1K 9A9

e-mail: [campbell@ete.inrs.ca](mailto:campbell@ete.inrs.ca)

To elicit a biological response from a target organism and/or to accumulate within this organism, a metal must first interact with a cell membrane. Metals generally exist in the aquatic environment in polar, hydrophilic forms, which are strongly hydrated and are unable to traverse cell membranes by simple diffusion. With few exceptions (e.g., some organometallics and neutral metal complexes – see Fig. 1, mechanism (iii); some metal complexes involving an assimilable ligand, such as thiosulphate – Fig. 1, mechanism (ii)), metal transport across respiratory or digestive membranes normally occurs by facilitated transport, involving either membrane carriers or channels (Fig. 1, mechanism (i); Campbell 1995). Such transport is normally a function not of the total dissolved metal but rather of the free metal ion concentration (Fig. 1, mechanism (i)). Once metals have entered the intracellular environment, a variety of mechanisms exists for their detoxification (Vijver *et al.* 2004; Luoma and Rainbow 2008). It follows that simple predictions of metal-induced toxicity on the basis of metal quotas or burdens, as is often done with organic contaminants, are rarely applicable. Metals bound to inducible metal-binding proteins such as metallothionein, or precipitated into insoluble concretions consisting of metal-rich granules, can be considered to be biologically detoxified metal, as compared to metals in metal-sensitive fractions such as organelles and heat-sensitive proteins. A corollary to this model of metal accumulation is that metal tolerance or resistance will be related to the ability of an organism to prevent "inappropriate" metals from binding to sensitive sites. The binding of an "inappropriate" metal to a metal-sensitive site, often termed "spillover", could be the precursor to the onset of metal-induced stress (Campbell and Hare 2009).

Within this general construct of metal-organism interactions, how might we develop guidance for determining whether metals are responsible for effects that have been confirmed in environments receiving mining effluent? For non-essential metals, a hierarchical approach might include the following steps:

- *Identify* appropriate biomonitor organisms that live both in the receiving environment and in the control or reference environment. Criteria for selecting biomonitor organisms are well established (Phillips and Rainbow 1993). If appropriate indigenous organisms are unavailable, caged organisms may be considered.
- For each biomonitor species, *choose* the appropriate target organ, e.g. gill or liver (for some small species, it may not be feasible to analyze metals at the organ level, in which case whole body concentrations could be used).



- *Compare* the levels of bioaccumulated metals in specimens collected in the receiving and reference environments. The observation of markedly increased metal levels in the exposed specimens is *prima facie* evidence that one or more of these metals is responsible for the effects observed in the receiving environment. Note that by relying on metal bioaccumulation (rather than on the determination of metal concentrations and metal speciation in the receiving environment), one can circumvent the need to estimate the bioavailability of the metals found in the water body receiving the mining effluent.
- To refine the interpretation of the bioaccumulated metal levels, *determine* the subcellular partitioning of the metals in the target organisms (Campbell and Hare 2009). However, this distinction between biologically detoxified metals and metals that have “spilled over” onto metal-sensitive sites would only be justified for biomonitor species that demonstrate a clear threshold response to increasing metal exposure – see discussion in Campbell *et al.* (2008) and Campbell and Hare (2009).

In the receiving environment, metals often occur as metal mixtures. To tease apart the relative importance of individual metals, the comparison of gene transcription in exposed and reference organisms shows some promise as a discriminatory biomonitoring tool to detect and differentiate among metal contaminants (Pierron *et al.* 2009; Walker *et al.* 2008).

### Bibliography

Campbell, P.G.C. 1995. Interactions between trace metals and organisms: a critique of the free-ion activity model. *In* Metal Speciation and Bioavailability in Aquatic Systems. *Edited by* Tessier, A. and Turner, D. J. Wiley & Sons, Chichester, UK pp. 45-102.

Campbell, P.G.C. and Hare, L. 2009. Metal detoxification in freshwater animals. Roles of metallothioneins. *In* Metallothioneins and Related Chelators. *Edited by* Sigel, A., Sigel, H., and Sigel, R.K.O. Royal Society of Chemistry, Cambridge, UK pp. 239-277.

Campbell, P.G.C., Kraemer, L.D., Giguère, A., Hare, L., and Hontela, A. 2008. Subcellular distribution of cadmium and nickel in chronically exposed wild fish: inferences regarding metal detoxification strategies and implications for setting water quality guidelines for dissolved metals. *Human Ecol. Risk Assess.* **14**: 290-316.

Phillips, D.J.H. and Rainbow, P.S. 1993. Biomonitoring of trace aquatic contaminants. Elsevier Applied Science Publishers, London, UK.

Pierron, F., Bourret, V., St-Cyr, J., Campbell, P.G.C., Bernatchez, L., and Couture, P. 2009. Transcriptional responses to environmental metal exposure in wild yellow perch (*Perca flavescens*) collected in lakes with differing environmental metal concentrations (Cd, Cu, Ni). *Ecotoxicol.* **18**: 620-631.

Luoma, S.N. and Rainbow, P.S. 2008. Metal contamination in aquatic environments: science and lateral management. Cambridge University Press, Cambridge, UK.

Vijver, M.G., Van Gestel, C.A.M., Lanno, R.P., Van Straalen, N.M., and Peijnenburg, W.J.G.M. 2004. Internal metal sequestration and its ecotoxicological relevance: a review. *Environ. Sci. Technol.* **38**: 4705-4712.

Walker, P.A., Kille, P., Hurley, A., Bury, N.R., and Hogstrand, C. 2008. An in vitro method to assess toxicity of waterborne metals to fish. *Toxicol. Appl. Pharmacol.* **230**: 67-77.

## Questions/Discussion:

Q: How is the spill-over estimated?

A: In this case, differential centrifugation was utilized, but other methods are also possible.

Q: Can we compare the results of the crab and fish acclimatization studies?

A: The current fish study utilizes chronic exposure, so it cannot be compared to the previous crab studies.

Q: Why are the toxicity values different from tissue reference values?

A: The data may reflect the fraction of the metal that is not bioavailable. The more relevant information is what metals are being taken up by the organism, and not necessarily what is floating in the water around the organism (that is not taken up by the organism).

## Investigating Selenium Toxicity Using an Environmental Effects Monitoring Approach

Palace, V.P.<sup>1</sup>

<sup>1</sup> Fisheries and Oceans Canada, Freshwater Institute, Winnipeg MB, R3T 2N6

### Introduction

In Canada, several types of mining activities mobilize selenium (Se) by disturbing soils. Selenium is typically found in sulfide deposits of Cu, Pb, Hg, Ag, U and Zn and loadings to nearby watersheds can increase during the mining and smelting of these ores (USEPA 2004, Wang *et al.* 1993). Selenium is usually present in uncontaminated waters ranging from 0.1 to 1 µg/L, but downstream from mining activity water concentrations have been detected in the 10s to 100s of micrograms per Litre, with elevated levels in resident fish tissues as well (Palace *et al.* 2003, 2007).

Selenium is an essential dietary element, being incorporated into more than 30 seleno-protein families (Stewart *et al.* 2010). At slightly elevated concentrations Se can accumulate in tissues of oviparous vertebrates, including fish, and induce reproductive toxicity arising from deposition of the element into eggs. During development, and just after yolk sac absorption has been completed, eggs/embryos with elevated concentrations of Se exhibit developmental abnormalities (terata) including spinal curvatures, edema, and cranio-facial deformities (Holm *et al.* 2005). Adult fish are normally not affected by exposure to even highly elevated waterborne and dietary Se concentrations. The most remarkable aspect of Se toxicity is its narrow range between levels required in the diet (0.1 to 0.5 mg/kg for fish) and concentrations at which terata begin to appear in offspring (> 1- 2 mg/kg) (Lemly 1993).

Examining potential toxicity of Se using traditional Environmental Effects Monitoring (EEM) methods is difficult because of selenium's complex geochemistry and because fish species that are most sensitive, and most often used for monitoring purposes, are highly mobile. As a result, there are concerns that typical EEM monitoring may not adequately detect the reproductive toxicity arising from Se exposure in resident fish populations. A brief review of some of those concerns, as well as recommendations for examining potential reproductive toxicity arising from Se exposure is discussed.

## Concerns Regarding the Use of EEM to Detect Se Toxicity

### *a) Aqueous Se is not a reliable indicator of toxicity*

In many cases, potential toxicity arising from exposure to an inorganic contaminant can be effectively screened based on effluent or receiving water concentrations. In May 2006 the EEM science committee initiated screening for Se releases from metal mines by recommending an analysis of the element as part of effluent characterization. However, because adverse effects are dominated by dietary exposure, modelling based on aqueous exposures is of limited value. In fact, the biogeochemistry of selenium and its incorporation into organisms in aquatic systems is extremely complex. In order to describe the relationships between waterborne Se and resultant concentrations in fish, it is important to empirically derive site specific algal enrichment factors as well as trophic transfer relationships for each level in a given food-web (Stewart *et al.* 2010). As a result, tissue based criterion are generally accepted as the most reliable indicator of potential Se toxicity in fish (Lemly 1993, Hamilton. 2002, USEPA 2004). Whole body guidelines range between 4 and 7.9 mg/kg on a dry weight basis (reviewed by Chapman. 2007).

### *b) Species differences in Se partitioning and toxicity*

Muscle or whole body Se-tissue criterion are preferable to aqueous exposure metrics for assessing the potential for Se-toxicity, but species differences in Se partitioning and toxicity also must be recognized. For example, mobilization of Se from muscle to egg during vitellogenesis is variable between fish species. Even among species that inhabit similar habitats (eg. rainbow trout and brook trout), the slope of the relationship between concentrations of Se in muscle and the amount of Se deposited into eggs can be quite different (NAMC 2008). Muscle can often be used as a surrogate for egg/ovary concentrations because strong correlations are often reported between these tissues. However, relationships vary depending on when samples were obtained during the reproductive cycle, and for many species, this basic information is not currently available (North America Metals Council (NAMC) 2008). Because of these concerns, the most reliable indicator of potential Se-toxicity is a measurement in eggs or ovary (Holm *et al.* 2005). However, here too, thresholds for Se-induced terata vary widely between fish species, requiring species-specific toxicity curves in order to achieve accurate risk assessments (NAMC 2008).

### *c) Fish mobility*

An analysis of potential reproductive toxicity arising from Se exposure within a given aquatic system can be confounded in highly mobile fish species. In fact, uncertainty regarding residency in the study area and the extent of exposure to a given effluent are generally major concerns for EEM. Key biological endpoints (body size, population size and structure, liver and gonad size) are used as indicators of overall organism health in wild fish captured from areas receiving effluent and in reference areas. Interpreting the data gathered from this national assessment program is limited by uncertainty of exposure to the effluents, especially in areas where fish are able to move between Se-exposed and reference areas. For example, we simultaneously observed elevated larval deformities in a system with a functional adult rainbow trout population (Holm *et al.* 2005). Later we provided evidence that reproductively active adults were immigrating to the system from other areas not exposed to selenium (Palace *et al.* 2007). A shift from a predominantly rainbow trout fish community structure to the less sensitive brook trout was only evident after several decades of population monitoring (Rassmussen *et al.* 2008). The EEM structure of biological monitoring is designed to detect such disturbances by assessing proportions of young of the year fish, species

abundance and recruitment (Environment Canada 2010). However, the time involved in such an assessment would be significant assuming typical two to three year EEM cycles. For fish with short windows of reproductive maturity (rainbow trout in cold water streams are reproductively active for two to five years (Nelson and Paetz 1992), in low productivity systems, and small population numbers, this time lag could allow a population to fall below the sustainable carrying capacity before corrective action was indicated.

### Conclusion and Recommendation

Evaluation of the potential for Se to induce toxicity in wild fishes requires a tissue based and species specific analysis. Egg or ovary are the preferred tissues for assessing risk and in the absence of an established species specific threshold, an analysis of Se concentration in one of these tissues should be combined with an analysis of deformities in embryos just after yolk sac absorption.

### References

Chapman, P. M. 2007. Selenium thresholds for fish from cold freshwaters. *Human Ecol. Risk Assess.* 13:20-24.

Environment Canada. 2010. About Environmental Effects Monitoring. <http://www.ec.gc.ca/eseec-eem/Default.asp?lang=En&n=4CDB9968-1>

Hamilton SJ. 2002. Rationale for a tissue based selenium criterion for aquatic life. *Aquat. Toxicity* 57:85–100

Holm J, VP Palace, P. Siwik, G Sterling, RE Evans, CL Baron, J Werner, K Wautier. 2005. Developmental effects of bioaccumulated selenium in eggs and larvae of two salmonid species. *Environ. Toxicol. Chem.* 24:2373-2381.

Lemly, A.D. 1993. Metabolic stress during winter increases the toxicity of selenium to fish. *Aquat. Toxicol.* 27:133-158.

Nelson, J.S. and M.J. Paetz. 1992. *Fishes of Alberta*. University of Alberta Press, Edmonton, AB. 437pp.

North America Metals Council. 2008. Selenium Tissue Thresholds: Tissue Selection Criteria, Threshold Development Endpoints, and Potential to Predict Population or Community Effects in the Field. 178pp. <http://www.namc.org/docs/00043675.PDF>

Palace, VP, CL Baron, RE Evans, K Wautier and JF Klaverkamp. 2003. Metals, Metalloids and Metallothionein in Tissues of Fish from A Canadian Freshwater System Receiving Gold Mining Effluents and in Fish from Nearby Reference Areas. *J. Physique IV* 107:1005-1008.

Palace VP, NM Halden, P yang, RE Evans, G sterling. 2007. Determining residence patterns of rainbow trout using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analysis of selenium in otoliths. *Environ. Sci. Tech.* 41:3679-3683.

Rasmussen, J. B., Peterson, S., Lele, S., Rosenfeld, J. [Eds]. 2008. *Experts workshop on selenium*

fish science in the Athabasca River, Alberta. Workshop summary report. 55pp.  
<http://environment.gov.ab.ca/info/library/8257.pdf>

Stewart, R., Grossell, M., Buchwalter, D., Fisher, N., Luoma, S., Mathews, T., Orr, P., Wang, W.-W. 2010. Bioaccumulation and trophic transfer of selenium. In. *Ecological Assessment of Selenium in the Aquatic Environment*. Chapman, P.M., Adams, W.J., Brooks, M.L., Delos, C.G., Luoma, S.N., Maher, W.A., Ohlendorf, H.M., Presser, T.S. and Shaw, D.P. [Eds]. SETAC Press, Pensacola, FL. pp. 93-140.

USEPA (U.S. Environmental Protection Agency). 2004. Draft Aquatic Life Water Quality Criteria for Selenium—2004. EPA-822-D-04-001. Office of Water, Washington, DC, USA

Wang D, Alfthan G, Aro A. 1993. Anthropogenic emissions of Se in Finland. *Appl Geochem Suppl* Issue 2: 87-93.

### **Questions/Discussion:**

Q: Were there differences noted in growth from the EEM in the field?

A: Yes, differences were noted, but the data will need to be assessed with the regions to determine an overall effect.

Q: Are the effects observed in eggs due to selenium concentration, or to species differences?

A: The range of variation between species is very narrow, so in general, the majority of the variation in the eggs can be attributed to the selenium concentration. In addition, the species sensitivity is irrelevant, since the concentration of selenium found in the field is so high anyway.

Q: How do spinal deviations occur due to oxidative stress?

A: This is unknown at the moment.

### **Cyanide speciation and fate in gold mine tailings: A case study**

Zagury G.J.

Department of Civil, Geological and Mining Engineering, École Polytechnique de Montréal, P.O. Box 6079, Station Centre-ville, Montreal, Quebec, Canada H3C 3A7

#### Introduction

The gold mining industries are among the largest consumers of cyanide due to its high affinity with gold. After the precious metal has been extracted from ore, cyanides are discarded as effluents or as contaminated solid tailings. Cyanides are highly toxic and their toxicity is related to their speciation. The free cyanide form (HCN, CN<sup>-</sup>) is classified as the most toxic because of its high metabolic inhibition potential, whereas the metal-cyanide complexes (e.g. Fe(CN)<sub>6</sub><sup>3-</sup>, Fe(CN)<sub>6</sub><sup>4-</sup>) are considered less toxic (Shifrin *et al.*, 1996).

The aim of the case study was to investigate the availability and fate of cyanide in aged (six to nine years) and fresh (three months) mine tailings collected at depths of 0.2 and 1.0 m from two gold mining sites in northern Quebec (Canada).

## Materials and Methods

Firstly, a detailed physicochemical characterization of the tailings was performed (Table 1), and leaching studies were conducted to evaluate the soluble cyanide fraction and its potential reactivity. The biodegradation of cyanide was also investigated. Solid samples were characterized for total cyanide ( $CN_T$ ) and weak acid dissociable cyanide ( $CN_{WAD}$ ) according to Standard Methods whereas the liquid portion of the samples was analyzed for  $CN_T$ ,  $CN_{WAD}$ , cyanate ( $CNO^-$ ), and thiocyanate ( $SCN^-$ ) (Table 2). To assess the bacterial populations, total heterotroph and cyanide-resistant bacteria counts were performed on the aged (AO(0.2), AO(1), BO(0.2), BO(1)) and fresh tailings (BF(0.2), BF(1)). Total heterotroph and cyanide-resistant counts (six replicates) were performed by a spread-plate counting method (Zagury *et al.*, 2004).

## Results

The physicochemical characterization showed that  $CN_T$  concentration was higher and the pH more alkaline in fresh tailings comparatively to aged tailings. Furthermore, elevated concentrations of  $CN_T$ ,  $CN_{WAD}$ ,  $CNO^-$ , and  $SCN^-$  were measured in the liquid phase of the more reactive fresh tailings whereas these species were not detectable in aged tailings. Accordingly, when leaching tests were performed on the fresh samples, solubilization yields of 70% and 23% of the total cyanide content were obtained for  $CN_T$  and  $CN_{WAD}$  respectively (Zagury *et al.*, 2004). On the other hand, when leaching tests were performed on aged tailings,  $CN_T$  and  $CN_{WAD}$  were not detectable in the aqueous phase suggesting that  $CN_{SAD}$  (strong metal-cyanide complexes) is the dominant form in aged tailings (Table 3). The enumeration of total heterotrophic and cyanide resistant bacteria in aged tailings showed an average population of  $10^5$  cfu/g but no growth was detected in fresh tailings. Nevertheless, cyanide mineralization tests indicated the presence, in both aged and fresh tailings, of a free cyanide degrading microflora (Oudjehani *et al.*, 2002).

## Conclusion

The results of this study revealed a difference in physicochemical properties (pH, CEC and buffer capacity), cyanide concentration and speciation, viable bacterial populations and cyanide leaching behaviour between aged and fresh solid tailings collected from two gold mining sites. The findings indicate that the more reactive cyanide species initially associated with the solid tailings have degraded within the mine tailings impoundment area. The  $CN_T$  decrease over time (average of  $19.5 \pm 2.0$  mg/kg in fresh tailings compared to  $3.2 \pm 0.7$  mg/kg in aged tailings) is believed to result primarily from volatilization (decrease in pH), leaching, and bacterial degradation. The results of this study also highlighted the role of microbial activity in the fate of cyanides in mine tailings. The higher proportion of stable cyanide species observed in aged tailings probably results from early dissociation of weak to moderately strong complexes. It is also possible that part of the free cyanide released was transformed into more stable forms such as ferro and ferri cyanide complexes during weathering.

**Table 1:** Selected physicochemical properties of aged and fresh solid tailings

<sup>a</sup>Results are presented as mean  $\pm$  standard deviation ( $n = 3$ )

Properties <sup>a</sup>	AO(0.2)	AO(1)	BO(0.2)	BO(1)	BF(0.2)	BF(1)
pH (water)	7.6 $\pm$ 0.1	8.3 $\pm$ 0.1	7.3 $\pm$ 0.1	7.4 $\pm$ 0.1	10.5 $\pm$ 0.1	10.6 $\pm$ 0.1
pH (CaCl <sub>2</sub> )	7.2 $\pm$ 0.1	7.5 $\pm$ 0.1	7.1 $\pm$ 0.1	7.1 $\pm$ 0.1	10.1 $\pm$ 0.1	10.0 $\pm$ 0.1
Total volatile solids (% w/w)	2.1 $\pm$ 0.3	0.8 $\pm$ 0.3	3.2 $\pm$ 0.2	3.0 $\pm$ 0.2	2.4 $\pm$ 0.1	2.6 $\pm$ 0.2
Water content (% w/w)	23.1 $\pm$ 0.2	20.9 $\pm$ 1.1	26.9 $\pm$ 0.1	24.3 $\pm$ 0.3	20.8 $\pm$ 1.1	21.7 $\pm$ 0.2
CEC (meq/100 g dry weight)	34.9 $\pm$ 9.9	54.0 $\pm$ 5.8	35.4 $\pm$ 6.1	24.3 $\pm$ 1.8	2.9 $\pm$ 0.3	2.3 $\pm$ 0.6

**Table 2:** Concentration of cyanide species in aged and fresh gold mill tailings

Solid phase <sup>a</sup>	AO(0.2) (mg/kg)	AO(1) (mg/kg)	BO(0.2) (mg/kg)	BO(1) (mg/kg)	<b>BF(0.2)</b> (mg/kg)	<b>BF(1)</b> (mg/kg)
CN <sub>T</sub>	4.8 $\pm$ 0.5	2.4 $\pm$ 0.3	2.4 $\pm$ 0.3	3.4 $\pm$ 0.3	17.0 $\pm$ 1.7	22.0 $\pm$ 1.0
CN <sub>WAD</sub>	< 0.5	< 0.5	< 0.5	< 0.5	3.1 $\pm$ 0.6	2.8 $\pm$ 0.5
Supernatant	AO(0.2) (mg/l)	AO(1) (mg/l)	BO(0.2) (mg/l)	BO(1) (mg/l)	BF(0.2) (mg/l)	BF(1) (mg/l)
CNO <sup>-</sup>	< 0.5	< 0.5	- <sup>b</sup>	-	93	98
SCN <sup>-</sup>	< 0.5	41	-	-	300	270
CN <sub>T</sub>	< 0.01	0.94	-	-	110	130
CN <sub>WAD</sub>	< 0.01	< 0.01	-	-	23	110

<sup>a</sup> Results are presented as mean  $\pm$  standard deviation ( $n = 3$ )

**Table 3:** Comparison between total cyanide content of solid tailings estimated by the standard 4500-CN<sup>-</sup> C method and total cyanide content released in NaOH

Samples	CN <sub>T</sub> 4500-CN <sup>-</sup> C (mg/kg)	CN <sub>T</sub> (NaOH 10 % w/v ) (mg/kg)	Weak to moderate cyanide complexes <sup>a</sup> (%)	Very strong cyanide complexes <sup>b</sup> (%)
AO(0.2)	4.8	31.2	15.4	84.6
AO(1)	2.4	8.6	27.9	72.1
BO(0.2)	2.4	13.7	17.5	82.5
BO(1)	3.4	10.4	32.7	67.3
BF(0.2)	17	37.7	45.1	54.9
BF(1)	22	41.6	52.9	47.1

<sup>a</sup> Calculated as:  $(\text{CN}_T \text{ 4500-CN}^- \text{ C} / \text{CN}_T \text{ NaOH}) \times 100$ ;

<sup>b</sup> Calculated as :  $100 - (\text{CN}_T \text{ 4500-CN}^- \text{ C} / \text{CN}_T \text{ NaOH}) \times 100$

## References

Oudjehani K, Zagury GJ and Deschênes L (2002) Natural attenuation potential of cyanide via microbial activity in mine tailings, Appl. Microbiol. Biotech., 58, 409-415.

Shifrin NS, Beck BD, Gauthier TD, Chapnick SD, Goodman G. (1996) Chemistry, toxicology and human health risk of cyanide compounds in soils at former manufactured gas plant sites. Regul. Toxicol. Pharmacol. 23, 106-116.

Zagury GJ, Oudjehani K and Deschênes L (2004) Characterization and availability of cyanide in solid mine tailings from gold extraction plants, Sci. Total Environ., 320, 211-224.

## Questions/Discussion:

**Q:** Is there a way to know the rate/timing of cyanide degradation?

**A:** No, not at the moment, because the times used in the study were three months and six years; due to the length of time, linear interpolation was not feasible.

## Mining Reagents and By-Products (e.g. Thiosalts) As Potential Toxicants in Mine Effluents.

Vigneault<sup>1</sup>, B., Desforages<sup>2</sup>, M. and McGeer<sup>3</sup>, J.

<sup>1</sup> Geological Survey of Canada, Natural Resources Canada.

<sup>2</sup> CANMET-Mining and Mineral Sciences Laboratories, Natural Resources Canada.

<sup>3</sup> Wilfrid Laurier University

## Introduction

In addition to regulated deleterious substances, potential toxicants in mine effluents often include additional inorganic and organic mine effluent constituents. Non-metallic effluent constituents include process reagents and wastewater treatment chemicals along with their by-products. Process reagents include frothing agents and collectors. Xanthates are commonly used as collectors during processing of sulphide ores by flotation. The flotation of sulphidic ore also results in the generation of thiosalts, which are composed of several sulfoxyanions: thiosalts thiosulphate, trithionate, tetrathionate and other polythionates. The most common process water treatment for metal mining is liming which is used to raise the pH and precipitate trace metals but resulted in elevated calcium and sulphate concentrations. Polymeric reagents specifically designed to precipitate out trace metals can also be used. Finally, flocculants can also be used in some cases to remove solids from mine waste water treatment, for example anionic polyacrylamide flocculents.

There are relatively few published studies on the potential contribution of non-metallic mine effluent constituents to effluent toxicity. De Rosemond and Liber (2004) have identified a cationic flocculant as the main source of toxicity to the invertebrate *Ceriodaphnia dubia* in the Ekati Diamond Mine effluent. Elphick *et al.* (2001) have identified a flotation reagent as the source of toxicity to *C. dubia* for the Eskay Creek Mine effluent. Finally, Stekoll *et al.* (2009) have suggested that the elevated calcium concentrations in the Red Dog Mine effluent could disrupt the fertilization process in salmonids.

Accordingly, testing was conducted to assess the toxicity of flotation and process agents to aquatic organisms, using MMER test species. The obtained toxicity endpoints were compared with expected concentration ranges in mine effluents to provide screening level data in the context of toxicity identification, evaluation, reduction and Investigation of Causes.

## Materials and Methods

Individual substances were tested using the *Metal Mining Effluent Regulations* test species and recommended methods. The conducted acute toxicity tests include lethality to the invertebrate *Daphnia magna* (48 hours(h)) and to *Oncorhynchus mykiss* (rainbow trout, 96h). The conducted sublethal toxicity tests include growth inhibition for the algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), 72h), for the plant *Lemna minor* (7 days(d)) and for the fish larvae *Pimephales promelas* (fathead minnows, 7d) as well as reproduction inhibition for the invertebrate *Ceriodaphnia dubia* (7d). Lethality variables (7day) for *C. dubia* are also reported. Tested substances include two xanthates (sodium isopropyl xanthate, NAX, and potassium amyl xanthate, KAX), two sulfoxyanions (thiosalts) species, (thiosulphate and tetrathionate) one flocculant (Magnafloc 10) and finally two chemicals used to precipitate metals (Nalmet 8702 and lime).

## Results and Discussion

### Process Reagents and by-products

Xanthates. While little information is available on residual xanthate concentrations in discharged effluent, the conducted toxicity tests and published data suggest that xanthates can be considered as potential toxicants. Both xanthates exhibited toxicity for the three tests species used, *C. dubia*, *P. subcapitata* and *L. minor*, within the expected concentration range in discharged effluent. The most

sensitive species was the algae *P. subcapitata* with a 72h- Inhibition Concentration (IC25) of about 0.5 mg/L for both xanthates while the least sensitive species was *C. dubia* with IC25 in the range of 3 mg/L for both xanthates (Figure 1). These toxicity results are comparable with the data available in the literature. Xu *et al.* (1988) reported an inhibition concentration for *L. minor* of less than 10 mg/L for sodium ethyl, sodium isopentyl, and sodium isobutyl xanthate. Xu *et al.* (1988) also reported 48h-EC50 for *Daphnia magna* of 0.35 mg/L for sodium ethylxanthate to 3.7 ppm for sodium isopropyl xanthate. It is difficult to estimate the potential exposure to xanthates in the aquatic environment. First, the half-life of xanthates in an ambient environment is approximately four days (Boening, 1998). In addition, there is limited information on the expected concentrations of these compounds in waters receiving effluent, or effluent itself, but reported concentrations range from 10 µg/L to 4.0 mg/L. These values suggest that the possibility of toxicity related to these compounds cannot be ruled out since all our measured IC25 are within this range.

Thiosalts. Thiosulphate ( $S_2O_3^{2-}$ ) was generally more toxic than tetrathionate as indicated by the lower IC25 values ( $S_4O_6^{2-}$ ) (Figure 2). Sensitivity to thiosalts ranged from no responses for rainbow trout to an IC25 of 59.4 mg  $S_2O_3$ / L for *C. dubia* (Figure 2). The acute toxicity of thiosulphate to *D. magna* was also greater than tetrathionate (Fig. 2, Effect Concentration (EC) 50 ~ 300 and 750 mg/L, respectively). By comparison, concentrations up to 800 mg/L were tested, as there are some reports that levels can reach as high as 700 mg/L in mine effluents. For all tests conducted with thiosulphate and tetrathionate, the thiosalt solutions were stable, ie. no pH depression was observed over the course of the test duration. To our knowledge, there is no literature available on the toxicity of thiosalts to these, or any other, aquatic organisms. The potential for thiosulphate toxicity is still quite low since inhibition concentrations will be in most cases higher than the expected concentrations in effluents. However, the relatively high toxicity of thiosulphate to *C. dubia* (7-d IC25<sub>reprod.</sub> = 59 mg/L) is of potential concern. In addition, some thiosalts species easily oxidize and the generation of acid in solution from thiosalts degradation has been observed both in laboratory toxicity tests and *in situ*. This potential for pH depression in the downstream environment is a possible mode of indirect toxicity that was not addressed in this study.

#### Waste Water Treatment Chemicals and by-products

Magnafloc 10. Sublethal endpoints showed higher sensitivity to Magnafloc 10 than did acute tests except for the least sensitive of the sublethal test organisms, *P. promelas* with a 7-d IC25 of 141 mg/L (growth, Figure 3). There is no data available in the literature regarding the sublethal toxicity of Magnafloc 10 or sublethal toxicity of any anionic polyacrylamide flocculent to *P. subcapitata*, *L. minor* or *P. promelas*. The 96-h lethal concentration of Magnafloc 10 for rainbow trout was 58 mg/L, which is in the same order of magnitude of the reported value of 18 mg/L (Ciba 2004). *Daphnia magna* was found to be the least sensitive of all our test organisms, with only 10 % mortality observed at the highest concentration tested (48-h LC50 >903 mg/L). This Lethal Concentration (LC)50 is higher than other reported EC50 values for *D. magna* (212 mg/L; Ciba 2003 and > 50 mg/L; Ciba 2004). The expected maximum concentration of flocculant in final effluent is in the range of 4 mg/L, based on the overly conservative assumption that all the added flocculent is discharged in the final effluent.

Nalmet. We performed sublethal toxicity tests with Nalmet on two species, *C. dubia* and *P. subcapitata*, which showed similar sensitivities (Figure 3). The 72-h IC25<sub>growth</sub> for *P. subcapitata*

was 4.2 mg/L and the 7-d IC<sub>25</sub><sub>reproduction</sub> for *C. dubia* was 3.9 mg/L. Lethality variables for *C. dubia* were also reported. For *C. dubia*, there was 100% mortality in the two highest concentrations of Nalmet (100 and 1000 mg/L). There is currently no data available in the literature to compare these toxicity values with residual concentrations for discharged mine effluent. The discharged concentrations could range from less than 2 mg/L up to 10-40 mg/L, which is the usual dosage for its use in wastewaters (NICNAS, 1996).

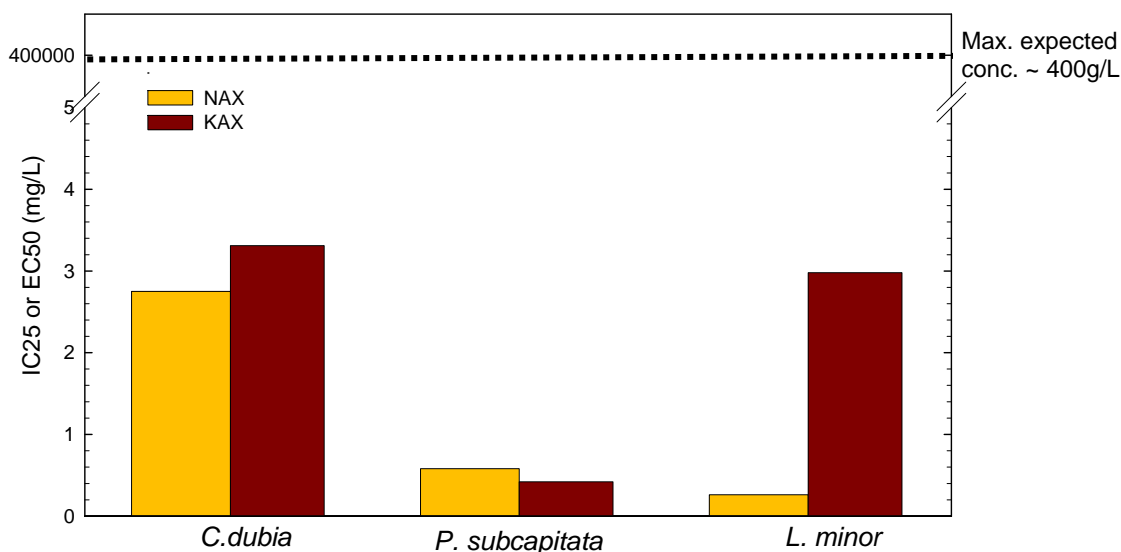
Liming. We tested elevated Ca levels (as CaSO<sub>4</sub>) on three sublethal test species, *C. dubia*, *L. minor*, and *P. subcapitata* at concentrations thought to mimic those in limed effluent. Results showed *C. dubia* was the least sensitive of the three species with a mean IC<sub>25</sub> of 460 mg Ca/L. *L. minor* was more sensitive to Ca, with a mean IC<sub>25</sub> of 59 and 106 mg Ca/L (frond count and dry weight, respectively). *P. subcapitata* has an intermediate sensitivity with an IC<sub>25</sub> of 492 mg Ca/L. Our toxicity results are in the same range than other Ca LC<sub>50</sub> reported by Goodfellow *et al.* (2000), where calculated acute values were as follows: *Daphnia pulex* 48-h EC<sub>50</sub> 499 mg/L; *P. promelas* 96-LC<sub>50</sub> 266 mg/L.

#### Conclusion:

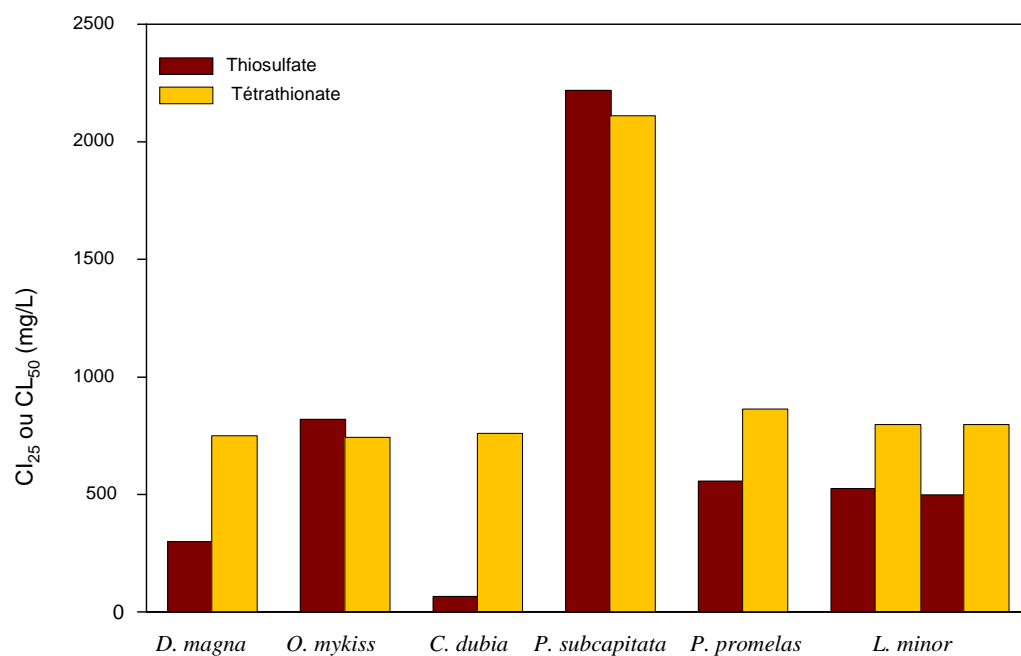
Taken together, available information suggests that non-metallic effluent constituents should be considered as potential causes of the foregoing effects. This could be particularly important since most of these constituents are not treated or are actually generated by conventional treatment of mine effluents. Finally, metal mining Investigation of Cause needs to go beyond MMER deleterious substances and EEM monitoring parameters.

#### Acknowledgments:

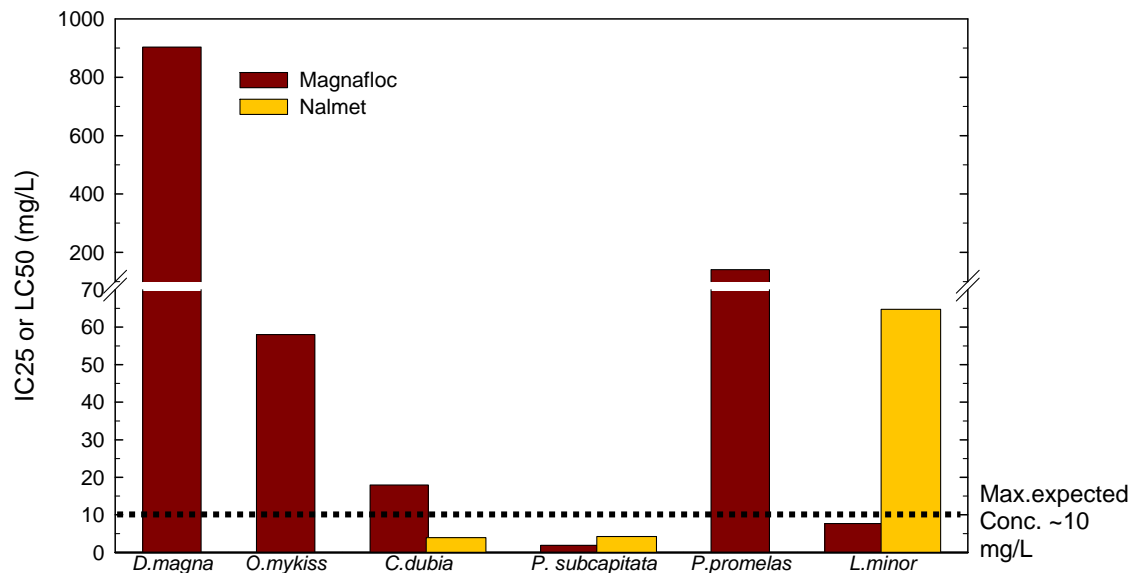
We would like to acknowledge the contribution of Jennifer Beyak (formerly CANMET-MMSL), Isabelle Gosselin (formerly CANMET-MMSL) and Morgan King for the toxicity testing. We also thank Carrie Rickwood for reviewing the draft version of this extended abstract. The support of the Thiosalts Consortium, Xstrata Nickel Canada and Iron Ore Company of Canada is also acknowledged.



**Figure 1:** Sublethal toxicity of sodium isopropyl xanthate (NAX) and potassium amyl xanthate (KAX) to *Ceriodaphnia dubia* (reproduction), *Pseudokirchneriella subcapitata* (growth) and *Lemna minor* (frond count).



**Figure 2:** Acute toxicity of thiosulphate and tetrathionate to *Daphnia magna* (lethality) and *Oncorhynchus mykiss* (rainbow trout lethality) and sublethal toxicity to *Ceriodaphnia dubia* (reproduction), *Pseudokirchneriella subcapitata* (growth), *Pimephales promelas* (fathead minnows, growth) and *Lemna minor* (frond count and dry weight).



**Figure 3:** Acute toxicity of Magnafloc 10 and Nalmet to *Daphnia magna* (lethality) and *Oncorhynchus mykiss* (rainbow trout lethality) and sublethal toxicity to *Ceriodaphnia dubia* (reproduction), *Pseudokirchneriella subcapitata* (growth), *Pimephales promelas* (fathead minnows, growth) and *Lemna minor* (frond count).

#### References

- Boening, D.W. 1998. Aquatic toxicity and environmental fate of xanthates. Min. Eng. 50: 65-68.
- Ciba 2003. Material Safety Data Sheet - Magnafloc 10. Ciba Speciality Chemicals Canada Inc.
- Ciba 2004. Material Safety Data Sheet - Magnafloc 10. Ciba Speciality Chemicals Canada Inc.
- De Rosemond, S. J. C., and Liber, K. (2004). Wastewater treatment polymers identified as the toxic component of a diamond mine effluent. Environ. Toxicol. Chem. 23: 2234-2242.
- Elphick, J.R., Bailey, H.C. and Murphy, P.M. (2001). Toxicity Identification Evaluation of effluent from a mine. In: 25th Annual British Columbia Mine Reclamation Symposium, September 24 - 27, 2001, Campbell River, BC.
- Goodfellow, W.L., Ausley, L.W., Burton, D.T. *et al.* (2000). Major ion toxicity in effluents: a review with permitting recommendations. Environ. Toxicol. Chem. 19: 175-182.
- National Industrial Chemicals Notification and Assessment Scheme, NICNAS (1996) Polymer in NALMET 8702. File No: NA/458. Camperdown, NSW, Australia.
- Stekoll, M. S., Smoker, W. W., Failor-Rounds, B. J. *et al.* (2009). Response of the early developmental stages of hatchery reared salmonids to major ions in a simulated mine effluent.

Aquacult. 298: 172-181.

Xu, Y., Lay, J.P., and Korte, F. (1988). Fate and effects of xanthates in laboratory freshwater systems. Bull. Environ. Contam. Toxicol. 41: 683-689.

**Questions/Discussion:**

Q: Are there non-metal concentrations that affect nutrient uptake in fish?

A: Yes, it is possible that the non-metallic effluent constituents could have an impact on nutrient uptake in fish. However, we have not collected any data in the current study to support this hypothesis. None of the toxicity data given/presented is necessarily related to algae & plant data.

Comment: Calcium is very reactive with this test, but may also affect fish nutrient intake.

Comment: Studies on xanthates should be published so that mines are more aware of the results and impacts of this on the metal mining industry.

### **Session 3: Regional Overview and Case Studies**

#### **Metal Mining Environmental Effects Monitoring in the Atlantic Region**

Maher, S.E.

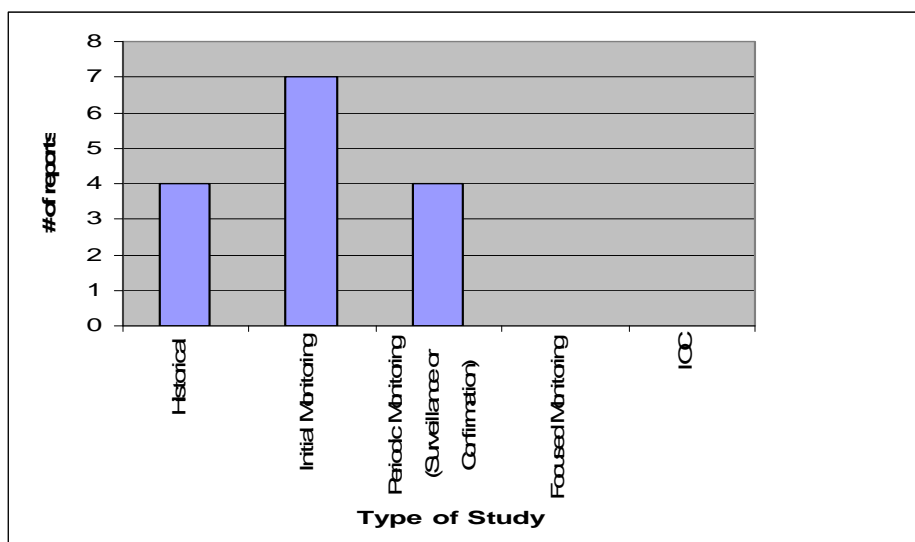
Environment Canada, Atlantic Region

At the time of the promulgation of the *Metal Mining Effluent Regulations* (MMER) in 2002, there were only four mines in Atlantic Canada that were subject to these regulations. By 2009, that number had increased to ten. Six of these mines are located in Newfoundland and Labrador, three are in New Brunswick and one is in Nova Scotia. As indicated in Table 1, the mine types include base metal, precious metal and ferrous mines, which extract both from open pits and underground. One mine deposits into a marine habitat while the other nine mines deposit into freshwater habitats.

**Table 1:** Breakdown of AR Mines by Mine Type and Receiving Environment

Mine Type	Number of Mines	Metal(s) Mined	Receiving Environment
Base Metal	6	2 zinc/lead	rivers
		2 zinc/lead/copper/silver	brooks
		1 zinc/copper	brook
		1 nickel/copper/cobalt	marine estuary
Precious Metal	2	Gold	brook, pond
Ferrous	2	iron	lake, river

Depending on when each mine became subject to the MMER, some of the mines in the Atlantic Region (AR) have completed two phases of environmental effects monitoring (EEM) whereas other mines that have been more recently captured under the MMER have yet to conduct their first biological EEM study. The status of the AR mines through the Metal Mining EEM program is depicted in Figure 1.



**Figure 1:**  
EEM Studies in the AR

Two of the four AR mines initially captured under the MMER have confirmed biological effects in the receiving environment and will be moving beyond periodic monitoring into focused monitoring (extent and magnitude) in the next phase of monitoring. Both mines had confirmed effects in both the fish and benthic invertebrate community (BIC) components. The specific endpoints for these confirmed effects are detailed below in Tables 2 and 3.

Table 2: Mine 1: Confirmed Biological Effects

Component	Effect Endpoint	Phases 1 & 2 Results
Benthic Invertebrate Community	Density	> CES of $\pm 2SD$ (exp < ref)
	Taxa Richness	> CES of $\pm 2SD$ (exp < ref)
	Bray-Curtis	> CES of $\pm 2SD$ (exp > ref)
Fish Survey (Lethal)	Lake Whitefish - Male: Size at age (weight)	SS (exp < ref)
	Longnose Sucker - Male: Size at age (weight)	> CES of $\pm 25\% SD$ (exp < ref)
	Longnose Sucker - Female: Size at age (weight)	> CES of $\pm 25\% SD$ (exp < ref)
	Longnose Sucker - Female: Condition	> CES of $\pm 10\% SD$ (exp < ref)

SS = statistically significant, CES = critical effect size, SD = standard deviation

Table 3: Mine 2: Confirmed Biological Effects

Component	Effect Endpoint	Phases 1 & 2 Results
Benthic Invertebrate Community	Taxa Richness	> CES of $\pm 2SD$ (exp < ref)
	Bray-Curtis	> CES of $\pm 2SD$ (exp > ref)
Fish Survey (Non-lethal)	White Sucker: Growth (length and weight of yong of the year)	SS (exp > ref)
	White Sucker: Condition	SS (exp < ref)
	White Sucker: Survival	SS

SS = statistically significant, CES = critical effects size, SD = standard deviation

Technical issues and/or confounding factors have been encountered during EEM at some of the mines in the AR. This resulted in either not all of the required effect endpoints being obtained or a degree of uncertainty in ascribing the observed effects to the mine effluent in the initial monitoring study. However, because of the experience and knowledge gained from the conduct of the initial EEM studies, many of the technical issues and/or confounding factors have been addressed through the subsequent re-design of the EEM studies. For the mines which modified their study designs between EEM phases, these mines must remain in periodic monitoring for the next EEM phase to allow for a confirmation study that uses the same study design. Table 4 illustrates some of the technical and confounding issues that have been encountered in the AR.

**Table 4: Technical and/or Confounding Factors Encountered during EEM in the AR**

Technical Issues	Confounding Factors
<ul style="list-style-type: none"> <li>● selection of appropriate finfish for EEM</li> <li>● timing of survey(s) inappropriate for obtaining reproductive measures</li> <li>● selection of alternate method if no finfish present or finfish available are not appropriate for EEM</li> <li>● differing fish collection methods used among study areas</li> <li>● selection of best habitat for EEM (i.e. river vs. pond habitat)</li> <li>● obtaining fish tissue sample in a non-lethal study</li> </ul>	<ul style="list-style-type: none"> <li>● historical contamination (most often result of the operation of the current mine)</li> <li>● difficulty in identifying appropriate reference area (e.g. reference not available in same watershed, reference area is a headwater branch of downstream impacted river)</li> <li>● habitat differences between exposure and reference areas (e.g. habitat type, substrate type, water depth, flow rate, vegetation, cover)</li> <li>● beaver dam impeding fish passage</li> </ul>

For the next phase of EEM in the AR, there will be two mines conducting focused monitoring (extent and magnitude), five mines will be conducting periodic monitoring (either surveillance or confirmation) and one mine will be conducting an initial monitoring study. Two of the ten mines currently captured under the MMER went into bankruptcy protection in 2009. Discussions regarding the regulatory requirements, including those of Schedule 5 (EEM) are on-going. There will be no mines conducting Investigation of Cause studies in the next phase of EEM in the AR. With respect to additional mines coming under the MMER in the next couple of years, it is possible that as many as six new mines may be captured under the regulations and thus be required to conduct EEM.

### **Metal Mining Environmental Effects Monitoring in the Quebec Region**

Chabot<sup>1</sup>, R.

<sup>1</sup>Environment Canada, Quebec Region

At the time of the promulgation of the *Metal Mining Effluent Regulations* (MMER) in 2002, there were 20 mines subject to the MMER in the Quebec Region. Currently, there are 27 mines that are under the MMER and they are categorized as base metal, precious metal or ferrous metal mines. The receiving environments for the mines in Quebec are in creek, river, marsh, pond and lake habitats. From the 20 mines that became subject to the MMER in 2002, 16 have completed two phases of environmental effects monitoring (EEM), whereas from the other 11 that have been recently triggered under the MMER, only four have finalized their first biological EEM study. There are six mines out of 16 that have reported confirmed biological effects and are moving beyond the periodic monitoring phase of EEM to focused monitoring. Some mines had to change one of the study zones in the second phase for different reasons and one mine had to change sentinel species. Habitat at some other mines was different between the exposure and the reference zones so no confirmation of effect could be made. Some confounding factors issues have proven to be more difficult to resolve and alternative monitoring approaches may be explored for the future phases of EEM at some mines.

### **Brief Overview of Metal Mining Environmental Effects Monitoring in the Ontario Region**

Plant<sup>1</sup>, W. and Audet<sup>1</sup>, D.

<sup>1</sup>Environment Canada, Ontario Region

### Mines In Ontario

When the *Metal Mining Effluent Regulations* were promulgated in 2002, there were twenty mines subject to the *Metal Mining Effluent Regulations* in the Ontario Region. There are currently thirty-five mines (Figure 1). Three additional mines may become regulated in the near future. Since 2002, one mine has received recognized closed mine status.

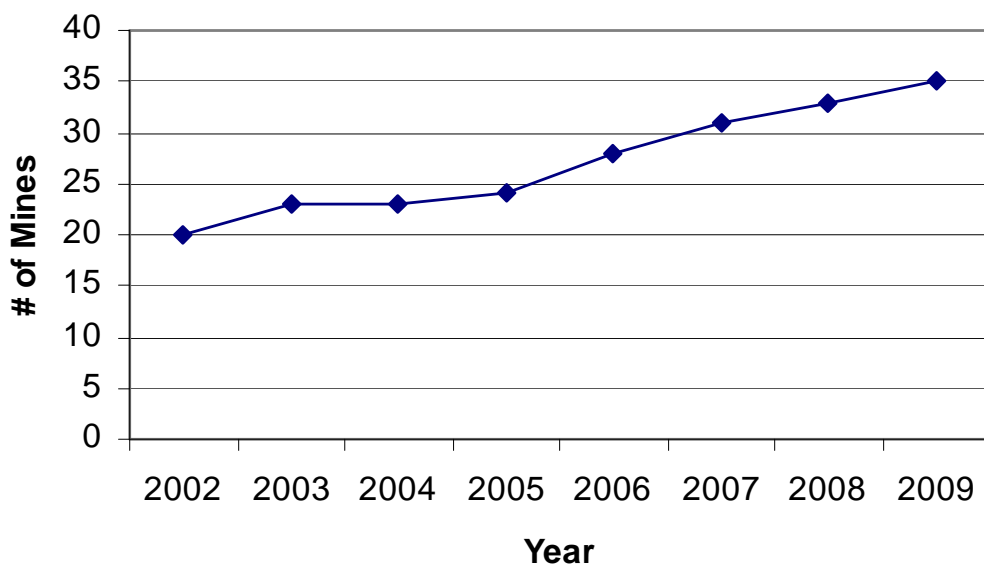


Figure 1: The number of mines in Ontario by year since the promulgation of the MMER in 2002

### Types of Mines in Ontario

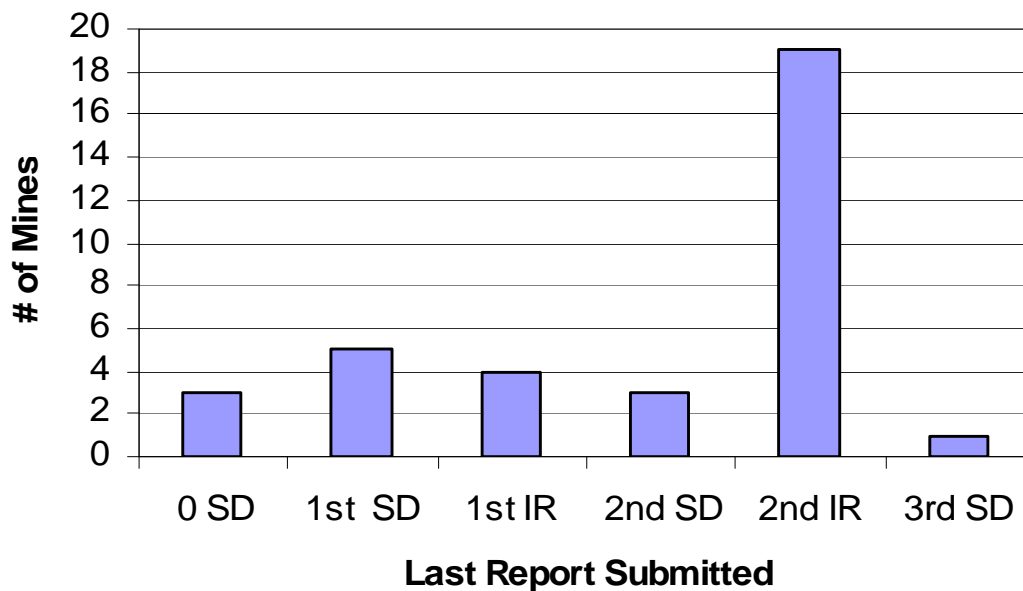
Ontario currently has only base and precious metal mines with roughly equal distributions of each.

### Receiving Environments

Creeks are the most common receivers (41%) for mine effluent discharge in the Ontario region, however, discharges to rivers (31%) and lakes (28%) are also very common.

### Mine Status

Most mines in the Ontario Region have recently submitted their second Metal Mining Effluent Regulation Environmental Effects Monitoring Interpretative Report. Some of the mines newer to the regulations have not proceeded as far (Figure 2). For Ontario mines which have completed two phases of Environmental Effects Monitoring, 12 mines (63%) will continue periodic monitoring, six mines (32%) will go into Focussed Monitoring (Extent and Magnitude or Investigation of Cause) and one mine (5%) will go to minimal six-year monitoring.



**Figure 2:** The last report submitted by Ontario Mines indicating their status within the *Metal Mining Effluent Regulations* Environmental Effects Monitoring program

#### Investigation of Cause Study

One Ontario Mine conducted an Investigation of Cause study looking at mine related effects on the benthic invertebrate community. The mine installed sediment traps to assess the current sediment deposition rates and the quality of the sediment being deposited. Sediment cores were collected for Lead-210 dating and general chemistry to determine the age and quality of sediments at various depths in order to understand whether exposure to elevated parameters of concern is primarily related to historical contaminant deposits or from more recent deposits. The mine assessed pore-water chemistry to get an indication of the bioavailability of metals within the sediments and the aqueous conditions which benthic invertebrates are exposed to. The mine installed data loggers to better understand the conditions affecting benthic invertebrates near the sediment/water interface over an extended time period. Receiving water quality samples were collected near the sediment/water interface monthly. Sediment samples were also submitted for toxicity testing using the fourteen day *Hyalella azteca* survival and growth test, the ten day *Chironomus riparius* survival and growth test and the twenty-eight day *Tubifex tubifex* survival, growth and reproduction test. Finally a benthic invertebrate community survey was conducted to compare to the previous two field studies.

#### Technical Challenges Encountered

Many mines have difficulties capturing sufficient numbers of fish to conduct a statistically significant study. This may be in part due to the small size and low productivity of many of the Northern Ontario receivers used by mining operations to dispose of their treated effluent.

Finding suitable Reference Areas to compare to a Mine's Exposure Area has proven difficult for a number of Ontario Mines. Many Ontario mines discharge to headwater lakes which are difficult to compare to.

It is challenging to get accurate age estimates for small bodied fish using scales. In the Ontario Region we have been requesting age estimates using calcified structures such as otoliths in order to improve age estimates.

Often, mine sites in the Ontario Region discharge to receiving environments that may be confounded by other anthropogenic effects. For example a mine may discharge to a receiver that accepts urban or agricultural runoff. There are often other mining facilities in the area, all of which discharge to the same watershed. There may be closed mines which still discharge from their tailings ponds in the area of a mine currently under the Metal Mining Effluent Regulations. Historical sediment contamination from mines may still be affecting the current receiving environment despite the mine meeting the current effluent standards.

#### Ontario Initiatives and Partnerships:

The Ontario Region worked in partnership with academic researchers, the Ontario Ministry of the Environment, other federal scientists and industry to design and develop the Reference Condition Approach for obtaining valuable benthic invertebrate reference data for mines without suitable Reference Areas. The objective was to develop a large network of reference sites to be used to assess mining effects by detecting any impairment in the benthic invertebrate community structure.

The Ontario Region also worked on a project to determine the best ageing structures for small bodied fish. Many mines are forced to conduct fish surveys using small bodied fish due to the small receivers that they discharge to. Mines were getting inconsistent results using scales to age fish. The objective of this project is to recommend the best aging structures for small bodied fish.

Ontario Region has worked with Lisa Taylor (Environment Canada, Method Development and Application Unit) and two mining companies to test a new sublethal toxicity method to be used as a tool for distinguishing current mine effects from past effects as a result of historical contamination. In Ontario there are many mine sites that have been active for many years, in some cases over 100 years. It is often very difficult to differentiate historical effects from current effluent effects. This sublethal toxicity method shows promise in helping a facility to demonstrate the quality of their current effluent.

#### Industry Compliance

In Ontario, the mining industry's compliance with Schedule 5 of the *Metal Mining Effluent Regulations* has generally been very good. Many mines have gone beyond the minimum requirements and provided extremely valuable additional information such as sediment chemistry data or they have sampled multiple Exposure and Reference Areas.

#### **Questions/Discussion:**

**Q:** What is the approach when there are problems finding appropriate reference areas, or in obtaining all EEM metrics?

A: This situation is site specific and should be discussed with your regional EEM co-ordinator.

Q: What about scheduling?

A: The EEM program is currently still learning more about each mine as time progresses. Generally, a helpful approach is to try to address all of the above (finding suitable reference areas and fish species, etc.) before the mine opens. When that is not an option, the post-opening study design tries to address (i.e. for existing mines) all the site-specific concerns.

### **The Metal Mining Environmental Effects Monitoring Program in the Prairie and Northern Region.**

Siwik, P., Boss, S., Kuczynski, E., Allen, E.  
Environmental Protection Operations Division, Environment Canada.

The Prairie and Northern Region includes the Northwest Territories, Nunavut, and the provinces of Manitoba, Saskatchewan and Alberta. Since the promulgation of the Metal Mining Effluent Regulations, 25 mines in this region have completed one or more Environmental Effects Monitoring (EEM) studies, one will complete their first biological study in 2010, and five have become Recognized Closed Mines (RCM). The facilities in this region extract and process a variety of ores including gold, rare earth elements, base metals and uranium. The receiving environments are predominantly freshwater (small head water streams, lakes and rivers) but did include marine areas during the initial sampling phase. Some of the challenges identified by facilities in this region during the initial phase of the program include: historical contamination, influence of other discharges, locating suitable reference areas, and identifying sentinel fish species. Many facilities were able to design subsequent studies to reduce the influence of potential confounding factors.

In 2009, ten facilities completed studies to delineate the extent and magnitude of their effects (six facilities) or to investigate the cause (IOC) of their effects (four facilities). The mines that submitted IOC study designs did so because they had existing information on the magnitude and extent of their effects, or had confirmed effects below the interim critical effect sizes. The 2009 IOC studies included two investigating confirmed effects on the benthic invertebrate community, one investigating confirmed effects on fish and one IOC to be conducted over two consecutive phases.

The first mine, triggered on benthic effects, hypothesized that effects could be due to increased trace element exposure, organic matter and nutrients, or habitat differences. The study included a standard EEM benthic survey plus collection of wild amphipods for analysis of metal body burdens, and expanded water, sediment and effluent sampling and analysis. The benthic data will be assessed at the lowest practicable level (LPL) and consider known sensitivities of taxa to trace elements, nutrients and/or habitat. The second mine triggered on benthic effects hypothesized that confirmed effects could be due to differences in habitat and/or possible nutrient enrichment as a result of effluent exposure. The study design proposed a standard benthic program with the inclusion of additional reference areas. Multivariate statistics will be used to identify the most suitable reference lake for future EEM sampling, and examine nutrients, metals, and other parameters in water and sediment from each lake. Benthic data will be analyzed at both Family and LPL.

The mine with confirmed fish effects included four hypotheses in their study design: historical sediment contamination, eutrophication/conductivity response, an effluent contaminant response, and habitat differences. As this mine is in a stage of long term remediation and has not discharged since September 2008, a weight of evidence approach was used. Study components included: young of the year growth survey, adult lethal fish survey, in-depth fish liver histology, sublethal toxicity tests using surface water collections, water quality, data from plume modeling, habitat comparisons, and a review of existing data. Each component contributes to testing one or more of the four hypotheses.

Finally, one mine is working on a two-phase IOC study. In this case, effects were confirmed but with no clear cut response pattern to direct a focused IOC study. This mine has an abundance of data collected for EEM and other regulatory programs, and has conducted a large amount of field based aquatic research. Therefore the first phase of the IOC is focussed on a detailed analysis and integration of these data to identify key response patterns of effects within the receiving environment, a knowledge gap analysis and the identification of potential mechanisms. The Interpretative Report will include the results of this detailed analysis and integration and identify key patterns and mechanisms to investigate further in the second phase of IOC study. The Phase 2 IOC work will focus on the key response patterns and potential mechanisms identified in phase 1 and undertake lab and/or field based studies to investigate these potential causes.

#### **Questions/Discussion:**

Q: What benthic endpoints are used to trigger IOC?

A: Bray-Curtis Index, richness, evenness, and density.

#### **Metal Mining Environmental Effects Monitoring in Pacific and Yukon Region**

Hagen<sup>1</sup>, M.E. and Lacroix<sup>2</sup>, D.

<sup>1</sup>Environmental Protection Operations, Environment Canada, Vancouver, BC

<sup>2</sup>Environmental Protection Operations, Environment Canada, Whitehorse, YT

##### Mines in Pacific and Yukon Region (PYR)

Five mines were captured by the MMER in 2002, all in BC. Five more mines have since become subject to the MMER, including two in Yukon. PYR also has three non-discharging, non-MMER mines and ten coal mines. Numerous projects currently in the Environmental Assessment process may become mines over the next few years (Figure 1).

Eight of the ten PYR mines are base metal mines, usually producing copper plus some combination of silver, gold, molybdenum, zinc, and/or lead. Two of the base metal mines produce only molybdenum. The other two mines are both precious metal mines producing gold. Six of the mines are open pit operations, four are underground.

Eight mines discharge to small, headwater creeks. These creeks are usually tributary to larger creeks or to lakes. One mine discharges to a creek that is tributary to a large river, and one discharges to a large river via a diffuser. One mine started out discharging to a small creek upstream of a lake, but now discharges directly to the lake via a diffuser.

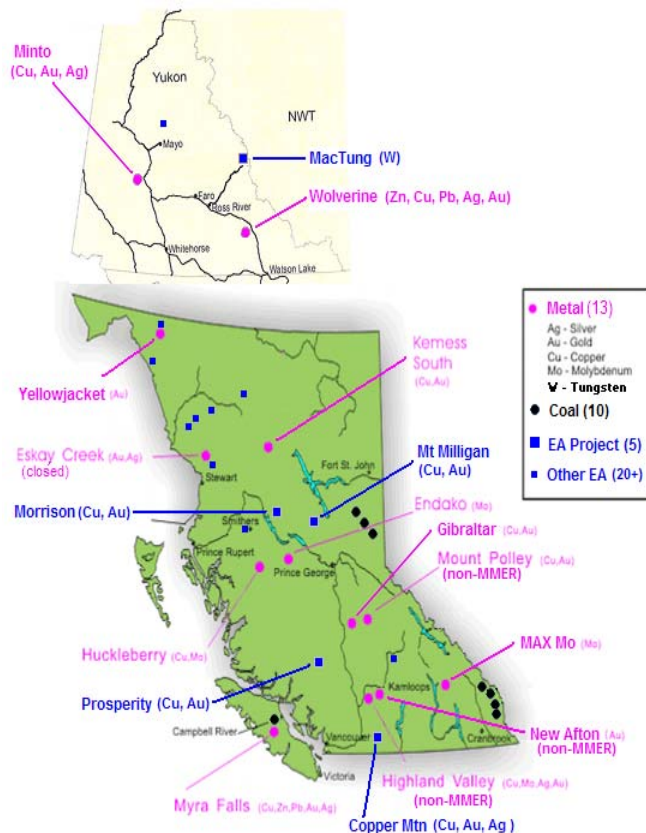


Figure 1: Mines in Pacific and Yukon Region

In short, the typical PYR mine is a 50,000 t/d open pit copper/gold operation discharging to small, headwater streams.

### EEM Challenges Associated with Small Creeks

Environmental effects monitoring (EEM) is faced with a number of challenges when effluent is discharged to small creeks. An upstream reference area is often not available and it is necessary to sample in another watershed or downstream. Habitat differences are hard to control. Geochemistry also varies.

Effluent in small creeks may comprise up to 100% of flow. 50% or more is not uncommon for long distances downstream. This may affect characteristics of the exposed stream to the point where reference streams simply are not available. Exposed streams may flow year-round, for example, while all potential reference streams of similar size and morphology have intermittent flow.

Small creeks may be hard to sample. It may be difficult to manipulate the equipment. Even if the creek is big enough for the sampler, there may not be the appropriate number of discrete pool or riffle habitats.

Small creeks may be high energy and/or subject to flow extremes. Mountainous terrain also has accessibility and safety issues. At one mine, a mesocosm fish survey designed to address fluctuating water levels and the risk of bivalves washing away was itself washed away.

Small creeks may have no fish, few fish, small fish, only juvenile fish, or show transient use. If there are no fish it may become necessary to go well downstream to find fish or to use an alternate method. If there are few fish, or only juvenile fish, then non-traditional fish surveys using non-lethal sampling may be done, but this raises challenges as some endpoints are not measured. Small fish are hard to measure. Alternate methods such as hatch boxes may be useful when effluent-exposed creeks are only used for spawning. Other alternate methods may be appropriate for other transient use creeks.

### Status of Mines in the EEM Program

Four of the five mines subject to the MMER in 2002 have completed two phases. Three of the four submitted historical reports. The fifth submitted a historical report, completed one phase, and closed. Their final program is in progress. One of the five mines captured by the MMER since 2002

has submitted a first phase report; four have yet to submit an EEM Interpretive Report.

Fourteen federal EEM reports have been submitted: four historic reports, six initial monitoring reports, and four periodic monitoring reports designed to confirm results of previous reports (Figure 2). Several mines have also submitted provincial EEM reports, sometimes annually, some pre-2002. No PYR mines have yet conducted focused monitoring programs designed to establish the extent and magnitude of effects, or to investigate the cause of effects.

The subsequent reports expected from the ten PYR MMER mines consist of four initial reports, four periodic reports (including one final), and two extent and magnitude survey reports.

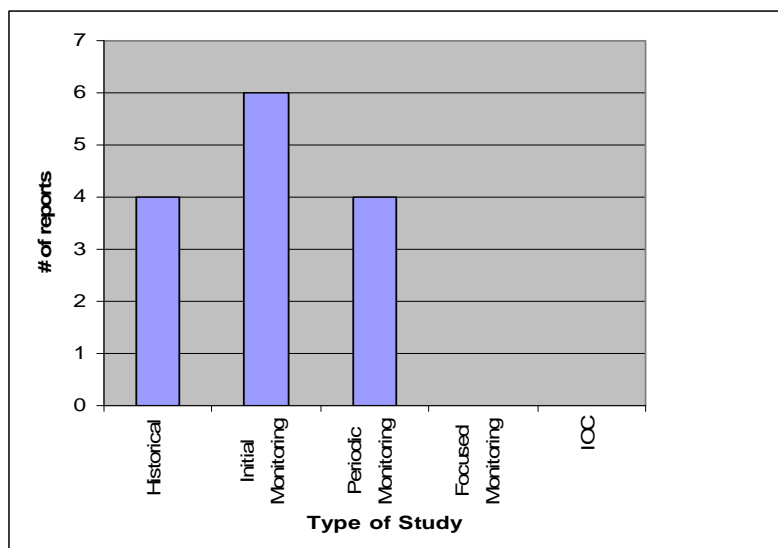


Figure 2: EEM reports submitted in Pacific and Yukon

Two of the four mines that have completed a second EEM monitoring phase did not confirm effects found in the first phase. Changes to the initial study designs were required due to confounded effects or differing exposed area. These mines repeat Periodic Monitoring for the next phase of monitoring.

Two mines confirmed phase one effects in their subsequent program. While each mine found statistically significant effects in both fish and benthos endpoints, the effects were less than critical effect sizes in the fish endpoints for one of the mines. In both cases, an enrichment effect of mine effluent was indicated, not an inhibitory effect. Both mines are conducting extent and magnitude surveys in their subsequent programs during 2010.

Both mines are challenged in that the small creeks they discharge to drain into lakes immediately below their current EEM stations. Their subsequent programs will need to monitor in lake habitats and determine whether any effects found (if any) are due to effluent or to the creek itself. Appropriate reference areas are needed. Potentially different fish, or different life stages, occurring in a lake than in the creek may also confound interpretation of results.

### Questions/Discussion:

**Q:** Are there any ecological reasons why there are more IOC studies being conducted in certain geographical regions (i.e. middle of Canada)?

**A:** Some mines already had historical data, so they moved directly into IOC. It is not necessarily due to the type of effluent or discharge. In their cases, having an extra (monitoring) cycle would not be helpful because those mines were already conducting EEM-like studies.

[Editor's note: In addition, in regions where there is a one-window approach, such as Prairie Northern Region, additional data concurrent to EEM, was collected to satisfy requirements of other regulators. As a result, effects were confirmed and M&E was delineated. These mines moved through the EEM program faster than the majority of other mines across the country.]

### **Investigation of Cause Study at the McArthur River Uranium Mine**

Stecko<sup>1</sup>, P. Orr<sup>1</sup>, P. Russel<sup>1</sup>, C. Stark<sup>2</sup>, S. and England<sup>2</sup>, K.

<sup>1</sup> Minnow Environmental Inc. <sup>2</sup> Cameco Corp.

Cameco Corporation's McArthur River Operation, located in the Athabasca Region of Northern Saskatchewan, has been in operation since 1999 and is the world's largest high grade uranium deposit. Two phases of Environmental Effects Monitoring (EEM) identified significantly greater benthic invertebrate density, taxon richness and Bray-Curtis distance at effluent exposed areas than at a reference area (located upstream in the same waterbody that receives effluent), triggering focused monitoring in the next monitoring phase. Both phases of EEM included far field monitoring that captured the magnitude and geographic extent of effects to benthic invertebrate communities. Therefore, the mine, in consultation with Environment Canada, chose to proceed with an Investigation of Cause (IOC) study. Minnow was retained by Cameco to develop an approach for the IOC study. A review of historical information indicated that differences in benthic invertebrate communities identified in EEM were also apparent prior to mine operation. Before-After-Control-Impact (BACI) analysis results were equivocal, but identified that some of the reference-exposure differences were greater after the initiation of mine operation than before. Overall, the review suggested that the observed differences may have been related to poor reference area suitability (including differences in sediment particle size and organic carbon content), nutrient enrichment, and/or other mine-effluent related constituents. An IOC study was designed and implemented in 2009 to investigate these factors and included a characterization of physical conditions, chemical conditions (water and sediment quality) and benthic invertebrate communities of effluent-exposed and reference water bodies in the vicinity of the McArthur River Operation (including six new reference areas) to support a determination of potential causes of spatial differences in benthic invertebrate communities.

### **Questions/Discussion:**

**Q:** Do the results from the fish survey corroborate the hypothesis? (The hypotheses to support spatial differences in benthic communities (increased density and diversity) were discussed. These included natural habitat, nutrient enrichment, mine-related contaminants (ions, metals, radionuclides). Results for fish, 1<sup>st</sup> study no effect, 2<sup>nd</sup> study—change in design. No consistent effects on two consecutive studies.).

**A:** To an extent – yes. Larger size is seen in the exposure, although the reason is questionable because it compares a lake environment with a creek environment.

### **Investigation of Cause of Effects on Benthic Invertebrates at the Kidd Metallurgical Site**

Weech<sup>1</sup>, S. Orr<sup>1</sup>, P. Russel<sup>1</sup>, C. Fedat<sup>2</sup>, L. and Yaschyshyn<sup>2</sup>, D.

<sup>1</sup> Minnow Environmental Inc. <sup>2</sup> Xstrata Copper - Kidd Metallurgical Site

The Kidd Metallurgical Site (Metsite) is a base metal processing operation, located near Timmins, Ontario, which is owned and operated by Xstrata Copper Canada. Effluent from the tailings management area is treated and discharged into the Porcupine River. Upstream of the Metsite, the Porcupine River also receives effluents and runoff from active and closed mines and sewage treatment plants. Since the MMER came into force, EEM studies have been conducted twice at the Metsite (2004 and 2007). In the benthic invertebrate surveys of both studies, statistically lower taxa richness and Simpson's Evenness, and greater Bray-Curtis distance were observed in downstream exposed communities relative to upstream ( $>2$  standard deviations difference). Therefore, the Metsite has proceeded to an investigation of the cause (IOC) of effects involving three downstream effluent-exposed areas and one upstream reference area, in a control-impact design. Specifically, the design included: collection of water and benthic invertebrate samples for assessment of current conditions; installation of sediment traps to assess within-year deposition rates and sediment quality; collection of sediment cores for lead-210 dating and chemical analysis to determine year-over-year sediment accumulation rates and the age and quality of sediments at various depths; collection of sediments for Sequential Extraction Analysis to evaluate the form and bioavailability of metals; installation of 'peepers' for collection of pore water to determine partitioning of metals between sediments and interstitial water; installation of data loggers to assess possible diurnal or seasonal changes in oxidation-reduction state and/or water quality; and a mineralogical assessment of sediment gypsum deposits. Finally, sediment toxicity tests using *Hyalella azteca*, *Chironomus riparius* and *Tubifex tubifex* were also completed to determine if sediment chemistry is associated with direct toxic effects on biota. Taken together, the data will contribute to a better understanding of the influence of current versus historical discharges from the Metsite on sediment chemistry and provide new insight into the relationship between sediment chemistry and benthic invertebrate community health.

#### **Con Mine: Investigation of Cause Study on fish livers – Challenges to designing a new Investigation of Cause study**

Sharpe<sup>1</sup>, R.L., Machtans<sup>2</sup>, H., Crowe<sup>2</sup>, J., Smith<sup>2</sup>, P., Patrick<sup>2</sup>, H., Chapman<sup>3</sup>, P.M., Connell<sup>4</sup>, R., Daniels<sup>5</sup>, E.

<sup>1</sup> Golder Associates Ltd. Edmonton <sup>2</sup> Golder Associates Ltd. Yellowknife <sup>3</sup> Golder Associates Ltd. Burnaby <sup>4</sup> Miramar Northern Mining Ltd., Con Mine, Yellowknife <sup>5</sup> Newmont Gold, Nevada

#### Introduction

Con Mine is a gold mine located adjacent to the City of Yellowknife on Great Slave Lake, in the south-central Northwest Territories. It was the first mine in production in Yellowknife in 1938. Miramar Northern Mining Ltd. (MNML) has owned and operated the mine since December 2007, when Newmont Mining Corporation (Newmont) purchased Miramar Mining Corporation, forming MNML. Underground mining ceased at the site in September 2003 at which time the underground workings were allowed to flood. At present, no water is pumped from the underground and the only activities carried out on site are clean-up, seasonal effluent treatment, and standard monitoring. A new water treatment facility is being designed in 2010 which will treat surface run-off, the only source of future effluent.

#### Phase 1 and 2 Summary

The first and second phases of the Environmental Effects Monitoring (EEM) program for Con Mine were completed in June 2005 and June 2008, respectively.

### Reference Area

Treated effluent is discharged from the water treatment plant into an unnamed creek that flows through a system of three shallow lakes: Meg, Keg and Peg. Outflow from Peg Lake enters a small, narrow bay on Great Slave Lake called Jackfish Bay. The effluent exposure area during mine operations included the Meg-Keg-Peg lakes system, Jackfish Bay and a portion of Great Slave Lake adjacent to Jackfish Bay, resulting in an exposure area about 7 kilometres long.

The reference area used for the majority of the EEM program and for the IOC study was Horseshoe Bay, a large sheltered bay on the south side of Horseshoe Island. It is located almost directly across from Jackfish Bay on the far side of Yellowknife Bay. The bay is a depositional area with substrate and extensive emergent macrophytes, and is highly similar to the habitat at Jackfish Bay. The predominant flow of water in Yellowknife Bay is from north to south; therefore, the reference area is not influenced by the effluent discharged to Con Mine.

### Effluent Characterization

Treated effluent from Con Mine had very elevated concentrations of major ions, reflecting the influence of deep Canadian Shield brine that contributed saline groundwater to the minewater. Conductivity of effluent ranged from 10,800 to 19,700 microSiemens per centimeter ( $\mu\text{S}/\text{cm}$ ) from 2000-2002. Chloride, the major ion present, and sodium and calcium concentrations were all elevated. The pH of treated effluent was slightly alkaline (7.4-7.8) and cyanide, ammonia and nitrate concentrations were elevated. Several metals/metalloids including arsenic, copper, lead, nickel, zinc and strontium were also elevated.

### Effluent Toxicity

Treated effluent was found to be acutely lethal to two aquatic test species in 2003: Rainbow Trout (*Oncorhynchus mykiss*) and a waterflea (*Daphnia magna*). A Toxicity Identification Evaluation (TIE) was conducted during Phase 1 monitoring and identified the likely toxic components of the effluent to be ammonia and major ions. During Phase 2 monitoring, the effluent was not acutely lethal to fish but remained toxic to *D. magna* and demonstrated sublethal toxicity to other aquatic species (e.g., *Lemna minor*).

### Benthic Invertebrates

A simple gradient design was initially used for the Phase 1 benthic invertebrate community study with samples collected in the marsh near the mouth of the Peg Lake outflow (conductivity 19,000  $\mu\text{S}/\text{cm}$ ) along a gradient through Jackfish Bay into the main body of Great Slave Lake (conductivity 250  $\mu\text{S}/\text{cm}$ ). Analysis of the gradient data collected in Phase 1 in 2004 did not detect changes in community structure. In Phase 2 monitoring, a control/impact design was conducted at Jackfish Bay (exposure) and Kam Bay (reference) to investigate benthic invertebrate community effluent effects. The data were summarized for four EEM effect endpoints (total invertebrate density, family level richness, Simpson's Evenness Index and Bray Curtis index) and tested for statistically significant differences. Simpson's Diversity Index (SDI), taxa presence/absence and community composition were also included as supporting information. All four effect endpoints were significantly different between the areas; the majority of the endpoints exceeded the critical effect sizes and were considered ecologically significant. Effluent and/or historical sediment contamination were identified as probably (based on correlation) responsible for the observed effects on the benthic community.

### Fish Study

The Phase 1 fish study consisted of a community survey of small-bodied fish species and a population study of Ninespine Stickleback (*Pungitius pungitius*). Fish from Jackfish Bay (exposure) and Horseshoe Bay (reference) were examined and measured for the standard parameters required under the EEM program, as well as additional supporting information including liver pathology, visceral tissue arsenic (composite sample) and gonad histology. The results showed liver weight and gonad weight of male fish were significantly greater in the exposure area. In Phase 2, a lethal fish health study was performed with Ninespine Stickleback at Jackfish and Horseshoe Bay. In addition to the standard EEM parameters, fish were examined for liver pathology and visceral (composite) and whole body arsenic concentrations, as well as gonad histopathology. Fish tissue mercury and arsenic were also analyzed in Northern Pike (*Esox lucius*) during the Phase 2 program. Increased liver and gonad weight in male Ninespine Stickleback in the exposure area was confirmed in the Phase 2 EEM fish survey. There were no significant differences in mean muscle mercury concentrations between exposure and reference areas in Northern Pike. Arsenic concentrations were elevated in the reference relative to the exposure area; however, all tissues were below the Health Canada arsenic guideline for human consumption (0.35 mg/kg ww). Due to the confirmation of effects (i.e., elevated liver and gonad weights in male fish) between the Phase 1 and Phase 2 programs, Miramar Northern Mining Ltd. was required to conduct an IOC within 24 months of the date of submission of the Phase 2 report.

### IOC Study Design

The question that directed the 2009 IOC study design was simple: what causes the observed response pattern in fish from Jackfish Bay and Horseshoe Bay? Four hypotheses were put forward to answer this question.

Hypothesis 1: The response pattern observed in the fish is due to sediment differences in the receiving environment.

Hypothesis 2: The response pattern observed in the fish is due to nutrients and ions in the effluent (eutrophication and/or conductivity response).

Hypothesis 3: The response pattern observed in the fish is due to contaminants in the effluent (contaminant response).

Hypothesis 4: The response pattern observed in the fish is due to habitat differences in the exposure area compared to the reference area.

The approved 2009 IOC study design at Con Mine focused on a fish program which included both the standard EEM program parameters (fork length, body weight, age, gonad weight, liver weight) and additional endpoints targeting liver pathology: liver arsenic, liver lipid analyses (triglyceride (TG) and glycogen) and liver, gonad and whole body histopathology. Liver histopathology included standard light microscopy (LM), as well as transmission electron microscopy (TEM). Additional data were collected for water quality and water temperature.

Preliminary results indicate fish were, on average, two years old at Jackfish Bay and two to three years old at the reference site. Liver analyses are ongoing, but preliminary analyses indicate triglyceride (TG) concentrations are lower at Jackfish Bay, while arsenic concentrations are higher. Preliminary histopathology of liver tissues suggested there may be alterations in the appearance of cell nuclei in fish from Jackfish Bay.

#### Challenges for Designing the IOC

The greatest challenge in designing an IOC at Con Mine in 2009 was the absence of effluent. The mine has capacity to hold surface run-off for a number of years prior to treatment and release; therefore during the summer of 2009 the mine did not discharge effluent while initiating the installation of a new water treatment facility. Designing an effective IOC program to determine the toxicity of an effluent in a year without an effluent presented real challenges. For instance, the study design could not follow the traditional Eutrophication Effect or Toxic Effect line of reasoning used in Pulp and Paper Mill Effluent (PPME) EEM programs because both conditions, excessive nutrients and metals, exist at the Con Mine location limiting the usefulness of this standard approach.

A number of additional challenges to developing an effective IOC at Con Mine are worth noting as at least some will be applicable to other mine operations:

A lack of high-quality, commercially available, specialist laboratory sub-contractors in Canada. For example, it was particularly challenging to find a liver histopathology expert with experience in metal and nutrient toxicity who could perform the services required of the IOC Con Mine design. In the end, the sub-contract was established with an academic laboratory in the United States. The logistics of finding and developing such relationships have the potential to introduce time delays and challenges to getting a study design approved and completed in 24 months. A significant elevation in cost should also be expected in association with acquiring specialist analyses.

The northern environment presents a substantial challenge to designing and completing an effective IOC program, especially for mines not in proximity to urban centres. It was challenging to acquire and maintain the integrity of perishables goods required for the IOC program (e.g., buffers and preservatives) and the conditions to hold samples (e.g., -80°C freezer) were not available. Significant additional cost was incurred to ensure the integrity of samples on dry ice (delivered daily) until shipping to sub-contracts for analyses.

The use of small bodied fish presents both a pro and a con in the context of designing an IOC. Their tendency to occupy small home ranges with high site fidelity provides confidence in the relevance of the data. The inherent challenge of using small bodied fish, however, is the necessity of combining individual samples to acquire sufficient sample sizes for analyses. Multiple analyses on the same individual are not often possible.

The late spring in the northern hemisphere presents a challenge to sampling fish species for biologically relevant endpoints. The most useful information would be collected at the time of spawning (i.e., spring); however, fish are inaccessible at this time as ice is present until early summer.

#### Acknowledgments

The helpful, responsive and relevant feedback of the Technical Advisory Panel (TAP) was valuable

during the 2009 IOC Program at Con Mine. The challenge of completing the IOC program was met largely due to the competence, commitment and experience of the field crew, the senior project management team and the management team of Miramar Northern Mining Ltd. and Newmont Mining Corporation. The success of the program was greatly benefitted by the active participation of all members of the team, including Miramar Northern Mining Ltd., Environment Canada, and the various sub-contractors.

### **Questions/Discussion:**

Q: One of the hypotheses mentions a link between habitat differences and toxicity test results as part of the study design. The toxicity tests were performed with water samples from the exposure and reference area. Can you elaborate on that? (The hypothesis presented was: The response pattern observed in fish is due to habitat differences in exposure area compared to reference area. Differences noted include: acutely lethal to Rainbow Trout and *Daphnia Magna*; sublethal toxicity to *Lemna minor*. Fish study results note increase in male liver and gonad weights, increase in viscera arsenic.)

A: The exposure water should be checked for whether or not it is toxic, as the result would change the interpretation of the data.

Q: How does young of the year growth and energy use link to sediment quality?

A: Although it links somewhat to historical data, the reasoning is that the sediment holds whatever is in the environment, and so growth and energy use should be linked with what is (available) in the environment.

## **Session 4: Tools and Approaches for Investigation of Cause Studies**

### **An update on the use of caged fish for Environmental Effects Monitoring**

Bandler, C.<sup>1</sup>, Baron, C.L.<sup>2</sup> and Palace, V.P.<sup>1,2</sup>

<sup>1</sup> Queen's University, Dept. Biology, Kingston ON K7L 3N6

<sup>2</sup> Fisheries and Oceans Canada, Freshwater Institute, Winnipeg MB, R3T 2N6

#### **Introduction**

Establishing exposure to effluents is a long standing issue for both metal mining and pulp and paper EEM programs. Situations with confounding influences due to multiple contaminant input sources have also been challenging. While alternative means for assessing potential impacts of effluents have been explored, caging has not been adopted because of concerns regarding the interactive effects of stress in captive fish (Courtenay *et al.* 2002). We had previously suggested that small-bodied fish with potentially smaller home ranges, may be less susceptible to stress and more appropriate for caging studies. Standardized methodologies were also outlined (Palace *et al.* 2005). At a workshop held in Fredericton, New Brunswick in 2004, the EEM science committee recommended that physiological stress be systematically evaluated in caged fish before the technique could be considered as a valid alternative to wild fish surveys for EEM programs. Demonstration of unimpeded growth in confined juvenile fish was suggested as an adequate measure. Therefore, two separate field studies conducted in 2004 and 2005 evaluated growth, condition, gonad development and diet in two small bodied fish species, pearl dace (*Margariscus margarita*) and finescale dace (*Phoxinus neogaeus*) caged in a freshwater system for short durations.

#### **Materials and Methods**

##### **a) Experiment 1**

In May 2004 juvenile pearl dace were captured from a freshwater reference system using hoop nets and baited minnow traps. After capture, fish were anesthetized, measured, weighed and randomly distributed among eight cages as previously described (Palace *et al.* 2005). Four of the cages were deployed at the reference site while the remaining four cages were deployed at a downstream site exposed to final effluent from active gold mining operations (Doebel 2006). Cages were deployed in contact with the sediment so that the top of each cage was approximately 0.5 m from the surface of the water. An additional 20 fish were immediately anesthetized, weighed and frozen on dry ice. Two weeks after the initial cage deployment, another eight cages were deployed using the same methods. Therefore, a total of 16 cages were deployed at the two sites (eight exposure, eight reference). Similarly, another 20 resident juveniles were obtained, weighed, measured and frozen as a measure of the condition of the source population at the beginning of the second caging period. Four weeks after deployment of the first set of cages, fish from all 16 cages were retrieved, anesthetized, weighed, measured and frozen. Thus, four cages at each site were deployed for two weeks and four cages at each site were deployed for four weeks. Twenty resident juvenile fish were again obtained at the termination of the caging period.

##### **b) Experiment 2**

Because pearl dace were not abundant at the time of Experiment 2, juvenile finescale dace were substituted and caged at the reference and exposure sites during the spring of 2005. All methods

were identical to experiment 1 except i) fish were held in cages for four weeks only ii) Two cages per treatment were deployed instead of four and iii) treatments consisted of two caging densities (five fish/cage or ten fish/cage) deployed at the reference and exposure sites, for a total of four treatments. Only two groups of resident fish were collected for this experiment (i.e. once when the cages were deployed and again when the cages were removed).

### c) Endpoints

Condition factor (K) was calculated as  $K = (\text{whole body weight}/(\text{length}^3)) \times 100$ . Percent weight change was calculated by using initial and final weights. Diet evaluations were conducted for experiment 1 only. Gut contents were analyzed for 12 resident pearl dace collected at the reference site at the beginning and end of the caging periods (36 fish total) as well as from 3 fish from each of the cages. For this analysis, the viscera were dissected and stored in 3 ml of 70% ethanol before weighing and enumeration to genus by an independent commercial laboratory (Doebel 2006).

### Results and Discussion

Relative to their initial weights at capture, fish lost weight at both sites and caging durations (Figure 1). Generally, losses of weight and condition were greater at the reference site than at the exposure site. There were no differences in K in fish caged at the exposed site between two and four weeks indicating that K appeared to stabilize after two weeks at this site.

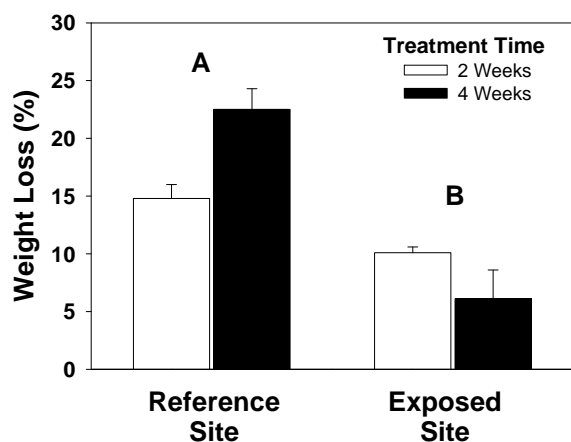


Figure 1: Mean  $\pm$  SEM percent weight loss in juvenile pearl dace caged at reference or mine effluent exposed locations for two or four weeks.

Lower condition and growth among caged fish did not appear to be a result of food limitation. In fact, weight of gut contents was higher in resident fish compared to both caging groups for the two week treatment, and the opposite was true for the four week treatment (Figure 2).

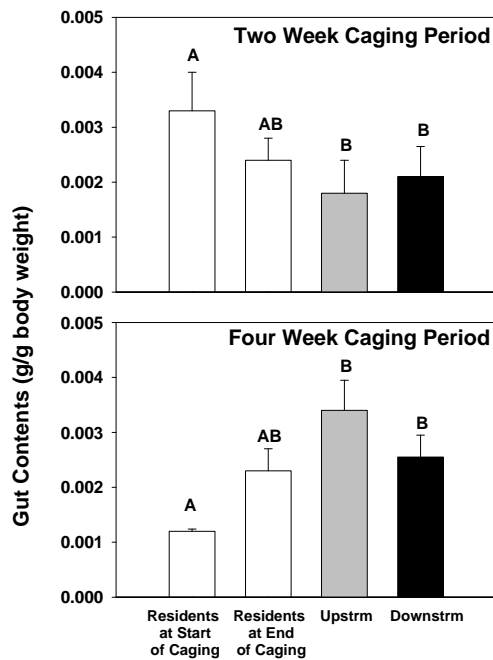


Figure 2: Mean  $\pm$  SEM weight of gut contents (in grams) per g of bodyweight in juvenile pearl dace captured from a reference site or caged at either a reference site or mine effluent exposed site for two or four weeks.

However, fish confined to cages may have had access to lower food quality than free ranging fish. Chironomid larvae were most abundantly identified in guts of resident fish and fish caged at the upstream site, but there was a higher proportion of Arachnida at the exposed site (data not shown). Whether this can account for the stabilization of K after two weeks in fish caged at the exposed site remains to be investigated.

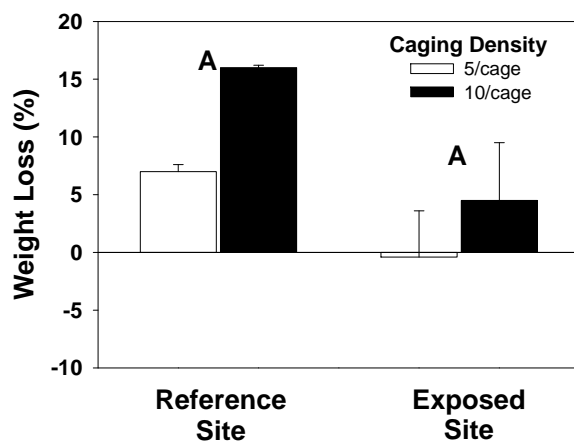


Figure 3: Mean  $\pm$  SEM percent weight loss in juvenile pearl dace caged at reference or mine effluent exposed locations for four weeks at densities of either five or ten per cage.

Similar to the results from Experiment 1, all caged fish lost weight in Experiment 2 (Figure 3). While it appears that the effect was more pronounced when there were ten fish per cage than when there were only five, there was no significant difference between the two caging durations. Future studies should clearly define potential dietary differences at caging sites over the duration of caging and also optimize densities to ensure adequate access to preferred prey items.

### References

Courtenay, S.C., Munkittrick, K.R., Dupus, H.M.C., Parker, R., Boyd, J. 2002. Quantifying impacts of pulp mill effluent on fish in Canadian marine and estuarine environments.: problems and progress. Water. Qual. Res. J. Can. 37:79-99.

Doebel, C. 2006. The Utility of the Caged Fish Method in the Canadian Metal Mining Environmental Effects Monitoring Program. Master's Thesis, Queen's University, Department of Biology. 97pp.

Palace VP, C Doebel, CL Baron, RE Evans, KG Wauiter, JF Klaverkamp, J Werner, S Kollar. 2005. Caging small bodied fish as an alternative method for environmental effects monitoring (EEM). Can. Wat. Qual. Res. J. 40:328-333.

### **Questions/Discussion:**

Q: Is there a reason why the lake chub had a greater survival and condition compared to the spottail shiner?

A: Larger fish retain confinement stress for a longer time.

### ***In situ* measurements of toxicity and contaminant bioaccumulation with caged amphipods exposed to water and sediment for Investigation of Cause in metal mining Environmental Effects Monitoring studies**

Grapentine, L.C.

Water Science and Technology Directorate, Environment Canada, 867 Lakeshore Rd., Burlington, ON, L7R 4A6.

### Introduction

#### ***Benthic assessments in EEM***

Assessments of ecological effects of industrial discharges on the receiving environment typically include measurements of water or sediment toxicity in laboratory tests, surveys of resident benthic invertebrate communities, and determinations of the health of individuals or populations of selected fishes and wildlife. While the endpoints for these methods allow a comprehensive characterization of responses to contaminants and other stressors, limitations can remain in linking specific stressors to responses under environmentally realistic exposure scenarios. For example, a component of Canada's national environmental effects monitoring (EEM) program for the metal mining industry is an assessment of conditions of benthic invertebrate communities in areas exposed to effluents from mining operations. Analyses of results from the first phase of assessments from across the nation

indicate a prevalence of inhibitory effects; i.e., reduced overall density of invertebrates, reduced taxon richness, and changes in community structure compared to conditions in reference areas or sites exposed to lower effluent concentrations (Lowell *et al.* 2007). Potential causes of these effects are not necessarily clear, but could include direct toxicity from effluents, habitat alteration, and indirect effects from impacts to food supply or predators.

Guidelines for the EEM program recommend that when the magnitude and geographic extent of an effect is known but the cause has not been determined, an Investigation of Cause (IOC) study should be conducted. Regarding the benthic community assessment, key questions to be addressed include:

- Identifying stressors that account for effects (e.g., metals via direct toxicity, other effluent contaminants, habitat alteration, and/or indirect effects), and
- Determining the important exposure and uptake pathways (i.e., water/sediment/diet) if contaminants account for benthic effects.

#### Linking stressors with biological effects

Evidence for understanding the causes of benthic disturbance is provided by the main EEM assessment components. The site and effluent characterizations and water and sediment quality monitoring can describe physicochemical disturbances to the receiving environment and identify potential stressors on biota in the field. Effluent toxicity testing measures biological responses to effluent exposure and, with effluent chemistry data, can further identify potential stressors on biota in the receiving environment. From the benthic community survey, a spatial correlation between biological effects and level of effluent exposure under receiving environment conditions can be determined. Alternative monitoring, such as mesocosm and caged bivalve studies, allows measurements for effects of effluent on biota under better control of exposure level and confounding factors.

In some situations though, the collective evidence from these studies may not be sufficient to establish a causal stressor – effect relationship in the receiving environment when it actually exists. This could be for several reasons, including:

- not detecting adverse effects in the receiving environment, due to a lack of suitable reference sites or high variability in the biological response to the stressor,
- the stressor in question being confounded with natural (habitat) factors or other stressors (as, e.g., effluents with multiple contaminants), and
- difficulties extrapolating laboratory results to the field because of differences in contaminant bioavailability or differences in responses of lab organisms and resident field organisms.

Field observation, as in the benthic community survey, is the most direct method of determining and understanding *in situ* effects, but it is not readily amenable to controlled experimentation, which provides more definitive proof of cause. On the other hand, controlled laboratory conditions may not sufficiently mimic *in situ* conditions to allow extrapolation of results to the receiving environment.

### In situ assessments

In situ effects assessment combines the environmental realism of field observations (re contaminant speciation and site-specific cofactor conditions) with the experimental control of laboratory studies (re spatial position and replication, stressor exposure level, and cofactor variability). Information from *in situ* assessments of effects of effluent exposure can provide the additional evidence required to determine the cause of adverse effects in EEM studies.

While caged bivalve studies are often valuable for *in situ* assessments of effluent effects in the receiving environment, the deployment of caged amphipods – specifically *Hyaella azteca* – is an approach that offers additional advantages for linking metal ecotoxicity with benthic community impacts. *Hyaella* is a highly studied metal toxicity test organism and a frequent member of resident benthic communities. It is robust to experimental manipulation. The caged amphipod methodology is well-developed, not technically difficult, addresses multiple contaminant exposure pathways, amenable to high experimental replication, incorporates multiple biological endpoints, and low to moderate in cost.

### Methodology

#### Applications of caged *Hyaella* procedures for metal ecotoxicology

The utility of *in situ* deployment of caged *Hyaella* for metal ecotoxicological studies has been established in studies involving various habitats and sources of metals, including:

- a northwestern Ontario lake used for a Cd addition experiment (Stephenson and Turner 1993);
- southern Ontario urban streams and ponds exposed to wet-weather runoff (Grapentine *et al.* 2004);
- Clark Fork River, Montana, exposed to Ag-Cu-Zn mine effluent (Burton *et al.* 2005);
- northern Saskatchewan lakes exposed to effluent from uranium operations (Robertson and Liber 2007); and
- northwestern Québec streams exposed to Cu-Zn metal mine effluent (Borgmann *et al.* 2007; Couillard *et al.* 2008).

The papers describing these studies document procedures for assessing toxicity and metal bioaccumulation for *Hyaella* exposed to overlying water, sediment, porewater and/or specific food supplies. While some differences exist in the experimental materials, design and procedures, all the studies involve placing groups of amphipods held in screened plastic cages in preselected locations in the receiving environment followed by recovery and analyses for survivorship, growth and/or concentrations of metals in tissues. Outlined below is a methodology initially described by Grapentine and Rosenberg (1992) and most recently applied in Borgmann *et al.* (2007) and Couillard *et al.* (2008).

### Overview of procedures

#### Test organism

*Hyaella azteca* is distributed across North America in streams, ponds and lakes (mainly littoral zone) and is a standard water and sediment toxicity test organism. *Hyaella* for *in situ* cage studies can therefore be obtained from either laboratory cultures or natural populations. The amphipod is

tolerant of laboratory conditions and handling, and adaptable to a range of environmental conditions. There is a large amount of data available on responses of *Hyaella* to metals and other environmental conditions to support interpretation of experimental results. Studies (e.g., Borgmann *et al.* 2004b) show that *Hyaella* is an effective monitor of metal bioavailability.

#### Cage structure

Cages are easy construct and can be designed for exposure to the water column, sediment and water column, or pore water. A cage for water column assessment (7.6 cm diameter × 7.6 cm length) is made of two sections of clear acrylic tube fitted securely together and sealed at each end by 500-μm (or smaller) mesh nylon screen (Fig. 1). Rubber bands wrapped around fasts and guides hold the cages closed. *In situ* positions are maintained by attachment to substrate anchors or floats.

#### Experimental design

Cages are deployable over most substrates. Assessment locations can therefore be preselected for exposure to stressors at levels and durations required by the study design. Low production cost and small size of the cages allow high sampling replication for quantifying spatial and temporal variability. Individual cages can be deployed and retrieved at the end of the exposure period, or revisited multiple times during the experiment to observe amphipod survival through time. Growth and tissue metal concentrations can also be measured through time for sites at which multiple cages are deployed. Several of these cages can be retrieved and the amphipods analyzed throughout the study period.

#### Set-up and deployment

Each cage can hold at least 15 amphipods without showing significant mortality after deployment. Individual juveniles or adults (four to ten weeks old) are randomly selected and allocated to cages which have been pre-assigned to the experimental treatments. This ensures similar size distributions for the amphipod groups and unbiased application of experimental treatments. Each cage also contains a 2.5 × 2.5-cm square of cotton gauze for substrate. If food quality is not an experimental factor, depending on deployment time, food used for culturing or natural organic material can be added at the start and during the experiment. Amphipods placed in cages can be held in buckets of culture water for several hours or days before deployment at field sites without mortality from handling and transportation. During initial deployment, exposure to site water should be gradual to allow adjustment to any differences in temperature or water quality. After immersion any air bubbles within the cages should be removed with a pipette. Deployment can be at least as long as eight weeks, which exceeds the generation time of *Hyaella*, but can be much shorter depending on experimental objectives. For example, at ~23°C concentrations of some metals in *Hyaella* reach steady state after seven days (Borgmann *et al.* 2004a). Longer response times to site conditions would be expected in cooler waters.

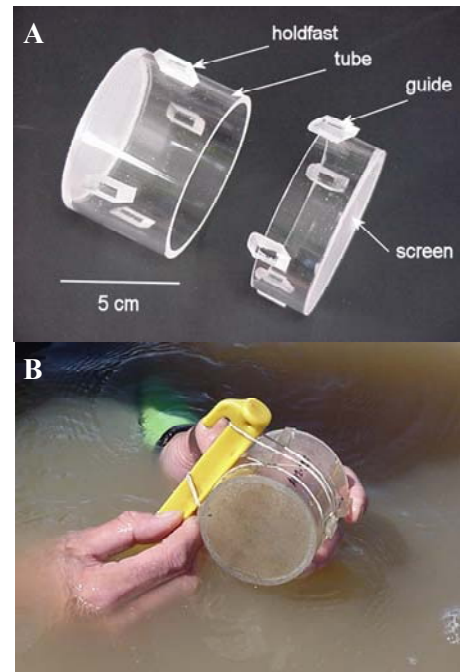


Figure 1. Cage for exposing amphipods to water column conditions: A. Structure. B. Attached to plastic tent peg for anchoring on substrate.

### Toxicity endpoints and data analyses

As in laboratory toxicity tests, the number of survivors for the group and mean individual growth over the exposure period can be determined after counting, drying and weighing the recovered amphipods (Fig. 2). If the mesh size of the cage screen is sufficiently small, reproduction could also be estimated. If the amphipods are allowed to depurate before drying, whole-body concentrations for a series of elements can also be obtained (e.g., Borgmann *et al.* 2007).

Data for these endpoints can be analysed to determine effects of location or stressor level on amphipod health and populations. Analyses of metal bioaccumulation data can also indicate whether particular metals are (a) bioavailable and (b) at concentrations in tissues associated with adverse effects for either *Hyaletella* or species that feed on the amphipods.

### Prediction of Toxicity from Metal Bioaccumulation

Laboratory studies by Borgmann *et al.* (2004a) and Norwood *et al.* (2007) determined critical body concentrations for a series of metals and metalloids (including As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Tl and Zn) for *Hyaletella*, above which lethal toxicity is likely to occur. These values appear applicable to field environments. *In situ* deployments of caged *Hyaletella* by Couillard *et al.* (2008) showed that bioaccumulation of most of these metals in metal-contaminated streams is similar to bioaccumulation in laboratory tests, and that dissolved metals were accumulated in a dose-dependent manner.

The critical body concentrations, together with metal bioaccumulation measurements for *Hyaletella* exposed to sediment from mining areas, were used to identify Ni and Cd as the metal most responsible for both amphipod toxicity and benthic community impairment in Sudbury area lakes (Borgmann *et al.* 2001) and Rouyn-Noranda area lakes (Borgmann *et al.* 2004b), respectively. Bioaccumulation data, derived from laboratory sediment tests or *in situ* caged amphipod studies are therefore potentially useful for identifying causes of benthic effects in metal-mining areas.

### Relevance for IOC Studies

*In situ* assessments of toxicity and metal bioaccumulation with caged *Hyaletella* provide ecologically relevant information for evaluating causes of benthic effects in EEM studies. Experiments can be designed to (a) test for direct effects of effluents or contaminated sediment, (b) link particular metals to toxicity and benthic impairment, (c) identify important contaminant exposure and uptake pathways, (d) quantify stressor-response relationships, and (e) examine effects of natural factors and other anthropogenic stressors. Although the studies cited here involve only *Hyaletella*, it may also be possible to use other amphipod species in estuarine and marine environments.

### Conclusions

The placement of groups of the freshwater amphipod *Hyaletella* held in screened plastic cages in preselected locations in the receiving environment is an established methodology for *in situ*



Figure 2. Recovery of amphipods from cages. A. Sorting amphipods from natural food supply and substrate. B. Surviving *Hyaletella*.

assessments of biological responses to environmental conditions. The methodology is applicable for monitoring effects of effluents from metal mining operations and for Investigation of Cause studies. Measurements of survival and growth indicate lethal and sublethal responses to stressors under site-specific conditions. Patterns of bioaccumulation can indicate not only metal bioavailability but also the most probable cause of metal-related toxicity. The use of caged amphipods in an EEM program would provide evidence on the cause of effects complementary to the standard components as well as offering an alternative means of assessing effects in problematic receiving environments.

## References

Borgmann, U., M. Nowierski, L.C. Grapentine and D.G. Dixon. 2004a. Assessing the cause of impacts on benthic organisms near Rouyn-Noranda, Quebec. *Environmental Pollution* 129:39-48.

Borgmann, U., W.P. Norwood and D.G. Dixon. 2004b. Re-evaluation of metal bioaccumulation and chronic toxicity in *Hyaella azteca* using saturation curves and the biotic ligand model. *Environmental Pollution* 131: 469-84.

Borgmann, U., W.P. Norwood, T.B. Reynoldson and F. Rosa. 2001. Identifying cause in sediment assessments: bioavailability and the Sediment Quality Triad. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 950-960.

Borgmann, U., Y. Couillard and L. C. Grapentine. 2007. Relative contribution of food and water to 27 metals and metalloids accumulated by caged *Hyaella azteca* in two rivers affected by metal mining. *Environmental Pollution* 145: 753-765.

Burton, G. A., Jr., M.S. Greenberg, C.D. Rowland, C.A. Irvine, D.R. Lavoie, J.A. Brooker, L. Moore, D.F.N. Raymer and R.A. McWilliam. 2005. In situ exposures using caged organisms: A multi-compartment approach to detect aquatic toxicity and bioaccumulation. *Environmental Pollution* 134: 133-144.

Couillard, Y., L.C. Grapentine, U. Borgmann, P. Doyle and S. Masson. 2008. The amphipod *Hyaella azteca* as a biomonitor in field deployment studies for metal mining. *Environmental Pollution* 156: 1314-1324.

Grapentine, L., Q. Rochfort and J. Marsalek. 2004. Benthic responses to wet-weather discharges in urban streams in southern Ontario. *Water Quality Research Journal of Canada* 39: 374-391.

Grapentine, L.C., and D.M. Rosenberg. 1992. Responses of the freshwater amphipod *Hyaella azteca* to environmental acidification. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 52-64.

Lowell, R.B., C. Tessier, S.L. Walker, A. Willsie, M. Bowerman and D. Gautron. 2007. National Assessment of Phase 1 Data from the Metal Mining Environmental Effects Monitoring Program. National EEM Office, Environment Canada, Ottawa, ON. December 2007.

Norwood, W. P., U. Borgmann and D. G. Dixon. 2007. Chronic toxicity of arsenic, cobalt, chromium and manganese to *Hyaella azteca* in relation to exposure and bioaccumulation. *Environmental Pollution* 147: 262-72.

Robertson, E.L., and K. Liber. 2007. Bioassays with caged *Hyalella azteca* to determine in situ toxicity downstream of two Saskatchewan, Canada, uranium operations. *Environmental Toxicology and Chemistry* 26: 2345-2355.

Stephenson, M., and M.A. Turner. 1993. A field study of cadmium dynamics in periphyton and in *Hyalella azteca* (Crustacea: Amphipoda). *Water Air and Soil Pollution* 68: 341-361.

### **Questions/Discussion:**

Q: How sensitive are amphipods to metals in the mining environment compared to other organisms/invertebrates?

A: They are somewhat sensitive, but not the most sensitive. They are probably within the fifth percentile for organic and inorganic [metals], and very sensitive to cadmium. If you collect these organisms in the field (sometimes this is possible), you can see if the metals are accumulating.

Comment: There was an open suggestion open to all presenters to provide references (i.e. citations, published reports, or data).

[Editor's note: The author subsequently indicated that none were provided by the audience.]

### **Experimental Designs Using Artificial Streams (Mesocosms) to Address Cause for Metal Mine Effluents**

Dubé<sup>1</sup>, MG, Driessnack M<sup>2</sup>, Rozon-Ramilo L<sup>2</sup>, Ouellet J<sup>3</sup>, Niyogi, S<sup>3</sup>, Rickwood C<sup>2</sup>.

<sup>1</sup> Canada Research Chair, Aquatic Ecosystem Health Diagnosis, School of Environment and Sustainability, University of Saskatchewan Saskatoon, SK

<sup>2</sup> Toxicology Centre, University of Saskatchewan, Saskatoon, SK

<sup>3</sup> Biology Department, University of Saskatchewan, Saskatoon, SK

#### Introduction

Over the past decade, in-field artificial stream systems have been used to test specific hypotheses about the effects of complex mixtures on different trophic levels in aquatic ecosystems. Their utility lies in the flexibility of different experimental designs and endpoints that can be measured to test specific hypotheses in a controlled and replicated manner and with increased environmental realism over highly controlled laboratory experiments. Initial investigations commenced using pulp and paper mill effluents to assess effects on fish (Dubé *et al.*, 2002; Pollock *et al.*, 2010). These investigations then expanded into Investigation of Cause studies (using different mill waste streams relative to final effluent exposures) to isolate causative streams affecting reproductive endocrine responses in different small bodied fish species (Rickwood et Dubé, 2007; Rickwood *et al.*, 2006a, 2006b; Dubé et MacLatchy, 2001).

Since 2000, application of mobile artificial stream systems expanded into the exposure assessment of metal mine effluents. While mining is similar to pulp and paper with respect to the diversity of mine

types, varied ore composition, and different receiving environments for treated effluent discharge, mining significantly differs from pulp and paper with respect to the spatial extent of operations. This of course affects the perspective and approach for Investigation of Cause studies. Applications of artificial stream systems to the mining sector have included experimental designs to examine altered discharge volumes (Dubé *et al.*, 2005), to tease apart effluent versus historical sediment contamination (Driessnack *et al.*, 2010), and the consequences of exposure through waterborne and foodborne pathways (Dubé *et al.*, 2006; Rickwood *et al.*, 2006c, 2008; Rozon-Ramilo *et al.*, 2010). Most recently experimental designs have examined the effects of single metal concentrations at effluent-equivalent doses to full strength effluent to assess potential causative metals (Dubé *et al.*, 2010). All studies have been conducted using small bodied fish species including slimy sculpin (*Cottus cognatus*) and fathead minnow (*Pimephales promelas*). In the case of fathead minnow, a partial lifecycle reproductive bioassay has been used. Studies involving full reproductive lifecycles of invertebrates have also been conducted using metal mine effluent in field-based exposure designs (Hruska et Dubé, 2004, 2005). Examining effects of metals and mine effluents on fish and invertebrate reproduction is critical, due to the propensity of some metals to be transferred through the parents to the offspring. Examination in a controlled experiment in the field provides for the opportunity to assess effects on adults, eggs, and larvae. In addition, due to the importance of diet in metal transformation and assimilation into higher trophic levels, artificial streams methodology was modified from exclusively waterborne exposures to fully sustaining multi-trophic studies (Rickwood *et al.*, 2006c, 2008; Rozon-Ramilo *et al.*, 2010; Dubé *et al.*, 2010). In these designs, a food base of *Chironomus dilutus* (previously named *Chironomus tentans*) is established in each stream and allowed to culture under exposure conditions to known densities. A breeding group of fathead minnow are then added to the streams and exposed to effluents for at least 21 days with no provision of an external food source.

The objective of this extended abstract is to outline some key changes in artificial stream methodology developed in the past decade specifically for metal mine effluent assessments and to identify key results and citations for reference. All studies were funded through partnership models with industry wherein academic researchers were able to leverage funds for matching through national funding bodies (such as NSERC Collaborative Research and Development Grants). Funding through the Canadian Foundation for Innovation and the Canada Research Chairs Program also supported this research including development of novel testing equipment. The research met the industry's needs for their Environmental Effects Monitoring (EEM) requirements under the *Fisheries Act* and in turn, the methodology and results were developed much more intensely than would normally be required for an EEM program in the interest of developing better tools and approaches for the industry, regulators and the science in general.

### Key Results

Artificial stream studies have illustrated that exposure to treated metal mine effluents has the potential to reduce gonad sizes in fish, alter reproductive hormone levels, and increase metal body burdens (Dubé *et al.*, 2006). Studies with invertebrates have also shown reproductive effects including decreased emergence, hatching success, survival, and increased metal burdens in body tissues (Dubé *et al.*, 2010; Hruska et Dubé, 2005). Effects of mine effluent on fish reproduction are affected by the route of effluent exposure (Driessnack *et al.*, 2010; Rickwood *et al.*, 2006c, 2008; Rozon-Ramilo *et al.*, 2010). In addition, the role of diet cannot be ignored in studies to examine effects either in the field or in the laboratory. As knowledge of metal speciation and

biotransformation increases, the importance of the food web and understanding how assimilation of metals at lower trophic levels transfers to higher trophic levels becomes increasingly important. It was this understanding that led my group to develop the multi-trophic test system. Further, the role of successful reproduction to population sustainability is well understood. Thus, development of a multi-trophic test that could assess metal mine effluent effects on multiple life stages of fish and invertebrates was critical. This was the basis for development of the invertebrate life cycle test in in-field artificial streams as well as the multi-trophic partial fathead minnow reproductive bioassay.

The work of Rickwood *et al.* (2006c) showed that greater numbers of eggs (more than 2 times) were produced by breeding fathead minnow in multi-trophic mesocosm exposures compared to waterborne exposures. In the laboratory, exposure to an environmentally-relevant effluent dilution reduced egg production by four to four and a half fold. In the field with the same effluent and same mesocosm system, Rickwood *et al.* (2008) reported even greater numbers of eggs in the multi-trophic control streams. The effects of effluent through waterborne exposure were the same as in the lab study. However, when exposed in the field through a multi-trophic system, the effects of the effluent on egg production were not observed as they had been in the lab study. These studies illustrated consistency in fathead minnow reproductive response and also illustrated the need for diet to be critical in the method for application of controlled test procedures including mesocosm studies. Further studies by Rozon-Ramilo *et al.* (2010) at this site using multi-trophic systems showed the significantly different effects that different mine effluents (all discharging to the same receiver) can have on fish reproduction. This study was important to separate and prioritize different effluents with respect to biological responses. The work of Driessnack *et al.* (2010) also illustrated the importance of diet for assessing effects of a uranium mine effluent. What was critical about this research is that it illustrated that fathead minnow egg production was affected differently in multi-trophic systems compared to when fish were fed a known amount of contaminant-raised *C. dilutus*. This again, illustrates the need for controlled methodologies to include a food web for investigation of mine effluent effects on fish. Most recently, Investigation of Cause studies using mesocosms have moved to comparatively evaluate effects of single metals at effluent-equivalent doses versus the complex mixture (Dubé *et al.*, 2010). So far, studies have focused on selenium, although further work is underway to examine metals such as copper and nickel. These causal studies have shown that while effects characteristic of selenium exposure did exist in the test fish, they did not match the effects observed after exposure to the final effluent.

### Conclusions

Controlled, manipulated, hypothesis-driven studies are essential to better understand cause. The more relevant the exposure protocols to the actual environment, the more useful the information. Our work has shown that studies in the laboratory, even under “constructed” dilution water to match field conditions, produce significantly different results than studies in the field using the same artificial stream systems. Furthermore, studies using an established food web generate different results than using waterborne exposures alone, and also generate different results compared to simply feeding fish the same diet (*C. dilutus*) raised under effluent-exposed conditions. Artificial stream systems have provided a significant opportunity for testing different hypotheses and for development of an approach that specifically addresses issues of concern for causal investigations in the metal mining sector (e.g., maternal transfer of metals, dietary exposure routes). We conclude that studies in the field are essential in multi-trophic experimental designs and using test species that allow for direct measurement of reproductive effects.

## References

- Driessnack, M.K., M.G. Dubé, L.D. Rozon-Ramilo, P.D. Jones, C.I.E. Wiramanaden et I.J. Pickering. 2010. The use of field-based mesocosm systems to assess the effects of uranium milling effluent on fathead minnow (*Pimephales promelas*). *Ecotoxicology*. In press.
- Dubé, M.G., A.J. Harwood, C.I.E. Wiramanaden and I.J. Pickering. 2010. Effects of treated uranium mine effluent and selenium on fathead minnow (*Pimephales promelas*) reproduction in self-sustaining multi-trophic artificial streams. *Aquatic Toxicology*. Submitted in March 2010.
- Dubé, M.G., D.L. MacLatchy, J.D. Kieffer, N.E. Glozier, J.M. Culp and K.J. Cash. 2005. Effects of metal mining effluent on Atlantic salmon (*Salmo salar*) and slimy sculpin (*Cottus cognatus*): Using mesocosms to assess existing effects and predict future consequences. *Science of the Total Environment*, 343:135-154.
- Dubé, M.G., D.L. MacLatchy, K.A. Hruska and N.E. Glozier. 2006. Assessing the responses of creek chub (*Semotilus atromaculatus*) and pearl dace (*Semotilus margarita*) to metal mine effluents using in situ artificial streams in Sudbury, Ontario, Canada. *Environmental Toxicology and Chemistry*, 25 (1):18-28.
- Dubé, M.G., and D.L. MacLatchy. 2001. Identification and treatment of a waste stream at a bleached kraft pulp mill that depresses a sex steroid in the mummichog (*Fundulus heteroclitus*). *Environmental Toxicology and Chemistry*, 20:985-995.
- Dubé, M.G., J.M. Culp, K.J. Cash, N.E. Glozier, D.L. MacLatchy, C.L. Podemski and R.B. Lowell. 2002. Artificial streams for Environmental Effects Monitoring (EEM): Development and application in Canada over the past decade (invited). *Water Quality Research Journal of Canada*, 37:155-180.
- Hruska, K., and M. Dubé. 2005. Comparison of a partial life cycle bioassay in artificial streams to a standard beaker bioassay to assess effects of metal mine effluent on *Chironomus tentans*. *Environmental Toxicology and Chemistry*, 24 (9):2325-2335.
- Hruska, K., and M.G. Dubé. 2004. Using artificial streams to assess the effects of metal-mining effluent on the life-cycle of the freshwater midge (*Chironomus tentans*) in situ. *Environmental Toxicology and Chemistry*, 23 (11):2709-2718.
- Pollock, M.S., M.G. Dubé and R. Schryer. 2010. Investigating the link between pulp mill effluent and endocrine disruption: attempts to explain the presence of intersex fish in the Wabigoon River, Ontario, Canada. *Environmental Toxicology and Chemistry*, 29 (4):952-965.
- Rickwood, C.J., and M.G. Dubé. 2007. Application of a pair-breeding fathead minnow (*Pimephales promelas*) adult reproduction bioassay to a pulp mill effluent. *Water Quality Research Journal of Canada*, 42 (2):82-90.
- Rickwood, C.J., M.G. Dubé, L.M. Hewitt, T. Kovacs, J.L. Parrott and D.L. MacLatchy. 2006a. Use of paired fathead minnow (*Pimephales promelas*) reproductive test: Part I: Assessing biological effects of final bleached kraft pulp mill effluent using a mobile bioassay trailer system. *Environmental Toxicology and Chemistry*, 25:191-201.
- Rickwood, C.J., M.G. Dubé, L.M. Hewitt, T. Kovacs, J.L. Parrott and D.L. MacLatchy. 2006b. Use of paired fathead minnow (*Pimephales promelas*) reproductive test: Part II: Source identification of

biological effects at a bleached kraft pulp mill. *Environmental Toxicology and Chemistry*, 25:202-211.

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

Rickwood, C.J., M.G. Dubé, L.P. Weber, S. Lux and D.M. Janz. 2008. Assessing effects of a mining and municipal sewage effluent mixture on fathead minnow (*Pimephales promelas*) reproduction using a novel, field-based trophic-transfer artificial stream. *Aquatic Toxicology*, 86:272–286.

Rozon-Ramilo, L.D., M.G. Dubé, C.J. Rickwood and S. Niyogi. 2010. Examining the effects of metal mining mixtures on fathead minnow (*Pimephales promelas*) using field-based multi-trophic artificial streams. *Environmental Toxicology and Chemistry*. Submitted in June 2010.

### **Questions/Discussion:**

Comment: U.S. EPA threshold numbers are based on the EC20, but we will see what happens with this value later.

Q: So nutrient effects mask toxicity?

A: Yes, nutrient enrichment mitigates toxic effects.

Comment: Use the existing information out there to save time & money when developing and conducting the study designs. It is also especially useful when generating good hypotheses for IOC.

### **Toxicity Reduction Evaluations as a Tool for Investigation of Cause - Mining Case Studies**

Novak<sup>1</sup>, L.J. and Holtze<sup>1</sup>, K.E.

<sup>1</sup>AquaTox Testing & Consulting Inc.

The *Metal Mining Effluent Regulations* (MMER) under the Fisheries Act requires that all Canadian metal mines produce an effluent that is non-acutely lethal to rainbow trout when tested in accordance with Environment Canada's test method, EPS 1/RM/13. Mine operations are also required to monitor the acute lethality of effluent to *Daphnia magna*. In the event of a toxicity failure (>50% mortality in 100% effluent), the mine must implement a plan to investigate the cause(s) of acute lethality. The most common investigative approach used is the Toxicity Reduction Evaluation (TRE), which is a site-specific study designed to identify the substance(s) responsible for toxicity, isolate the source, evaluate the effectiveness of control options, and confirm the reduction in acute lethality of the final effluent. Prior to the implementation of the MMER regulations, commonly recognized mining effluent related toxicants included ammonia, metals, TDS and acidic pH. However, as more facilities were required to conduct toxicity testing, additional toxicants responsible for acute lethality have emerged. Through the use of actual TRE case studies, some of the recently identified metal mining related toxicants were discussed in the context of the workshop, including thiosalts, xanthates, sulphide and process chemicals.

The author provided the following references to supplement the abstract:

Holtze, KE, GG Gilron, LJ Novak and B Zajdlik. 2002. Guidance document for acute lethality testing of metal mining effluents. Prepared for TIME (Toxicological Investigations of Mining Effluents) Network. 70 p.

Novak, LJ and KE Holtze. 2005. Overview of Toxicity Reduction and Identification Evaluations for use with Small-Scale Tests. In Small-scale Freshwater Environment Toxicity Test Methods (C. Blaise and J.F. Férard, editors); Kluwer Academic Publishers (Dordrecht, The Netherlands)

Novak, LJ, KE Holtze, R Wagner, G Feasby and L Liu. 2002. Guidance document for conducting Toxicity Reduction Evaluation (TRE) investigations of Canadian metal mining effluents. Prepared for TIME (Toxicological Investigations of Mining Effluents) Network. 85 p.

US Environmental Protection Agency. 1989. Generalized methodology for conducting industrial toxicity reduction evaluations. EPA-600/2-88/070.

US Environmental Protection Agency. 1991. Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures. EPA-600/6-91/003.

US Environmental Protection Agency. 1993a. Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity. EPA-600/R-92/080.

US Environmental Protection Agency. 1993b. Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. EPA-600/R-92/081.]

### **Fathead Minnow Lifecycle Assays for Assessment of Complex Effluents**

Parrott<sup>1</sup>, J.P., Hewitt<sup>1</sup>, L.M., McMaster<sup>1</sup>, M.E.

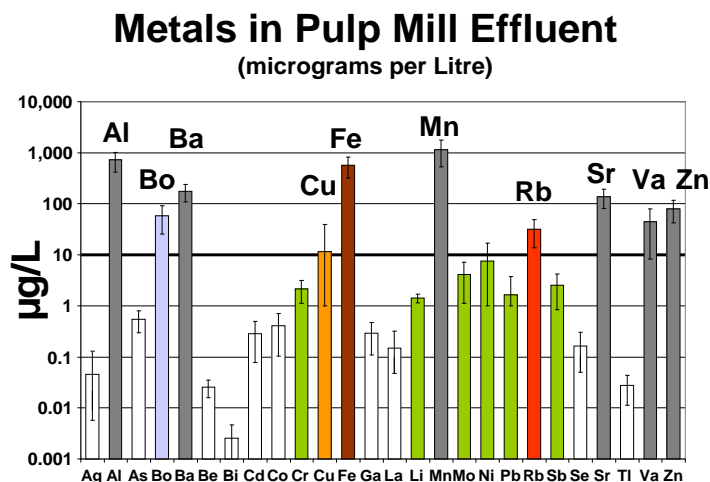
<sup>1</sup> Aquatic Ecosystem Protection Research Division, Water Science and Technology Directorate, Science and Technology Branch, Environment Canada, 867 Lakeshore Rd., Burlington, ON L7R4A6

Predicting and assessing effects in wild fish from standard laboratory tests is difficult. Standard short fish exposure bioassays, developed in the 1970's and 1980's, work well for assessing acute toxicity and immediate effects of compounds and effluents. In Canada, fortunately, many of the effluents we regulate are no longer acutely toxic. Some compounds, effluents and discharges, however, do have long-term impacts on fish health. To assess the effects of long term exposures, we have used the fathead minnow lifecycle bioassay for several pure compounds and mixed effluents.

Lifecycle tests encompass all "critical windows" of exposure; egg, larvae, developing and maturing juvenile, reproduction of adult fish, and survival of the F1 generation. Lab lifecycle exposures to municipal wastewater effluents (MWE) show changes in secondary sex characteristics and decreased breeding, despite increased growth. Wild fish exposed to MWE show similar effects on

sex characteristics. Effects of pulp mill effluents on fish growth and reproduction in lifecycle studies also mirror effects seen in some wild fish.

Data on plant compounds and metals in pulp mill effluent show that effluents can vary significantly week to week. This may be due to variation in the mill process, and in the species and origin of the trees pulped. Analysis of metal concentrations in pulp mill effluent (Figure 1) showed some metals in the 10 to 1,000 µg/L range.



**Figure 1:** Mean (µg/L; ± standard error) concentrations of metals in bleached kraft pulp mill effluent.

Lifecycle tests of metal mining effluents could provide useful data to indicate long-term effects in the environment, especially in cases where the capture of wild fish is difficult. The lifecycle tests have the disadvantages of being expensive and lengthy. However, for mimicking effects of real environmental exposures, and for use in risk assessments, these tests provide valuable data that are difficult or impossible to obtain using shorter lab fish exposures.

### Questions/Discussion:

**Q:** For gonad size as reproduction, is the decrease in egg production a behavioural response, and how does that relate to gonads being a good indicator?

**A:** It may be behavioural, but at this point, it is not known yet. It would be worth looking into though. Even with normal gonads, effects may still occur.

**Comment:** Metals content in pulp and paper effluent is much higher than the limits set in EEM for metal mining.

[Editor's note: Author's correction: In fact, they were not higher than the limits set in EEM for metal mining. The concentrations presented at the workshop showed mg/L for metals, but the actual concentration units were µg/L. The axes were mislabelled.]

## **Using bioaccumulation models for predicting dissolved metal toxicity**

McGeer<sup>1</sup>, J., Ng<sup>1</sup>, T., and Wood<sup>1</sup>, C.

<sup>1</sup>Wilfred Laurier University; <sup>2</sup> Dept of Biology, McMaster University, Hamilton, ON. L8S 4K1.

### Introduction

Within environmental effects monitoring (EEM) investigations of cause (IOC), it is generally assumed that bioaccumulation can be “used to directly infer the cause of toxicity”<sup>1</sup>. The use of bioaccumulation as an indicator of toxicity is known as the tissue residue approach (TRA) and it relies on establishing a dose-response relationship to link tissue residues with toxic effects. Using bioaccumulation as a measure that is indicative of the toxicity of metals presents an interesting dichotomy. In concept it appears relatively straightforward as the accumulation of metal is required in order to induce deleterious effects<sup>2</sup>. Short term accumulation can be directly correlated with bioavailability and thus accounts for the variability associated with factors in the exposure medium that influence metal speciation and uptake (see section below on the biotic ligand model). However, links between short term accumulation and acute toxicity are only available for a few metals and in general short term accumulation is poorly correlated with chronic long term effects. Indeed, even chronic accumulation in an organism, usually measured on a whole body or tissue basis, often does not equate to chronic toxicity.

Toxicity is induced by the buildup of contaminant at the specific site(s) of toxic action. Not all metal that accumulates in an organism interacts at the site(s) of toxic action and significant amounts can be sequestered or otherwise detoxified. Detoxification by the metal binding protein metallothionein is a good example. Processes such as these complicate the interpretation of effect relationships. The following discussion gives an overview of metal bioaccumulation in relation to interpreting effects, outlines and comments on the current metal mining EEM guidance on using bioaccumulation, and gives some details on alternative approaches being developed for linking metal bioaccumulation to toxicity.

### Bioaccumulation of metals

The presence of metals is elemental and they are ubiquitous in all environmental media. As such, all biota will have some concentration of metals in their tissues. Through the course of evolution organisms have developed the capacity to deal with accumulated metal and avoid adverse impacts, at least up to some point along the exposure continuum. In the case of metal exposures which induce toxicity, whole body and/or tissue concentrations are an aggregate measure of all forms of bioaccumulated metal. It is generally not possible to distinguish metal acting at the site of toxicity from that which is accumulated prior to exposure conditions, required for normal functioning (in the case of essential metals), or detoxified or otherwise not contributing to toxicity (in the case of all metals).

There are two recent publications that offer succinct and up-to-date discussions on the science of bioaccumulation. One arose from the comprehensive multistakeholder process developed by the US

---

<sup>1</sup> Metal Mining EEM Guidance 2002 (also see later in this discussion).

<sup>2</sup> There are exceptions, for example precipitation of Al on respiratory surfaces in aquatic environments with low pH values.

EPA and is known as the *Framework for Metals Risk Assessment*<sup>3</sup>. This work includes a series of white papers on science issues related to metals, including bioavailability and bioaccumulation<sup>4</sup>. The recent publication of Adams *et al.* (2010) is a synthesis of the work of the metals subgroup of the recent SETAC Pelston Workshop on the Toxicity Residue Approach. This workshop brought together approximately 40 of the leading experts on contaminant bioaccumulation. The first paragraph of the Adams *et al.* (2010) paper provides a useful summary of the TRA for metals.

“A goal of the tissue residue approach (TRA) for toxicity assessment is to determine the dose-response relationship for various toxicants and evaluate the range of tissue residues that would likely lead to adverse effects. Although considerable effort has been expended over the years to relate tissue metal residues that result from waterborne or dietary exposure, to effects, these efforts have achieved only limited success. Interpretation of results has been confounded by a limited understanding of the manner in which aquatic organisms store, detoxify or eliminate metals, and by lack of standardized exposure periods, standardized test protocols and specified test species. In particular, advancement in the science of using tissue residues has been hampered by a failure to recognize that metals in whole body or muscle do not reflect the biologically or metabolically active portion of metal that is available to contribute to toxicity at the site of action and, further, that total metal is at best a surrogate for the fraction of metabolically active metal at the site of action.”

This work concluded that the TRA (i.e. metal bioaccumulation) for assessment of metals is not supported and this is unlike organic contaminants where a generalized conceptual framework on bioaccumulation can be applied to estimate effects. Some exceptions were noted including organo-metal forms of metal and selenium. The authors also recognized that there could be some metal and species combinations where the TRA approach may work but that these would require field validation.

#### EEM Guidance on the Use of Metal Bioaccumulation

The following excerpts are taken from the sections related to Investigation of Cause within the 2002 *Metal Mining Guidance Document for Aquatic Effects Monitoring*. In section 5.23.3 on methods for determining biomarkers and metal body burdens it indicates that:

“Significant increases in metal body burdens or in biomarker responses (either over time or relative to reference sites) will indicate that metals are bioavailable in the receiving environment. If data on the relationship between bioaccumulation and toxicity are available, metal bioaccumulation can be used to directly infer the cause of toxicity.”

and following some discussion on metallothionein (MT) it continues with:

“Appropriate monitoring species for MT measurements and metal body burdens include freshwater and marine bivalves, benthic insect larvae and amphipods. Indigenous specimens can be collected at exposure sites, or they can be transplanted from reference locations to these exposure sites. Bioaccumulation is particularly useful for identification of bioavailability of non-essential metals (Cd, Pb, Tl, etc), but copper and zinc in invertebrate tissues may be regulated to varying degrees, and their background concentrations may be high. This may limit the usefulness of copper and zinc measurements in invertebrates.”

---

<sup>3</sup> Available at: <http://www.epa.gov/raf/metalsframework/index.htm>

<sup>4</sup> Available at: <http://www.epa.gov/raf/publications/pdfs/BIOFINAL81904.PDF>

In the same section the ease of bioaccumulation measurement is highlighted.

“Body metal and MT concentrations are easy and relatively inexpensive to measure, and these analyses are available in the private sector (body metal more than MT).”

In the section discussing lethal and sublethal toxicity testing (section 5.23.4) the guidance states:

“....metal concentrations in invertebrates exposed to sediments can be a direct measure of bioavailability, and if data on the relationship between bioaccumulation and toxicity are available, metal bioaccumulation can be used to directly infer the cause of toxicity.”

In section 8.4.5.7 within guidance on the use of bioaccumulation in test organisms from sediment toxicity testing it states that:

“Measurement of metals in test organisms from sediment toxicity testing can be used for the quantification of bioavailable metal, and the identification of cause of sediment toxicity. Metal concentrations in benthic invertebrates exposed to sediments are a direct measure of bioavailability and correlate much better with metal-induced sediment toxicity than total metals in the sediment (Borgmann and Norwood 1997a, 1997b). If data on the relationship between bioaccumulation and toxicity are available, metal bioaccumulation can be used directly to infer cause of toxicity (Borgmann and Norwood 1997b).”

and then continues with:

“Animals for bioaccumulation measurement can be obtained from the same tests used to measure sediment toxicity. A gut clearance .....etc..... If data on the relationship between bioaccumulation and toxicity are not available, these data may be obtained using sediment spiking experiments as part of an identification of cause study (e.g., Borgmann and Norwood 1997a, 1999).”

Section 8.4.6 provides guidance on the application of data from the fathead minnow 14 day test as part of guidance on sediments quality testing. It includes the following:

“Juvenile fathead minnows used in the 21-day test can be preserved for future whole body residue analysis to determine bioaccumulation of chemicals from the sediments.”

These excerpts from the *Metal Mining Guidance Document for Aquatic Environmental Effects Monitoring* demonstrate two important considerations. The first is that the understanding of metal bioaccumulation in relation to toxicity was limited when the EEM document was developed. Guidance consistently refers to “if data on the relationship between bioaccumulation and toxicity are available” but there are no specifics on threshold concentrations associated with effect and no examples given (i.e. nothing on when a relationship between bioaccumulation and toxicity occurs). There are no criteria provided for evaluating when inferences on causes of toxicity can be drawn from bioaccumulation and toxicity relationships (i.e. any bioaccumulation to toxicity relationship will do). As well and more importantly, the current state of the science (as developed by the Adams *et al* (2010) and US EPA Metals Framework activities) indicates that the relationship between bioaccumulation and toxicity is confounded by physiology.

Guidance indicating that bioaccumulation can be used to infer cause of toxicity seems ill-advised.

Further, according to Adams *et al* (2010), the EEM guidance may actually be hampering the advancement of science on metal bioaccumulation because it fails to highlight that there is a distinction between accumulated metal that contributes to toxicity at the site of action and metal that is in metabolically inactive forms. While correlations between metal bioaccumulation and toxicity can be demonstrated in single species and single metal exposures, these are unlikely to hold across different environmental conditions. Correlations do not always indicate a cause and effect relationship particularly when the relationship is confounded, as is the case for metal bioaccumulation and toxicity. Extrapolating correlations from single metal exposures in lab conditions to complex effluents with a mix of potential contaminants, even if it is within the same species, would require extensive validation. Extrapolating relationships across species is not warranted without validation. These qualifications and cautions on applying bioaccumulation within IOC appear to be completely lacking in the EEM guidance document (with the one minor but important exception on Cu and Zn bioaccumulation which is limited to invertebrates only).

Much of the research cited within the EEM guidance is based on studies using *Hyalella azteca*. The cited studies are excellent, of high quality and offer important understandings on bioaccumulation and toxicity relationships within that species. The review of Adams *et al.* (2010) includes consideration of these studies and indicates that bioaccumulation relationships developed for *Hyalella* may be relatively unique. Additionally, the recent work of Wang *et al* (2004) provides an indication that interpretation of data derived from *Hyalella* studies, particularly for contaminants in sediment, may not be straightforward. EEM guidance on application of bioaccumulation within IOC currently does not provide this level of discussion.

Therefore, it would seem reasonable and advisable that the EEM guidance on using bioaccumulation in the context of IOC be revised, updated and expanded.

#### Alternative Bioaccumulation Approaches: the Biotic Ligand Model for Acute Metal Toxicity<sup>5</sup>

The biotic ligand model (BLM) provides one example of when metal bioaccumulation can be applied successfully as an indicator of impact. The BLM is based on predictions of the binding of metal at the site of toxic action and the approach integrates metal interactions along the exposure – uptake – accumulation – and toxicity pathway. Metal uptake within the BLM is based on estimates of the relative bioavailability of different dissolved forms (species) of metal. These interactions between metal species and the biotic ligand (BL) are incorporated into the equilibrium modeling framework. The strength of biotic ligand models is that they simultaneously account for the geochemical speciation as well as the relative binding of metal species (or not) to the site of toxicity. Application of the BLM in acute water quality criteria or guidelines is unique because it is a site specific method based on bioaccumulation, and the predicted bioaccumulation versus toxicity relationship has been previously validated by experimentation in a range of different water chemistries.

The BLM has recently been adopted for application as a tool for setting acute water quality guidelines and criteria on a site specific basis. It has also been promoted for application in risk assessment and for setting site specific discharge objectives. Its application with the EEM program would not extend to inferences on identification of cause. However, the model is simple to use and may be useful in providing information on the bioavailability of metals and prioritizing detailed

---

<sup>5</sup> For more details see Niyogi and Wood, 2004 and McGeer *et al.* 2010.

investigation strategies. The advancement of the BLM over the last 15 years offers a useful guideline on approaches for the development of tools for environmental protection and the importance of a mechanistic understanding of toxicity, multistakeholder involvement, interdisciplinary research, teamwork, research investment and vigorous validation.

### Alternative Bioaccumulation Approaches

Alternative approaches to using bioaccumulation to toxicity relationships for metals are much less developed compared to the BLM. One active area of research is the application of subcellular fractionation techniques. These methods characterize bioaccumulation on a tissue or whole organism basis and through the use of homogenization, centrifugation and heat treatments, distinguish between metal in detoxified and metabolically active forms within a cell. Subcellular partitioning of bioaccumulated metal has the potential to provide valuable information on metal toxicity. Partitions include metal-rich granules, cellular debris, organelles (e.g. mitochondria, microsomes and lysosomes), heat-sensitive proteins, and heat-insensitive proteins. Studies have grouped subcellular compartments into metal-sensitive pools such as mitochondria and heat sensitive proteins (where metal toxicity may occur) and metabolically inactive pools such as granules and heat-insensitive proteins ( where metal accumulation is benign)<sup>6</sup>.

Another and different approach to bioaccumulation and toxicity was suggested by Adams *et al* (2010). In this approach a relationship between bioaccumulation and toxicity is established, but not within the same organism. Bioaccumulation is assessed in a metal-resistant organism that strongly and reliably accumulates. Toxicity is assessed in a metal-sensitive (and therefore toxicologically relevant) organism and the two measures are linked through a common exposure. The bioaccumulation by the resistant organisms provides a measure of bioavailability and the toxicological endpoint could be at any level, from physiological, to organism toxicity to community indicator. A conceptual diagram of this approach is given in Figure 1.

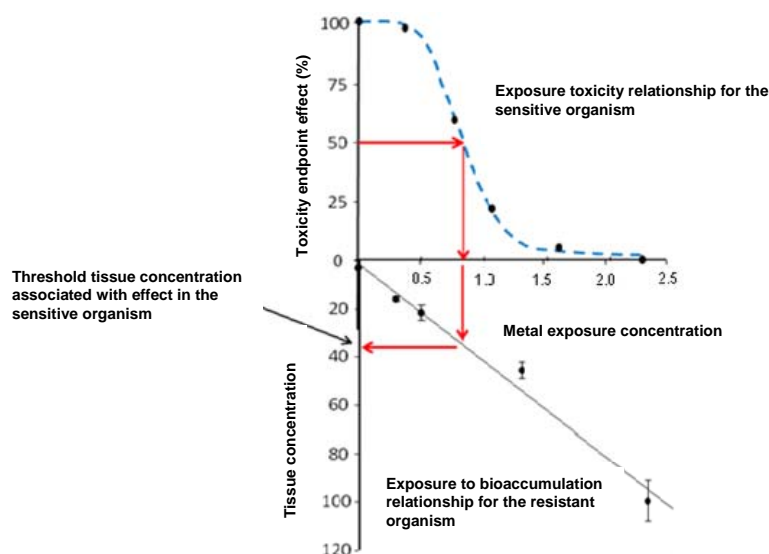


Figure 1. Conceptual diagram of the alternative approach that develops a tissue threshold for bioaccumulation in resistant organisms as indicators of toxicity in sensitive organisms.

The solid lined arrows show the progression from sensitive endpoint (for a 50% inhibition effect level (e.g. growth)) to exposure concentration through to the accumulation in the resistant organism. Numbers on the axes are arbitrary.

<sup>6</sup> See the following works for an overview: Wallace *et al.* 2003, Kraemer *et al.* 2005, and Campbell *et al.* 2008.

## References

Adams, W.J., R. Blust, U. Borgmann, K.V. Brix, D.K. Deforest, A.S. Green, J.S. Meyer, J.C. McGeer, P.R. Paquin, P.S. Rainbow and C.M. Wood. 2010. Utility of tissue residues for predicting effects of metals on aquatic organisms. *Integrated Environmental Assessment and Management*. Sous presse. PMID: 21046571.

Borgmann U. and Norwood W.P. 1997a. Toxicity and accumulation of zinc and copper in *Hyaella azteca* exposed to metal-spiked sediments. *Can. J. Fish. Aquat. Sci.* 54: 1046-1054.

Borgmann U. and Norwood W.P. 1997b. Identification of the toxic agent in metalcontaminated sediments from Manitouwadge Lake, Ontario, using toxicity-accumulation relationships in *Hyaella azteca*. *Can. J. Fish. Aquat. Sci.* 54: 1055-1063.

Borgmann, U. and Norwood, W.P.. 1999. Assessing the Toxicity of Lead in Sediments to *Hyaella azteca*: The Significance of Bioaccumulation and Dissolved Metal. *Canadian Journal of Fisheries and Aquatic Sciences*, 56(8): 1494 1503.

Peter G. C. Campbell; Lisa D. Kraemer; Anik Giguère; Landis Hare; Alice Hontela. 2008. Subcellular Distribution of Cadmium and Nickel in Chronically Exposed Wild Fish: Inferences Regarding Metal Detoxification Strategies and Implications for Setting Water Quality Guidelines for Dissolved Metals . *Human Ecol. Risk Assess.*, 14(2): 290-316.

Lisa D. Kraemer, Peter G.C. Campbell, Landis Hare. 2005. Dynamics of Cd, Cu and Zn accumulation in organs and sub-cellular fractions in field transplanted juvenile yellow perch (*Perca flavescens*). *Environ. Pollut.*, 138 : 324-337.

McGeer *et al.* 2010. Chapter 8 in N. Bury et R. Handy (ed.). *Surface Chemistry, Bioavailability and Metal Homeostasis in Aquatic Organisms: An Integrated Approach*. London (Royaume-Uni), Society for Experimental Biology.

Niyogi, S., and C.M. Wood. 2004. Biotic Ligand Model, a Flexible Tool for Developing Site-Specific Water Quality Guidelines for Metals. *Environ. Sci. Technol.*, 38: 6177-6192.

Wallace WG, Lee BG, Luoma SN. 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. « Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). *Mar. Ecol. Prog. Ser.*, 249: 183-197.

Feiyue Wang, Richard R. Goulet, Peter M. Chapman. 2004. Testing sediment biological effects with the freshwater amphipod *Hyaella azteca*: the gap between laboratory and nature. *Chemosphere*, 57: 1713-1724.

## Additional reference

Paul R. Paquin, Joseph W. Gorsuch, Simon Apte, Graeme E. Batley, Karl C. Bowles, Peter G. C. Campbell, Charles G. Delos, Dominic M. Di Toro, Robert L. Dwyer, Fernando Galvez, Robert W. Gensemer, Gregory G. Goss, Christer Hogstrand, Colin R. Janssen, James C. McGeer, Rami B. Naddy, Richard C. Playle, Robert C. Santore, Uwe Schneider, William A. Stubblefield, *et al.* « The biotic ligand model: a historical overview. 2002. *Comp. Biochem. Physiol.*, 133C: 3-35.

## **Questions/Discussion:**

Comment: Use data analysis to feed into biotic ligand model (BLM). Note that some of the data

must be obtained from unconventional sources.

Comment: Exceptions to BLM include:

- thiosulfate can increase the availability of metals
- xanthates can complex with metals

Therefore, any other ligands that may be present must be considered.

### **Quantifying the Cumulative Effects of Multiple Stressors: Using Redundancy Analysis**

Somers<sup>1</sup>, K.M., Sarrazin-Delay<sup>2</sup>, C.L., and Keller<sup>2</sup>, W.

<sup>1</sup>Ontario Ministry of the Environment, Dorset Environmental Science Centre,

<sup>2</sup>Laurentian University, Cooperative Freshwater Ecology Unit

#### Abstract:

Most cumulative effects assessments are descriptive rather than quantitative. Statistical tools to tease apart the cumulative effects of multiple stressors are generally lacking. Recent interest in multiple stressors has underscored the need for tools to quantitatively evaluate the cumulative effects of two-or-more stressors. Using data based on metal mine and pulp-mill environmental effects assessments, we utilize multivariate multiple regression, or redundancy analysis, to partition the cumulative effects of two stressors. Variation in four benthic community metrics is partitioned among natural habitat features and the separate effects of mining and pulp-mill effluents. This approach quantifies the individual and combined effects of different stressors that potentially affect the benthic community, providing a quantitative tool for cumulative effects assessment, and permitting an objective evaluation of the separate impacts of multiple stressors.

#### Introduction:

Under the federal Environmental Effects Monitoring (EEM) Program of the *Pulp and Paper Effluent Regulations* and the *Metal Mining Effluent Regulations* of the *Canadian Fisheries Act*, pulp and paper mills and metal mines are required to evaluate the potential impacts of their discharges on fish populations and benthic invertebrate communities (Dumaresq *et al.* 2002, Walker *et al.* 2002, 2003). The most common experimental design used in EEM assessments is the upstream-downstream or control-impact design which is occasionally confounded by upstream sources that affect conditions at the reference site (Glozier *et al.* 2002, Lowell *et al.* 2002). As a result, potentially impacted areas below the discharge are exposed to the cumulative effects of the upstream source as well as the discharge of interest. Although the concept of cumulative effects assessment has a long history in environmental impact assessment (e.g., Smit and Spaling 1995, Duinker and Greig 2006), there is widespread recognition that cumulative effects assessments in EEM are largely inadequate.

Most studies investigating the cumulative effects of multiple stressors use univariate analyses to evaluate one biological response at a time relative to several predictor variables (e.g., Lowell and Culp 1999, Culp *et al.* 2000). Multivariate approaches are used less often, despite the fact that most data sets include a number of biological response variables (e.g., Scrimgeour and Chambers 2000). A variety of multivariate approaches could be used, including redundancy analysis (RDA), also known as multivariate multiple regression, to partition variation in multiple response variables among a number of different predictor variables.

In this presentation, we use RDA to quantitatively partition variation in 4 EEM benthic invertebrate endpoints (total invertebrate density, taxon richness, Simpson's evenness index and Bray-Curtis index) into components associated with: (1) uncontrolled background habitat variation, (2) an upstream point source, and (3), a downstream facility's effluent as well as the two-way and three-way interactions.

#### Methods:

Two large-river EEM data sets from separate sites in northern Ontario were combined to create a hypothetical situation with a pulp mill located upstream of a metal mine. Both data sets involved five replicate benthic invertebrate samples collected with a petit Ponar grab from each of upstream reference, near-field, far-field, and far-far-field areas, although the pulp-mill assessment included an additional very-far-field area. Water and sediment samples were also collected from each area. Habitat conditions were characterized by water depth, percent sand, and percent clay. The upstream pulp-mill point source was reflected by sediment total organic carbon (TOC), as well as total nitrogen (TN) and total phosphorus (TP) concentrations in water. By contrast, the downstream metal mine discharge was characterized by river-water concentrations of copper (Cu), zinc (Zn), and molybdenum (Mo). Because the pulp-mill reporting requirements did not include metal concentrations, values for the five pulp-mill areas were estimated as one half of the concentration reported for the upstream reference area for the mine discharge.

Total variation among the four EEM endpoints was initially quantified with a principal components analysis (PCA) using the correlation matrix. The sum of the eigenvalues from this PCA indicated the total variation in the set of 4 response variables. A series of seven RDAs was used to analyze the combined data set (all nine predictors) and partition variation in the four EEM endpoints according to three main factors: habitat, nutrients, and metals, where each main factor was represented by three variables. Variance components were expressed as the proportion of the total variation explained by each of the main effects and their two-way and three-way interactions (e.g., see Qinghong and Brakenhielm 1995, Anderson and Gribble 1998, Okland 2003). All numerical analyses were completed in Excel<sup>®</sup> (Microsoft Corporation 2003) using the Biplot add-in (Lipkovich and Smith 2001).

#### Results:

All three factors explained 48.4% of the variation in the four EEM endpoints (Figure 1). Natural habitat variation accounted for 13.2% of the variation, whereas the separate pulp-mill and metal mine effects explained 21.5% and 36.9% of the EEM metric variation, respectively. Because of correlations between the nine predictors, there was considerable overlap among the three sets of predictors. As a result, the combined effects for the pulp mill and metal mine accounted for 35.3% of the variation when the interacting habitat effects were removed or partialled out (i.e.,  $15.9 + 15.0 + 4.4$ ). Individually, the metal-mine effluent uniquely accounted for 15.9% of the variation in the EEM endpoints, which was substantially larger than the 4.4% associated with the unique effects of the pulp-mill discharge suggesting that the metal mine had a greater effect on the benthos than the pulp mill. However, the shared effects of pulp mill and mine discharges explained 15% of the EEM metric variation. Given that the pulp mill discharge was upstream of the metal mine, the pulp mill accounted for 19.4% of the EEM metric variation (i.e.,  $4.4 + 15.0$ ) whereas the unique (additional) variation associated with the metal-mine discharge was 15.9%.

### Discussion:

Two EEM data sets were cobbled together in this analysis to illustrate how to quantify cumulative effects with “real” rather than simulated data. That is, RDA was used to partition variation among the four EEM response variables according to 3 different influences (e.g., habitat, mine and pulp-mill discharges). Each influence was characterized by three variables that were associated with each factor in order to distinguish the separate impacts. Although we used nine predictors to balance the number of variables among the three factors and to keep the analysis simple, more predictor variables would normally be used in real-world applications (e.g., Scrimgeour and Chambers 2000). Additionally individual measurements should be obtained for each replicate sample within each area to provide better estimates of the underlying stressor-response relationships.

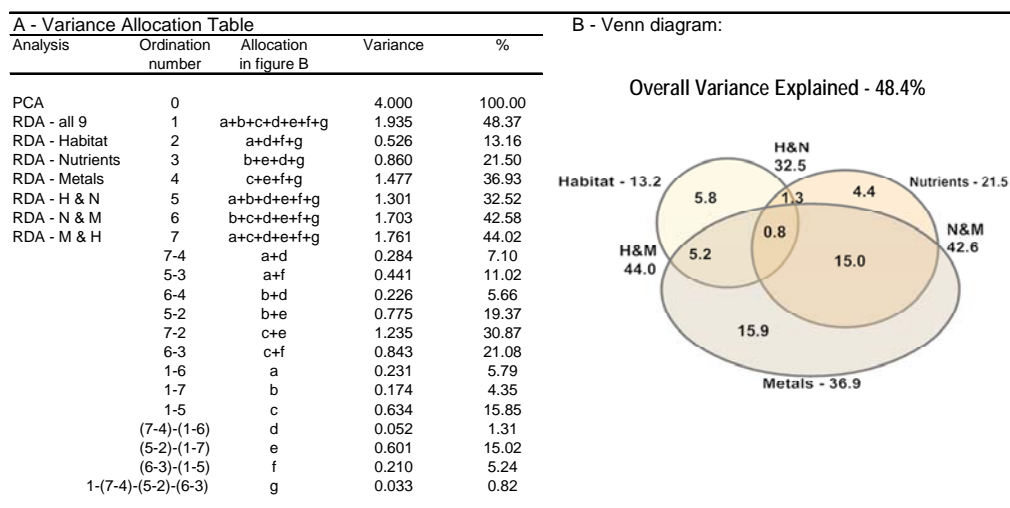
Within the context of a cumulative effects assessment, variance partitioning using RDA indicated that habitat-related effects (13.2%) were less important than pulp-mill effects (21.5%) which were less important than metal-mining effects (36.9%). Although all 3 factors explained 48.4% of the variation in the four EEM endpoints, the combined pulp-mill and metal-mine signals accounted for 35.3% of the variation when interacting habitat effects were removed. Interestingly, the shared effects of the pulp-mill and metal-mine effluents explained 15.0% of the variation in the EEM endpoints. Although the metal-mine effluent uniquely accounted for 15.9% of the variation in the EEM metrics, the upstream pulp-mill discharge explained 19.4% of the EEM endpoint variation suggesting that the downstream metal-mine had a smaller effect on the benthos than the pulp mill.

In this cumulative effects analysis we assumed that the two discharges and associated effluents could be characterized by predictor variables that were readily measured along the length of the study area. For best results, some of these predictors should be uniquely associated with each discharge in order to characterize (and distinguish) the unique impacts of each effluent. In future studies, signature variables for other anthropogenic influences (e.g. urbanization) will need to be identified. Additionally, a better understanding of stressor-response curves (e.g., Gray 1989) and the influence of habitat factors on these responses will help us to better interpret cumulative effects assessment results. Given that RDA can be used to tease apart the cumulative effects of multiple stressors, we now need several real-world data sets involving multiple discharges with an appropriate experimental design and suitable upstream control or reference sites to further illustrate the applicability and widespread utility of this approach for cumulative effects assessments.

### References

- Anderson, M.J. and N.A. Gribble. 1998. Partitioning variation among spatial, temporal and environmental components in a multivariate data set. *Austral. J. Ecol.* 23: 158-167.
- Culp, J.M., K.J. Cash and F.J. Wrona. 2000. Cumulative effects assessment for the Northern River basins Study. *J. Aquat. Ecosystem Stress Recovery* 8: 87-94.
- Duinker, P.N. and L.A. Greig. 2006. The impotence of cumulative effects assessment in Canada: ailments and ideas for redeployment. *Environ. Manag.* 37: 153-161.
- Dumaresq, C., K. Hedley and R. Michelutti. 2002. Overview of the metal mining environmental effects monitoring program. *Water Qual. Res. J. Can.* 37: 213-218.
- Glozier, N.E., J.M. Culp, T.B. Reynoldson, R.C. Bailey, R.B. Lowell and L. Trudel. 2002. Assessing metal mine effects using benthic invertebrates for Canada's environmental effects program. *Water Qual. Res. J. Can.* 37: 251-278.
- Gray, J.S. 1989. Effects of environmental stress on species rich assemblages. *Biol. J. Linnean Soc.*

- 37: 19-32.
- Lipkovich, I. and E.P. Smith. 2001. Biplot and singular value decomposition macros for Excel. J. Stat. Software 7(5): 1-13.
- Lowell, R.B. and J.M. Culp. 1999. Cumulative effects of multiple effluent and low dissolved oxygen stressors on mayflies at cold temperatures. Can. J. Fish. Aquat. Sci. 56: 1624-1630.
- Lowell, R.B., K. Hedley and E. Porter. 2002. Data interpretation issues for Canada's environmental effects monitoring program. Water Qual. Res. J. Can. 37: 101-117.
- Okland, R.H. 2003. Partitioning the variation in a plot-by-species data matrix that is related to n sets of explanatory variables. J. Vegetat. Sci. 14: 693-700.
- Qinghong, L. and S. Brakenhielm. 1995. A statistical approach to decompose ecological variation. Water, Air Soil Pollut. 85: 1587-1592.
- Scrimgeour, G.J., and P.A. Chambers. 2000. Cumulative effects of pulp mill and municipal effluents on epilithic biomass and nutrient limitation in a large northern river ecosystem. Can. J. Fish. Aquat. Sci. 57: 1342-1354.
- Smit, B. and H. Spaling. 1995. Methods for cumulative effects assessment. Environ. Impact assess. Rev. 15: 81-106.
- Walker, S.L., K. Hedley and E. Porter. 2002. Pulp and paper environmental effects monitoring in Canada: an overview. Water Qual. Res. J. Can. 37: 7-19.
- Walker, S.L., S.C. Ribey, L. Trudel and E. Porter. 2003. Canadian environmental effects monitoring: experiences with pulp and paper and metal mining regulatory programs. Environ. Monit. Assess. 88: 311-326.



**Figure 1:** A - Variance allocation table, and B - associated Venn diagram (not to scale) with percent of variance partitioned among the 3 factors and their two- and three-way interactions.

### Questions/Discussion:

**Q:** Does the analysis account for the total surface area in the study? How does this explain the variance?

**A:** The use of multiple regression analysis should account for this. E.g. since the metal mine is

downstream of a pulp and paper mill (in the example within the presentation), then the metal influence does not extend upstream.

Q: What are the data requirements to conduct this analysis?

A: Each study has its own sites. However, this introduces a new problem, as the degrees of freedom then increase in the multiple regression model.

Q: How are the variables chosen?

A: They are chosen by looking at the variables that were above detection overall, or are common to all the sampling locations.

Q: Are there any studies that include wastewater effluents?

A: No, there are none that we know of. Municipal point sources are more relevant, but the number of point sources analysed should be limited to lower the degrees of freedom (i.e. minimize the error/variability due to chance to see if the effect was correlated with the effluent source).

### **Separating Current Effluent Quality from Historical Contamination Using a Laboratory-Based Monitoring Tool for Investigation of Cause**

Taylor, LN<sup>1</sup>, Novak, L<sup>2</sup>, Holtze, K<sup>2</sup>, Ali, N<sup>3</sup>, Scroggins, R<sup>1</sup>

<sup>1</sup>Biological Methods Section, Environment Canada

<sup>2</sup>AquaTox Testing & Consulting Inc.

<sup>3</sup>Environmental Protection Operations-Ontario, Environment Canada

#### Introduction

Environment Canada's Environmental Effects Monitoring (EEM) program currently requires the source of confirmed field effects, the cause of the effects to be identified during the phase called "Investigation of Cause" (IOC). As more facilities enter the IOC phase of EEM, the need for better investigation tools has become a priority. One potential tool which has been under evaluation by the Biological Methods Section (BMS) is a test method for measuring aquatic and sediment toxicity using the freshwater amphipod *Hyaella azteca*. This sediment dwelling organism has been routinely used in both field and laboratory studies to investigate the source and cause of sediment toxicity (Shuhaimi-Othman *et al.*, 2006; Borgmann *et al.*, 2004; Couillard *et al.*, 2008; Ingersoll *et al.*, 2000; Borgmann and Norwood, 2002; Nowierski *et al.*, 2006). The BMS has an existing standard method for this organism (EPS 1/RM/33: *Test for Survival and Growth in Sediment Using the Freshwater Amphipod Hyaella azteca*), which was originally published in 1997 and is currently slated to be updated in 2010. Over the last two years, two mines (with confounding factors) volunteered samples of their effluent and sediment for experiments with *H. azteca* in hopes of developing this tool for IOC. In both cases, the EEM Interpretive reports indicated that it was not possible to separate the effects of the current effluent discharge from that of historical accumulation of metals in the sediment. The results of this research will eventually be integrated into the revised EPS 1/RM/33 method, such that a standardized approach to IOC can be implemented.

#### Materials and Methods

##### Mine 1

The first study involved a small headwater lake that currently receives treated mine effluent, but also

receives deposits from industrial activities carried out over the past 100 years. Sediment samples (~ 5 L of testable sediment) were collected from the near-field site at two locations approximately 8 m apart using a ponar sampler (~ depth of 5 to 10 cm) and from a single location at the reference lake. Water samples (2 x 23 L containers) from the near-field and reference site locations were collected from ~ 1 meter above the lake bottom. In addition, a sample of mine effluent (23 L) was collected and tested for sublethal toxicity to *Ceriodaphnia dubia* (Environment Canada, 2007) for comparison to historical sublethal toxicity data and in order to confirm current effluent quality was not a serious variable in the present study. Sediment tests following EPS 1/RM/33 were conducted with each sediment type: 1) contaminated sediment downstream of the mine effluent discharge (Exposure Site), 2) field-collected reference sediment (Field Reference), and 3) laboratory reference sediment (Lab Reference). For each sediment type, different types overlying water were used. Water hardness differences between the reference site water (Reference Water), field-collected receiving water mixed with effluent (Lake Water) and the laboratory dilution/culture water (Standard Lab Dilution Water) were accounted for by decreasing the water hardness of the Standard Lab Dilution Water to match that of the Reference or Lake Water. Accordingly, water hardness of the Standard Lab Dilution Water (initially 300 mg/L as CaCO<sub>3</sub>) was decreased to a hardness of either ~ 90 or 140 mg/L to be comparable to that of the Reference and Lake Water, respectively. Aqueous-only toxicity tests were also conducted with each water type. For each test and reference sediment, a control consisting of clean sediment was established using the appropriate control water (i.e., standard control or one of the hardness-adjusted controls) as the source of overlying water. Minimum sediment characterization was also conducted on all samples (i.e., total organic carbon (TOC), particle size and moisture content).

### Mine 2

The second mine was comprised of two underground gold mines that discharge treated effluent to a common lake receiver. Sediment samples (~ 6 L of testable sediment) were collected from the near-field site and from the reference site using a Ponar sampler (~ depth of 7 cm). Water samples (2 x 23 L containers) from the near-field and reference site locations were collected by mine staff (~ 1 meter above the lake bottom). Sediment tests following EPS 1/RM/33 were conducted with each sediment type: 1) contaminated sediment downstream of the mine effluent discharge (Exposure Site), 2) field-collected reference sediment (Field Reference), and 3) laboratory reference sediment (Lab Reference). For each sediment type, different types of overlying water were used. Water hardness differences between the reference site water (Reference Water) and laboratory dilution/culture water (Standard Lab Dilution Water) were accounted for by raising the water hardness of the site water to match that of the laboratory water. Accordingly, water hardness of the Reference Water (initially 30 mg/L as CaCO<sub>3</sub>) was increased to a hardness of ~ 300 mg/L to be comparable to that of the Standard Lab Dilution water. This was done in order to minimize any potential stress on the test organisms that may have been caused by the low water hardness of the Reference Water. Water hardness was adjusted according to the Environment Canada (1990) *Daphnia magna* test method. Aqueous-only toxicity tests were also conducted on each water type. For each test and reference sediment, a control consisting of clean sediment was established using the appropriate control water (i.e., standard control or one of the hardness-adjusted controls) as the source of overlying water. Minimum sediment characterization was also conducted on all samples (i.e., TOC, particle size and moisture content). In addition, total metals were also measured in all sediment samples and total and dissolved metals were analyzed in all water samples.

#### 14-d Sediment Exposure Using *Hyaella azteca*

The 14-day static survival and growth test using *H. azteca* (2 to 9-d old) were conducted following Environment Canada Method EPS 1/RM/33 (Environment Canada, 1997). Overlying water was continuously aerated during the entire duration of the test. The tests were conducted using high-form 300 mL glass beakers filled with 100 mL of sediment. Overlying water (175 mL) was added to each replicate test and control chamber. A total of 50 test organisms were exposed to each sediment-water combination (i.e., ten organisms per each of five replicate test vessels). Twenty-four hours prior to addition of the organisms, aeration was applied to each test chamber at the rate of ~ two to three bubbles per second. At the start of the test, organisms were randomly assigned to the test beakers until each beaker contained ten organisms. Yeast, Cerophyll™ and Trout chow (YCT) (containing ~2.7 g/L solids) was provided at a rate equal to 1.5 mL/d. Test results were based on mean percent survival and mean dry weight of surviving organisms. The test was considered invalid if the survival for the control treatment was <80% or if the average dry weight for the replicate control groups was <0.1 mg per surviving organism.

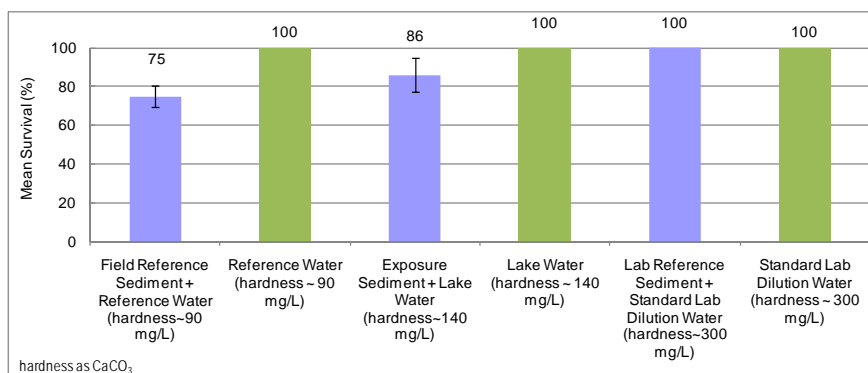
#### 14-d Aqueous-Only Exposure Using the *Hyaella azteca*

Aqueous-only tests were based on Environment Canada Test Method EPS 1/RM/33 (Environment Canada 1997) and a draft method for an aqueous-only method developed by Borgmann *et al.* (2005). The latter method was designed strictly for evaluating toxicity due to ammonia in a static test conducted over a 10-day period, with four replicates with 20 organisms (1 to 11 days old) and survival was the only endpoint of interest, so this method was modified for our purposes. In comparison, the current aqueous-only test design was based on a 14-d static-renewal exposure examining both survival and growth of the test organisms. The overlying water was renewed three times weekly. Tests were conducted in the absence of sediment. However, a small amount of nytex screening was added to each test vessel which acted as a suitable substrate for the test organisms. The overlying water was not aerated, since it was expected that renewal of the test solutions would maintain dissolved oxygen concentrations at an acceptable level. Test results were based on mean percent survival and mean dry weight of surviving organisms.

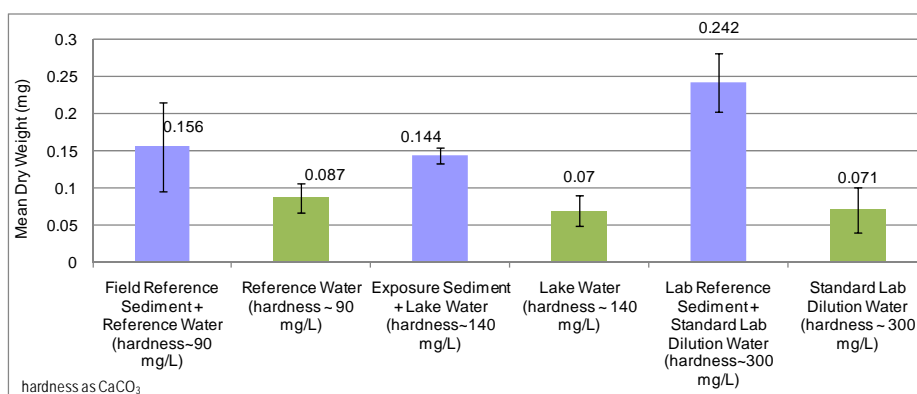
#### Selected Results and Conclusions:

##### Mine 1

*H. azteca* survival and growth results from the sediment and aqueous-only tests were compared and are presented in Figures 1 and 2, respectively. There was no significant difference in organism survival between the Lab Reference Sediment test and the Standard Lab Dilution Water aqueous-only test (100% survival in both tests). In comparison, there was significantly lower survival in the Field Reference Sediment test (75% survival) and the Reference Water aqueous-only test (100% survival). Similarly, significantly lower survival (86%) was observed in the Exposure Sediment test compared to the Lake Water aqueous-only test (100% survival). All aqueous-only tests resulted in significantly lower organism growth when compared to the sediment tests. However, these results need to be interpreted cautiously since none of the aqueous-only tests met the minimum growth criteria (mean dry weight was < 0.1 mg in all samples).



**Figure 1:** *H. azteca* survival data for Mine #1 – sediment vs. aqueous-only exposures



**Figure 2:** *H. azteca* growth data for Mine #1 – sediment vs. aqueous-only exposures

Initial testing with sediment and aqueous-only exposures with Mine #1 were inconclusive due to the high degree of variation and inability of the aqueous-only tests to meet the test validity criteria for growth. Consequently, further investigations were undertaken in an attempt to improve test outcome and reduce variability of the growth endpoint during aqueous-only testing. Results from these tests are not discussed here.

### Mine 2

Results from studies to improve the outcome (specifically, organism growth) in aqueous-only tests were inconclusive, and further investigations were required to define optimal conditions for conducting these tests. In the interim, a new IOC study design (that did not solely rely on aqueous-only tests) was developed and implemented with samples from a second mining facility. The new study design involved various sediment and overlying water combinations, as follows:

	<b>Standard Lab Dilution Water</b>	<b>Reference Water</b>	<b>Lake Water</b>
<b>Exposure Site Sediment</b>	√	√	√
<b>Field Reference Sediment</b>	√	√	√
<b>Lab Reference Sediment</b>	√	√	√

This study design allowed for a clear distinction to be made between biological impacts resulting from current effluent quality from those due to historical sediment contamination. The combination of Exposure Site Sediment with Lake Water represents the most realistic and current receiving environment conditions as it evaluates the combined effects of historically contaminated sediment and current effluent quality when combined with receiving water. Results from these tests can be compared to either the Field Reference Sediment with Lake Water or the Lab Reference Sediment with Lake Water to determine if the Lake Water itself may be contributing to reduced organism survival or growth in the absence of historically contaminated sediment. Inclusion of the Field Reference Sediment with Reference Water provides a field control in the absence of contaminated sediment. Importantly, by using a combination of field and lab sediment and water samples the study design also takes into account the influence that site-specific water chemistry (e.g., dissolved organic carbon (DOC) in receiver) may have on toxicity.

Sediment test results are summarized in Figure 3. Complete (100%) mortality was observed in all Exposure Site Sediment tests, regardless of the type of overlying water (Standard Lab Dilution Water, Reference Water or Lake Water). Complete survival was observed in all Field Reference Sediment tests and Lab Reference sediment tests, regardless of the type of overlying water. There was no significant difference in organism growth when either Lake Water (0.259 mg mean dry weight) or Standard Lab Dilution Water (0.287 mg mean dry weight) was added to Lab Reference Sediment. However, compared to the Lab Reference Sediment + Lab Dilution Water combination (0.287 mg mean dry weight), significantly lower growth was observed when Reference Water was added to the Lab Reference Sediment (0.154 mg mean dry weight). When Reference Site Water was used as overlying water, there was no significant difference in organism growth when either Field Reference Sediment (0.128 mg mean dry weight) or Lab Reference Sediment (0.154 mg mean dry weight) was tested. However, the lowest overall organism growth was observed in these test scenarios. Significantly lower organism growth was observed in the Field Reference Sediment + Reference Water combination (0.128 mg mean dry weight) compared to the Field Reference Sediment + Lab Dilution Water combination (0.255 mg mean dry weight). Yet no significant difference was observed when Lab Water was used as the overlying water in tests with Field Reference Sediment (0.255 mg mean dry weight) or Lab Reference Sediment (0.287 mg mean dry weight).

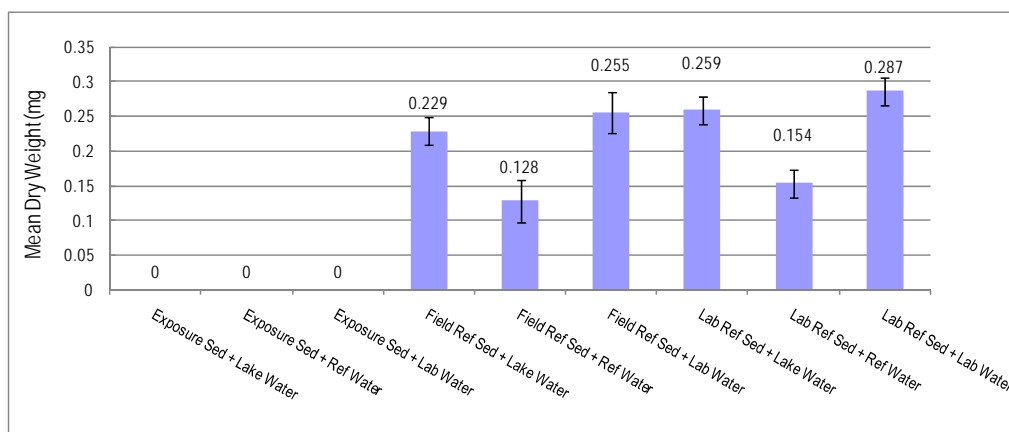


Figure 3: *H. azteca* growth data for Mine #2 – sediment exposures

Although optimal aqueous-only test conditions have not been established, tests were still conducted with each of the Lake Water and Reference Water samples used in sediment testing (tests also included a Standard Lab Dilution Water). All of the aqueous-only tests met the minimum test validity criteria for survival (survival was  $\geq 80\%$ ). Although there were no significant differences in organism survival or growth when comparing the three water samples (Standard Lab Dilution Water, Reference Water and Lake Water), the tests either did not meet or only barely met the minimum growth criteria (mean dry weight ranged from 0.092 to 0.102 mg).

The results from tests with Mine #2 indicated there was no significant difference when either Lake Water or Standard Lab Dilution Water was added to Lab Reference Sediment, suggesting the current Lake Water did not impart toxicity to *H. azteca*. However, complete mortality was observed in all Exposure Site Sediment tests, regardless of the type of overlying water (Standard Lab Dilution Water, Reference Water or Lake Water). These results strongly suggest historical sediment contamination contributed to current impacts on benthic invertebrate communities. Testing also suggested the Reference Water sample may have contributed to reduced *H. azteca* growth since significantly lower organism growth was observed when Reference Water was added to the Field Reference Sediment (compared to when Lab Dilution water was added to Field Reference Sediment). Although overlying water was renewed 3x weekly in the sediment tests (to replenish contaminant loss that may have occurred during testing), further refinement of the aqueous-only test methodology would be beneficial by allowing another approach to be used to distinguish between effects of historically contaminated sediment from the effects of the overlying river water currently receiving effluent downstream of the mine discharge point.

### Summary and Recommendations

Combined results from the current study indicate the Environment Canada *H. azteca* test method (EPS 1/RM/33) is a quick, cost-effective, readily available screening tool that, when combined with benthic surveys and chemical analysis, can be used to isolate the biological effects of historical contamination in sediment from current effluent quality. However, temporal variability in effluent constituents must be taken into consideration when deciding if current effluent quality may impact benthic invertebrate communities. In other words, a single sampling event represents a ‘snap-shot’ in time and would be insufficient to confirm whether current effluent quality alone is adversely impacting benthic invertebrate populations. Additionally, Borgmann *et al.* (2004) reported the

combination of benthic community changes, sediment toxicity, sediment chemical composition, *H. azteca* body burdens and overlying water concentrations (including threshold effect concentrations) provided the strongest indication of effects and their cause at metal-impacted lakes. To this end, future studies should include analysis of metals in overlying water from the sediment tests to determine if water concentrations are associated with toxicity. The use of metal body burdens in *H. azteca* may also assist with interpretation of the source and cause of toxicity, particularly if more subtle responses are observed (e.g., where organisms survive, but sediment impacts growth), though this may require the use of additional replicates in order to obtain sufficient tissue mass for chemical analysis. The current study also provided useful information for improving the outcome of aqueous-only tests with *H. azteca*. However, further testing would be required to determine the optimal conditions for conducting these tests (particularly for the growth endpoint). Several factors should be considered when deciding if further pursuit of an aqueous-only test method for IOC is needed. In one respect, the use of *H. azteca* might not be appropriate under these conditions as they are inherently sediment-dwelling organisms. Conversely, the development of a standardized aqueous-only method for application in IOC studies would have its advantages. The interactions between sediment and water are extremely complex, yet an aqueous-only test clearly eliminates interferences that a sediment sample may have on overlying water quality, and in turn, on organism response. The study design used with Mine #2 was effective in identifying impacts due to historical sediment contamination rather than a result of current effluent/receiving water quality. However, further research would be needed to establish if this same design would show if the opposite response exists (i.e., current effluent/receiving water is toxic, while there is no impact due to historical sediment contamination).

## References

- Borgmann U., Ingersoll CG, Mathyk S, and Lennie-Misgeld P. 2005. Draft biological test method: Test for survival in 10-day water-only exposures using the freshwater amphipod, *H. azteca*, with emphasis on detection of toxicity due to ammonia.
- Borgmann U, M Nowierski, LC Grapentine and DG Dixon. 2004. Assessing the cause of impacts on benthic organisms near Rouyn-Noranda, Quebec. *Environmental Pollution* 129: 39-48.
- Borgmann, U and WP Norwood. 2002. Metal bioavailability and toxicity through a sediment core. *Environmental Pollution*. 116: 159-168.
- Couillard, Y, IC Grapentine, U Borgmann, P Dolye and S Masson. 2008. The amphipod *H. azteca* as a biomonitor in field deployment studies for metal mining. *Environmental Pollution*. 156: 1314-1324.
- Environment Canada. 1990. Biological Test Method: Acute Lethality Test Using *Daphnia spp.* Environmental Protection Service, Environment Canada. Ottawa, Ontario, Report EPS 1/RM/11, July 1990. 57pp.
- Environment Canada. 2007. Biological test method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*. Environment Canada, Conservation and Protection. Ottawa, Ontario. Report EPS 1/RM/21 2<sup>nd</sup> Edition, 74 pp. February 2007.
- Environment Canada. 1997. Test for survival and growth in sediment using the freshwater amphipod *H. azteca*. Conservation and Protection, Ottawa, ON, EPS 1/RM/33.

Ingersoll, CG, CD Ivey, EL Brunson, DK Hardesty and NE Kemble. 2000. Evaluation of toxicity: Whole-sediment versus overlying-water exposures with amphipod *H. azteca*. Environmental Toxicology and Chemistry 19: 2906-2910.

Nowierski, M, DG Dixon, U Borgmann. 2006. Lac Dufault sediment core trace metal distribution, bioavailability and toxicity to *H. azteca*. Environmental Pollution 139: 532-540.

Shuhaimi-Othman, M, DPascoe, U Borgmann and WP Norwood. 2006. Reduced metals concentrations of water, sediment and *H. azteca* from lakes in the vicinity of the Sudbury metal smelters, Ontario, Canada. Environmental Monitoring and Assessment. 117: 27-44.

### **Use and Application of Benthic-Transplant-Devices BTDs® in Environmental Effects Monitoring Investigations**

Dr. Gregory P. Thomas  
G3 Consulting Ltd.  
206-8501 162<sup>nd</sup> Street, Surrey, BC, V4N 1B2  
(604) 598-8501  
gthomas@g3consulting.com  
www. g3consulting.com

#### Overview:

Metal Mining Environmental Effects Monitoring (MM EEM) is tiered to provide for more extensive monitoring where there are effects and less monitoring where there are not. If effects are present then mines are to determine magnitude and extent. If magnitude and geographic extent are determined and the cause of the effect is still not known, the mine must then investigate the cause of the effect. This investigation employs a “tiered” approach and is employed to focus successive studies.

#### Determining Effects

Under the federal *Fisheries Act*, EEM is to assess if current effluent discharges are having an effect(s) on fish, fish habitat and fisheries resources. Effects, and the extent and magnitude of those effects, is to be established, then an Investigation of Cause (IOC) undertaken. “Effect”, as understood in the *Metal Mining Effluent Regulations* and the *Pulp and Paper Effluent Regulations*, is a “statistical difference in indicators of adult fish exposed and not exposed to effluent” or, “a statistical difference in indicators of the benthos exposed and not exposed to effluent”. Critical Effect Size (CES) in this instance is a predetermined value that would be considered to be an effect of sufficient magnitude to be of ecological significance (e.g., +/- 2 SD).

#### Challenges

Investigating the potential causes of an identified effect is challenging. Typical designs (e.g., Before-After, Control-Impact) rely on adequate reference areas (conditions). Ambient ecological condition in these types of studies is often not well defined and in many cases prohibitively expensive to undertake effectively. Historical legacies, confounding influences, system variability, challenging sampling conditions/locations confound and complicate this challenge in complex and dynamic systems (e.g., rivers, lakes, streams, and ocean).

The question to ask then is: Can a program be designed to:

- reduce confounding/historical influences on study results; and,
- establish meaningful assessment of current industrial influences (spatially and temporally)?

#### Benthos as a tool

Benthic invertebrates are evaluated in standard monitoring tools used in EEM studies. They are used as an “indicator” of fish habitat condition. Sediments, and their constituent benthic invertebrates (benthos), have long been seen as a valuable tool for assessing environmental “effects” associated with industrial discharge. Benthos are favoured for this purpose as they are ubiquitous, respond to perturbations in variety of habitats, produce a range of measurable responses, are relatively sedentary and long-lived and enable an assessment of change with space and time.

Typically in these types of programs, differences in “Core” indices assessed between Reference and Exposure areas are used to quantify effects. Typically these indices can include 1) total density (# orgs/unit area); taxon richness (# taxa); Simpson's evenness (evenness of taxa distribution); and Bray-Curtis Index (community composition). Benthos communities also have their limitations and don't always respond well to all types of perturbations and their distribution and abundance can be affected by many factors. That said, with an effective sample design, many of these issues can be eliminated or reduced.

#### Design Considerations

To reduce the many the limitations of IOC studies, longer-term, multispecies, spatial, time-series experiments using indigenous communities may be an effective means by which to conduct an IOC. Planning an effective, robust and scientifically-defensible program design must necessarily consider practicality, cost, time, effort, access, safety, interpretive ability and reproducibility. Can we develop a new method by which benthic communities can be used to assess these complex and dynamic environments?

#### Benthic Transplant-Device (BTDs)<sup>®</sup>

A novel approach to the use and study of IOC, using benthic invertebrate communities and their associated sediments, employs a new type of sampling method. This method, developed by Dr. Gregory Thomas of G3 Consulting, is designed to reduce the artificial sampler bias inherent in other types of benthos samplers. This method evolved from spatial and gradient designs in mining and pulp and paper EEM studies conducted in rivers, creeks, reservoirs and complex marine environments over many studies. The approach employs the use and relocation (transplant) of indigenous benthos populations and associated substrate/habitats and has been effective in elucidating comparative trends in benthos communities and substrate quality with space and time. The Benthic Transplant Device (BTD<sup>®</sup>) enables identification of specific benthos species to accurately represent ambient ecological condition and indicators of specific industrial discharge effects through the direct exposure of indigenous benthos to current discharge while isolating from anthropogenic influences and historic confounding effects. This method collects sediment, and associated benthos, transplanted from one area and places them in another area of direct effluent exposure. BTD<sup>®</sup> samplers are left for a prolonged, pre-determined length of time then retrieved (after many months of exposure) and the properties of benthos communities and sediments are then assessed. The sample sizes used are comparable to those of previous programs, given that the

method of collection employs the same devices (Ponar or SmithMac, etc.). Sample sizes can be varied depending on program requirements and location. This approach is adaptable and found to be equally effective in marine and freshwater environments (rivers, lakes, and reservoirs).

The BTDS<sup>®</sup> are filled with background (source) sediment (and associated benthos) from randomly distributed replicates. The comparability (homogeneity or similarity) of source sediment and benthos communities is assessed between replicates (*a priori*). Source sediments are analyzed for sediment quality and benthos community structure at time zero ( $t=0$ ). Transplants, once placed into the sampling device (BTDS<sup>®</sup>), are subsequently relocated to exposure sites. In some studies conducted with this device, samplers were placed with increasing distances from discharge sources, either linearly or in radial patterns and observation related to proximity to source observed over time. Similarly, returning some of the BTDS back to reference areas also can facilitate a comparison between reference and exposure areas (depending on program need and design). During deployment, the BTDS<sup>®</sup> lid is closed, and then opens once on the bottom (to enable exposure of substrates and benthos communities to overlying waters). Upon retrieval the lid then self-closes under tension to protect the sample. Samples are then processed as they typically would be for other benthic programs (sieving, sediments, taxonomy, etc.).

#### Outcomes Relevant to EEM IOC

A key feature of this approach is the ability to tease out subtle differences between sites (biophysical, chemistry and benthos). Key indicator species have provided important clues to the condition of the receiving system and collectively the results have suggested several important findings. Namely: changes with exposure/distance measured; benthos abundance/diversity/species; sediment quality (chemistry/physical measures/appearance); biomass changes; bacterial presence and composition; and, benthic deformities/symmetry. These observations, often not possible in previous approaches, are now possible using the BTDS method. Some results of trial programs using this device correlated well, others not as much (depending on study and parameter); however, specific species have been identified as indicators of change and were the direct result of the BTDS<sup>®</sup> method developed and employed. Without this approach, system variability and current and historical influences tended to mask specific and important effects (positive and negative). BTDS<sup>®</sup> have been successful in assisting to define local ecological condition and distinguish localised and current effluent-related effects, through the use of statistics and weight of evidence (WOE) analysis to correlate effluent, sediment and benthic invertebrate data.

#### BTDS<sup>®</sup> (Advantages & Disadvantages)

The Benthic Transplant Device (BTDS<sup>®</sup>) can be an effective method by which to separate historical and current effluent effects from other natural and human-created confounding sources and variability. This method provides an alternative method for both the Investigation of Cause and Solutions as required under the pulp and paper EEM and Investigation of Cause for metal mining EEM. The device is adaptable to a variety of study conditions and locations and is particularly suited to complex environments. The BTDS<sup>®</sup> provide replicates with comparable sediment and benthic characteristics that can be strategically placed in Near, Mid and Far Field Areas (as well as Control or Reference areas). The BTDS<sup>®</sup> serves as an integrative tool that can be used to test and/or corroborate different test scenarios (e.g., connection between field vs. lab, field vs. mesocosm, etc.). The method is cost-effective, reusable, and repeatable and avoids bias in abundance and diversity estimates of artificial substrates. The time required for colonisation is much shorter than for artificial

substrates and given its portability and design, it can be used to undertake studies at any size (power) or time (logistics/life-cycles).

The colonization dynamics and ability of vagile organisms to enter the BTD<sup>®</sup> devices are restricted to the water column. In many programs this is desirable, but can also be seen as a disadvantage in some scenarios. Given this is a new and developing method, applied to a variety of scenarios, conditions and study designs, it is still experiencing a learning curve and would benefit from further testing in new scenarios as well as integrative work.

### Summary

BTDS<sup>®</sup> are a promising new approach for Pulp and Paper EEM IOCs and IOS and Metal Mining IOC. This approach enables the assessment of subtle, yet important, system changes specifically associated with point source discharges with space and time. The method is cost-effective and is useful in separating historical/confounding and natural variability from current discharge effects. The merits (and limitations) of using individual-level endpoints (e.g., fitness parameters) to support community structure assessments (e.g., biomass, species abundance and richness) currently being investigated with this method as well as its use in combined lab/field exposures to see if correlations/validations can be established.

### **Questions/Discussion:**

Comment: This methodology is similar to caging experiments but allows for access to community rather than individual effects. It is also similar to mesocosms.

Q: What is the size of the device? Would it be usable in creeks/streams?

A: The device can be different sizes. The problem is usually a weighting issue; it is important to ensure that the device stays where the sampling is meant to take place.

### **Should Biological Recovery Trigger an Investigation of Cause Study, in the context of Historical Impacts and Upcoming Mine Closure?**

LePage<sup>1</sup>, P. Russel<sup>1</sup>, C. Stecko<sup>1</sup>, P. and Prairie<sup>2</sup>, R.

<sup>1</sup>Minnow Environmental Inc. <sup>2</sup>Xstrata Zinc

The Brunswick No. 12 Mine, operated by Xstrata Zinc, is a zinc-lead mine located in northern New Brunswick. The mine has operated since 1964 and, based on current ore reserves estimates, mining/milling operations are expected to extend to 2011. Brunswick Mine ore originates from massive sulphide deposits and as a result, mill tailings tend to be acid generating with high thiosalt levels. Despite mine effluent generally meeting MMER limits, its release into the receiving waters historically resulted in degraded water quality characterized by seasonal pH depression and elevated metal levels (due to downstream thiosalts oxidation) which, in turn, severely impacted benthic invertebrate and fish communities. To address this issue, the Brunswick Mine commissioned an effluent treatment plant in 1993, upgrading this system in 2004 to include seasonal thiosalt treatment. Recognizing that severe impacts to the biological community within the receiving environment appeared related to historical mine practices, an alternative EEM program was implemented in 2005 and 2008/2009 with the specific objective of documenting biological recovery

of the system over time. Two cycles of EEM have shown clear, progressive improvements in benthic invertebrate and fish communities of the effluent receiver, especially relative to historical data. Despite these improvements, some significant differences in EEM biological endpoints between effluent-exposed and reference areas were observed through both EEM cycles. Because biological conditions continue to improve, historical confounding factors still exist, and the mine is scheduled for closure, this presentation focuses on potential goals for future site-specific EEM studies and how an IOC may fit into this scenario.

### **Questions/Discussion:**

Q: What if the market price of the commodity changes (goes up)? Wouldn't that lead to keeping the mine open?

A: Yes, that would be a factor in this decision.

Q: Are there any studies done on salmon species?

A: The Atlantic salmon species is mostly wiped out; that is not expected to change.

Q: Could you explain more about the non-point sources of zinc?

A: Other possible sources of zinc include buffers, which may affect the pH in the groundwater. This could then be mine-related. In terms of historical sources, sludge stirred up by the moving water or by beavers building dams could also uncover some zinc.

Q: What sort of brook trout is used in the mercury testing for this study?

A: The brook trout are residents, aged to be approximately three years old.

Comment: The fact that there is recovery in the fish and benthos studies shows that the EEM design is working.

## **DISCUSSION TO IDENTIFY CHALLENGES, AND RESEARCH NEEDS**

The presentations provided a solid background on potential causes of effects and also information on new tools and approaches that could potentially be applied to Metal Mining IOC. There were many detailed examples of current IOC studies and case studies. There were question and answer periods after each presentation (found after each abstract), and as well, a discussion after Session 1 (found after the Session 1 abstracts). Following the last presentation in Session 4, there was a discussion to identify gaps and research needs. The workshop closed with a wrap up and a path forward.

### **What Did the Workshop Accomplish?**

The workshop presentations provided valuable background information and mini-courses on potential causes of effects. New tools and approaches that could potentially be applied to Metal Mining IOC studies were also highlighted, as well as detailed examples of current IOC in action, via case studies. Information from the Proceedings will be used to update guidance for Metal Mining IOC. The workshop also provided participants the opportunity to share examples of what tools and approaches have worked; and what tools and approaches need further work, but have potential.

### **Challenges and Research Needs Identified**

After the presentations the participants of the workshop were asked to submit their written anonymous concerns, questions or issues surrounding IOC. After these were submitted, the co-chairs summarized the issues in a presentation and reviewed these during the wrap up session of the workshop. The discussion is summarized below.

Administrative and technical challenges in Metal Mining EEM Investigation of Cause were discussed in context of lessons learned and what tools and approaches to could be applied (i.e. what works and what doesn't). The intent of the workshop was to discuss policy, process and scientific aspects of the Investigation of Cause stage (including environmental studies, potential causes of effects, regional perspectives and case studies, and tools and approaches for IOC). The challenges discussed were reorganised slightly, in order to highlight challenges that were specifically related to the Investigation of Cause stage of the MM EEM program.

### **Investigation of Cause Related Challenges**

1. Industry re-iterated their concern regarding the regulatory time frame of 24 months for IOC and magnitude and extent studies, and recommended to change the reporting period from 24 months to 36 months.
  - Given the complexities of IOC, the two year cycle does not give sufficient time for study design, EEM program review, study completion & interpretive reporting, and necessary analysis and reflection, as well as timely response from Environment Canada. Other challenges and supporting rationales were discussed. Industry would endeavour to provide Environment Canada with additional examples to illustrate the challenges and concerns outlined.
2. The pertinence of IOC requirements in the context of a government approved environmental assessment or environmental impact statement (EA/EIS) was discussed. If environmental

assessment or an environmental impact assessment is done, why is an IOC needed?

- IOC has proved to be useful even if an AE/EIS has already been done (for example, benefits to the uranium industry were discussed).
  - In the context of EEM, IOC is necessary to understand if the effects are due to the metal mining effluent or some other reason, and to understand what is significant to that site.
3. The sensitivity of the trigger endpoints for initiating IOC (Bray-Curtis Index and fish endpoints) were discussed in context of the regulatory framework.
    - It was proposed that the CES could be used to evaluate the level of effort to put into IOC.
  4. Should Extent and Magnitude be completed before IOC?
    - It was pointed out that Extent and Magnitude will be needed later on (see third bullet below).
    - Some mines already have that data from previous studies, so it's redundant to do it again.
    - If an effect has been confirmed but is below the critical effect size (CES), the mine would not be expected to find a larger effect further away or at a higher level. The EEM program can then consider that "the results of the previous biological monitoring study indicate the magnitude and geographic extent of an effect" and that the mine may go directly to IOC.
  5. What is the level of precision required to gain sufficient knowledge to move to surveillance monitoring? i.e.: When has the IOC question been answered well enough to be able to proceed with the next step?"
    - To date, experience is limited; however, experience will bring greater knowledge and a more concrete answer to this question.
    - [Editor's note: This will be determined on a site-specific basis.]
  6. What are the challenges of IOC in the context of complex and/or multiple effluents situations? How do we address them?
    - It was recognized that complex/multiple effluents are challenging. Keith Somers proposed a multivariate approach to deal with multiple data.
  7. Should historical effects be addressed in a standard EEM survey or in an IOC survey?
    - In many studies, it is thought that there are historical effects. A standard survey is preferable, but an IOC survey is permissible if appropriate.
  8. Availability of complex tools and approaches should not disallow use of simple IOC study designs.
    - As long as the regulatory requirements are satisfied, the methodologies need not be complex. Keep the approach simple.
  9. What is the pertinence of initiating an IOC study when causes are known and solutions are being implemented?

- For metal mining, this has not been discussed.
- It was noted that IOC and IOS are combined in *Pulp and Paper Effluent Regulations* (PPER).
- Further discussion arose from this point including the Investigation of Solutions and six year monitoring provisions that currently exist in the PPER.

[Editor's note: According to Subsection 22(1) of Schedule 5 of the MMER, if causes are known, and have been specified in an interpretive report, three year monitoring is permitted to be resumed].

10. A thorough review of previous study designs should be conducted before triggering IOC.
  - If previous studies (standard surveys) had design problems, then there may not be adequate confirmation of effect (and hence the need for IOC is not yet determined). In that situation, it was suggested to re-evaluate the study designs and conduct additional standard surveys before going into IOC.
11. Site-specific problems
  - There must be flexibility to accommodate site specific problems.
  - Tangible examples of problems and solutions are needed.
    - i. Several examples were reported by regional officers in the case study presentations.
    - ii. Consult with Regional EEM Co-ordinators while preparing IOC study design.

### **Program Challenges Not Specific to Investigation of Cause**

During the workshop, other challenges, concerns, and comments were raised on several topics not specifically related to the policy, process, and scientific considerations of IOC studies, and are organised in point form below.

1. Consistency of EEM program between regions.
2. Inter-agency harmonization was encouraged.
3. Streamlining EA and EEM was encouraged.
4. Historical effects and natural variability as confounding factors.
5. Variability (in endpoints & between sites).
6. Study design decision making when there are fluctuating conditions or circumstances
7. Relevance of mercury measurement in fish
8. Statistical differences vs. Critical Effects Size
9. Ecological significance of statistical differences
10. Value of proactive pre-EEM studies
11. Consequence of poor study design
12. Co-operation/collaboration between mines; break isolation (between mines)
13. Decommissioning, low effluent, no discharge: regulatory need (or not) to continue EEM monitoring studies, including IOC.

## **Program Review Update**

In December 2005, Environment Canada initiated a review and established the Metal Mining EEM Review Team which consisted of a group of experts from government, industry, and environmental and aboriginal groups. The Review Team produced a final report which provides an overview of the program review and resulting recommendations for consideration by Environment Canada (*Review Team Report from the Metal Mining Environmental Effects Monitoring Program*, August 2007). This report is available at <http://www.ec.gc.ca/esee-eem/Default.asp?lang=En&n=7EBEA95C-1#Executive%20Summary>. During the workshop, an update of the Review Team was given by Charles Dumaesq. There was a brief discussion following the update, and the following points were raised:

1. Some recommendations may be partially addressed in the proposed amendments to the MMER
2. More discussion/interaction with the Science Committee was requested.
3. A closer look at how Technical Advisory Panel works in each jurisdiction was requested.
4. Pertaining to the ecologically significant effect:
  - a. What is ecologically different?
  - b. It was noted that ecologically significant, as it relates to CES, was documented by Munkittrick. [Munkittrick, K.R., Arens, C.J., Lowell, R.B., Kaminski, G.P. 2009. A review of potential methods of determining critical effect size for designing environmental monitoring programs. *Environmental Toxicology and Chemistry*: 28(7):1361–1371.]
5. What are the wider objectives (performance indicators) of EEM?
6. It was noted that a multi-stakeholder session would be helpful.
7. The importance of communication both with and within the review team was noted.

## **PATH FORWARD**

- The National EEM Office will use these Proceedings to develop the IOC Chapter (focusing on methodologies) in the *Metal Mining Technical Guidance Document for Environmental Effects Monitoring*.
- Concerns and issues raised during the workshop will be the object of subsequent discussions.

## **APPENDICES**

### **Appendix 1: Agenda**

#### **Environmental Effects Monitoring Workshop on Investigation of Cause for Metal Mining**

**December 8 and 9, 2009**

**Holiday Inn – La Chaudière, Gatineau, QC (Nation BC Room)**

<b>Ordre du jour / Agenda</b>
-------------------------------

#### Workshop Objectives

1. To provide background information and a forum for discussion for stakeholders on policy, process and scientific aspects of the Investigation of Cause stage including environmental studies, potential causes of effects, regional perspectives and case studies, and tools and approaches for IOC.
2. The information presented will be used to help develop detailed workshop proceedings and a guidance document for conducting Investigation of Cause studies for metal mining EEM

## Agenda – Day 1 - December 8, 2009

7:30 Inscription / Registration

<b>Session 1: Introduction, Background and Overview of Environmental Studies</b>		<b>Chairs: Robert Prairie (Xstrata Zinc Canada) and Rick Lowell (EC)</b>
8:00-8:05	Welcoming Remarks	Josée Lanctôt (EC)
8:05-8:15	Workshop Introduction and Objectives of Workshop	Robert Prairie (Xstrata Zinc Canada) and / et Rick Lowell (EC)
8:15-8:35	National Assessment of Effluent Effects on Fish and Invertebrates over the First Two Phases of the Metal Mining EEM Program	Rick Lowell (EC)
8:35-8:55	Update on National Investigation of Cause Project on Reduced Gonad Size in Fish	Mark Hewitt (EC)
8:55-9:10	Overview of Existing Guidance for Investigation of Cause in the Metal Mining EEM Program	Bonna Ring (EC)
9:10-9:25	Review of Regulatory Requirements and Policy for Metal Mines and Investigation of Cause Phase of the EEM program	Sherry Walker (EC)
9:25-9:35	Overview of Proposed Amendments to EEM Provisions of the Metal Mining Effluent Regulation	Charles Dumaesq (EC)
9:35-10:00	Discussion	Tous / All
10:00-10:20	<b>Pause santé / Health Break</b>	
<b>Session 2: Potential Causes of Effects / Causes potentielles des effets</b>		
10:20-10:45	Ecotoxicology of trace metals in the aquatic environment	Peter Campbell (Institut national de la recherche scientifique -Eau, terre et environnement)
10:45-11:10	Investigating Selenium Toxicity Using an EEM Approach	Vince Palace (DFO – MPO)
11:10-11:35	Cyanide Speciation and Fate in Mine Tailings: a Case Study	Gérald J. Zagury (École Polytechnique.)
11:35-12:00	Mining reagents and by products (e.g. thiosalts) as potential toxicant in mine effluents	Bernard Vigneault (NRCan)

12:00-1:30	<b>Lunch</b>	
<b>Session 3: Regional Overview and Case Studies</b>		<b>Chairs: Kent England (Cameco) and Jim McGeer (Wilfrid Laurier University)</b>
1:30-1:45	Metal Mining EEM in the Atlantic Region	Sue Ellen Maher (EC)
1:45-2:00	Metal Mining EEM in the Quebec Region	Raymond Chabot (EC)
2:00-2:15	Metal Mining EEM in the Ontario Region	Wes Plant (EC)
2:15-2:30	The Metal Mining Environmental Effects Monitoring Program in the Prairie and Northern Region.	Paula Siwik (EC)
2:30-2:45	The Metal Mining Environmental Effects Monitoring Program in the Pacific and Yukon Region	Mike Hagen (EC)
2:45-3:05	<b>Pause santé / Health Break</b>	
3:05-3:35	Investigation of Cause Study at the McArthur River Uranium Mine	Pierre Stecko (Minnow Environmental)
3:35-4:05	Investigation of Cause of Effects on Benthic Invertebrates at the Kidd Metallurgical Site	Shari Weech (Minnow Environmental)
4:05-4:35	Con Mine: Investigation of Cause (IOC) Study on fish livers– the good and the not so good elements of an designing a new IOC study	Rainie Sharpe (Golder Associates)
4:35-4:45	Wrap-up	Robert Prairie/Rick Lowell

### **Agenda - Day 2 - December 9, 2009**

8:30-8:45	<b>Opening Remarks and Review of Previous Day</b>	<b>Chairs: Robert Prairie (Xstrata Zinc Canada) and Rick Lowell (Environment Canada)</b>
<b>Session 4: Tools and Approaches for IOC Studies</b>		<b>Chairs : Jean-François Doyon (Mines Agnico-Eagle Cadillac) and Mark McMaster (Environment Canada)</b>
8:45-9:00	An update on the use of caged fish for EEM	Vince Palace (DFO – MPO)
9:00-9:25	In situ measurements of toxicity and contaminant bioaccumulation with caged	Lee Grapentine (EC)

	amphipods exposed to water and sediment	
9:25-9:50	Experimental Designs Using Mesocosms to Address Cause for Metal Mine Effluents	Monique Dubé (University of Saskatchewan)
9:50-10:10	<b>Health Break</b>	
10:10-10:35	Toxicity Reduction Evaluations as a Tool for Investigation of Cause - Mining Case Studies	Lesley Novak (AquaTox Testing and Consulting)
10:35-11:00	Fathead Minnow Lifecycle Assays for Assessment of Complex Effluents	Joanne Parrott (EC)
11:00-11:25	Using bioaccumulation models for predicting dissolved metal toxicity	Jim McGeer (Wilfrid Laurier University)
11:25-11:50	Using Redundancy Analysis to Quantify the Cumulative Effects of Multiple Stressors	Keith Somers (OMOE)
11:50-1:20	<b>Dîner / Lunch</b>	
1:20-1:45	Separating Current Effluent Quality from Historical Contamination Using a Laboratory-Based Monitoring Tool for Investigation of Cause	Lisa Taylor (EC)
1:45-2:10	Use and Application of Sediment-Benthic-Transplants in EEM Investigations	Greg Thomas (G3 Consulting)
2:10-2:35	Should biological recovery trigger an Investigation of Cause study, in the context of historical impacts and upcoming mine closure?	Paul Lepage (Minnow Environmental)
2:35-3:00	<b>Pause santé / Health Break</b>	
3:00-4:20	Discussion to identify gaps and research needs	Tous / All
<b>Session 5: Wrap Up and Path Forward</b>		<b>Chairs: Robert Prairie and Rick Lowell</b>
4:20-4:30	Closure	

## Appendix 2: Participant List

Name	Affiliation
Allen, Erik	Environment Canada, EEM, Prairie Northern Region
Audet, Debbie	Environment Canada, EEM, Ontario region
Baron, Chris	Department of Fisheries and Oceans, Mining Impacts
Belles-Isles, Jean-Claude	Association Minière du Québec
Berthelot, Debbie	Rio Algom, BHP Billiton PLC
Billau, Pascal	Environment Canada, Mining and Processing
Boss, Shelly	Environment Canada, EEM, Prairie Northern Region
Bugslag, Colleen	Tantalum Mining Corporation of Canada LTD.
Campbell, Peter	INRS-ETE
Chabot, Raymond	Environment Canada, EEM, Quebec Region
Coumans, Catherine	Mining Watch Canada
Cull, Andrea	Indian and Northern Affairs, Waste Management
Dart, Jeremy	Williams Mine
Davies, Martin	Hatfield Consultants, West Vancouver
Dowsley, Barb	CNSC
Doyon, Jean-François	Mines Agnico-Eagle Cadillac (Québec)
Dubé, Monique	University of Saskatchewan
Dumaresq, Charles	Environment Canada, Mining and Processing
Duncan, Bill	Teck Metals Ltd.
England, Kent	Cameco
Farara, Dennis	EcoMetrix
Fedat, Leah	Xstrata
Flood, Kenneth	Environment Canada, Ontario region
Fraser, Brian	EcoMetrix
Gagnon, Mario	IAM Gold - Mine Doyon
Gardiner, Elizabeth	Mining Association of Canada
Grapentine, Lee	Environment Canada, Aquatic Ecosystem Impacts Research Division
Hagen, Mike	Environment Canada, EEM, Pacific and Yukon Region
Hamilton, Dave	Golder Associates Ltd.
Hart, Ramsey	MiningWatch Canada
Henson, Elisabeth	HydroQual Laboratories Ltd.
Hewitt, Mark	Environment Canada, Ecosystem Health Assessment
Hopky, Glen	Department of Fisheries and Oceans
Huebert, David	Tetres Consultant Inc.
Hunt, Carolyn	Vale Inco
Irving, Elaine	Golder Associates Ltd.
Ji, Kevin (Changhai)	CNSC

Johns, Mike	Stantec Consulting Inc.
Johnson, Korin	Claude Resources Inc.
Kilgour, Bruce	Kilgour & Associates
Kuczynski, Eva	Environment Canada, EEM, Prairie Northern Region
Laflamme, Donald	Rio Tinto, Fer et Titane
Lapierre, Guillaume	AECOM - Tecsalt
Lancôt, José	Environment Canada
Leduc, Julie	Environment Canada, EEM, Quebec Region
Legault, Erin	North American Palladium
LePage, Paul	Minnow
Lowell, Rick	Environment Canada, National EEM Office
Machlans, Hilary	Golder Associates Ltd.
Maher, Sue Ellen	Environment Canada, EEM, Atlantic Region
Martin, Carl	GENIVAR
Marwood, Christopher	ChemRisk
Matteau, Isabelle	Environment Canada, EEM, Quebec Region
McDonald, Dave	Birchtree, Mb (Vale Inco)
McGeer, Jim	Wilfred Laurier University
McKee, Malcolm	Canadian Nuclear Safety Commission
McMaster, Mark	Environment Canada, Ecosystem Health Assessment
Merla, Allison	Vale Inco Limited Copper Cliff, ON
Munkittrick, Kelly	UNB
Ng, Tania	McMaster University
Nilsen, Joel	Hudson Bay Mining and Smelting Co., Limited
Novak, Lesley	Aquatox
Ollila, Harri	Lake Shore Gold
Orr, Patti	Minnow Environmental
Palace, Vince	Fisheries and Oceans Canada, Environmental Science Division
Parkman, Judy	Recycling Organization Against Rubbish
Parks, Derek	AECOM
Parrott, Joanne	Environment Canada, Priority Substances Effects
Penn, Alan	Grand Council of the Crees
Petkovich, David	Access Consulting (EEM consultant for the Minto Mine),
Plant, Wes	Environment Canada, EEM, Ontario Region
Portt, Cam	C. Portt and Associates
Poon, Donna	Environment Canada
Pouliot, Sandra	Mine Agnico-Eagle, division Laronde
Prairie, Robert	Xstrata zinc
Ramilo, Lisa	University of Saskatchewan, Saskatchewan Research Council
Rees, Cassandra	CanNorth

Ring, Bonna	Environment Canada, National EEM Office
Robertson, Erin	Cameco
Rosaasen, Arden	AREVA Resources Canada Inc.
Russell, Cynthia	Minnow Environmental
Ryan, Mike	Wardrop, Winnipeg
Scroggins, Rick	Environment Canada, Biological Methods
Serben, Kerrie	Golder, Saskatoon
Sferrazza, John	AMEC Earth & Environmental Ltd.
Sharpe, Rainie	Golder Associates Ltd.
Sherry, Jim	Environment Canada, Ecosystem Health Assessment
Siwik, Paula	Environment Canada, EEM, Prairie Northern Region
Somers, Keith	OMOE
Sonneberg, Helga	Stantec
Stark, Sheri	Cameco
Stecko, Pierre	Minnow
Stephenson, Malcolm	Stantec
Taylor, Lisa	Environment Canada, Method Development and Application Section
Thomas, Greg	G3 Consulting
Tremblay, Thierry	Iamgold
Vanriel, Peter	Canada North Environmental Services
Vigneault, Bernard	Natural Resources Canada (Environmental Geoscience program)
Walker, Sherry	Environment Canada, National EEM Office
Weech, Shari	Minnow Environmental Inc.
Zagoury, Gérard	École Polytechnique de Montréal

### Appendix 3: Acronyms List

AETE	Aquatic Effects Technology Evaluation
Ag	Silver
AO(0.2)	Aged tailings sample, closed site (A), depths of 0.2 m
AO(1)	Aged tailings sample, closed site (A), depths of 1 m
AQUAMIN	Assessment of the Aquatic Effects of Mining in Canada
AR	Atlantic Region
As	Arsenic
BACI	Before-After-Control-Impact
BC	British Columbia
BF(0.2)	3-month-old tailings, operational site (B), depths of 0.2 m
BF(1)	3-month-old tailings, operational site (B), depths of 1 m
BIC	Benthic invertebrate community
BL	Biotic ligand
BLM	Biotic ligand model
BMS	Biological Methods Section
BO(0.2)	9-year-old tailings, operational site (B), depths of 0.2 m
BO(1)	9-year-old tailings, operational site (B), depths of 1 m
BOD	Biochemical oxygen demand
BTD	Benthic Transplant Device
CANMET	Canada Centre for Mineral and Energy Technology
CaSO <sub>4</sub>	Calcium sulfate
Cd	Cadmium
CEC	Cation exchange capacity
CES	Critical effect size
C:N	Carbon to nitrogen ratio
CN <sup>-</sup>	Cyanide
CNO <sup>-</sup>	Cyanate
CN <sub>SAD</sub>	Cyanide strong acid dissociable
CNSC	Canadian Nuclear Safety Commission
CN <sub>T</sub>	Cyanide total
CN <sub>WAD</sub>	Cyanide weak acid dissociable
Co	Cobalt
Cr	Chromium
Cu	Copper
d	day
DFO	Department of Fisheries and Oceans
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EA/EIS	Environmental assessment or environmental impact statement
EC	Environment Canada
EC50	Median effective concentration
EEM	Environmental Effects Monitoring
EPS 1/RM/33	Environmental Protection Series Report

$\text{Fe}(\text{CN})_6^{3-}$	Ferricyanide anion
$\text{Fe}(\text{CN})_6^{4-}$	Ferricyanide ion
HCN	Hydrogen cyanide
h	hour
Hg	Mercury
IC25	Inhibitory concentration 25%
INRS-ETE	Institut national de la recherche scientifique, Eau Terre Environnement
IOC	Investigation of Cause
IOS	Investigation of Solution
K	Condition factor
KAX	Potassium amyl xanthate
LC50	Median lethal concentration
LM	Light microscopy
LPL	Lowest practicable level
MAC	Mining Association of Canada
MM	Metal Mining
MMER	<i>Metal Mining Effluent Regulations</i>
MMLER	<i>Metal Mining Liquid Effluent Regulations</i>
MMSL	Mining and Mineral Sciences Laboratories
Mn	Manganese
MNML	Miramar Northern Mining Ltd.
Mo	Molybdenum
MPO	Ministère des Pêches et des Océans
MT	Metallothionein
MWWE	Municipal wastewater effluents
NA	National Assessment
NAMC	North America Metals Council
NaOH	Sodium hydroxyde
NAX	Sodium isopropyl xanthate
Ni	Nickel
NRCan	Natural Resources Canada
NSERC	Natural Sciences and Engineering Research Council of Canada
NICNAS	National Industrial Chemicals Notification and Assessment Scheme (Australia)
OMOE	Ontario Ministry of the Environment
Pb	Lead
PCA	Principal components analysis
PPER	<i>Pulp and Paper Effluent Regulations</i>
PPME	Pulp and Paper Mill Effluent
PYR	Prairie and Yukon Region
RCM	Recognized Closed Mines
RDA	Redundancy analysis
$\text{SCN}^-$	Thiocyanate
SD	Standard deviation
SDI	Simpson's Diversity Index
Se	Selenium

SEM	Sequentially extracted metals
SEM/AVS	Simultaneously Extracted Metals to Acid Volatile Sulfide Ratio
SETAC	Society of Environmental Toxicology and Chemistry
$S_2O_3^{2-}$	Thiosulphate
$S_4O_6^{2-}$	Tetrathionate
SS	Statistically significant
TAP	Technical Advisory Panel
TEM	Transmission electron microscopy
TDS	Total dissolved solids
TG	Triglyceride
TIE	Toxicity Identification Evaluation
TI	Thallium
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
TRA	Tissue residue approach
TRE	Toxicity Reduction Evaluation
TSS	Total suspended solids
U	Uranium
UNB	University of New Brunswick
USEPA	United States Environmental Protection Agency
WOE	Weight of evidence
YOY	Young of the year
YCT	Mixture of Yeast, Cerophyll <sup>TM</sup> and Trout chow
YT	Yukon Territory
Zn	Zinc

**WWW.ec.gc.ca**

Additional information can be obtained at:

Environment Canada

Inquiry Centre

10 Wellington Street, 23rd Floor

Gatineau QC K1A 0H3

Telephone: 1-800-668-6767 (in Canada only) or 819-997-2800

Fax: 819-994-1412

TTY: 819-994-0736

Email: [enviroinfo@ec.gc.ca](mailto:enviroinfo@ec.gc.ca)