AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE) PROGRAM

Technical Evaluation of Determining Mining Related Impacts Utilizing Benthic Macroinvertebrate Population Level Fitness Parameters

AETE Project 2.1.5

DETERMINING MINING RELATED IMPACTS UTILIZING BENTHIC MACROINVERTEBRATE POPULATION LEVEL FITNESS PARAMETERS

Discussion Paper

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AQUATIC EFFECTS TECHNOLOGY EVALUATION PROGRAM

Notice to Readers

Technical Evaluation of Determining Mining Related Impacts Utilizing Benthic Macroinvertebrate Population Level Fitness Parameters

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program between the Canadian mining industry, several federal government departments and a number of provincial governments; it is coordinated by the Canada Centre for Mineral and Energy Technology (CANMET). The program was designed to be of direct benefit to the industry, and to government. Through technical and field evaluations, it identified cost-effective technologies to meet environmental monitoring requirements. The program included three main areas: acute and sublethal toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring.

The technical evaluations were conducted to document certain tools selected by AETE members, and to provide the rationale for doing a field evaluation of the tools or provide specific guidance on field application of a method. In some cases, the technical evaluations included a go/no go recommendation that AETE takes into consideration before a field evaluation of a given method is conducted.

The technical evaluations were published although they do not necessarily reflect the views of the participants in the AETE Program. The technical evaluations should be considered as working documents rather than comprehensive literature reviews. The purpose of the technical evaluations focused on specific monitoring tools. AETE committee members would like to stress that no one single tool can provide all the information required for a full understanding of environmental effects in the aquatic environment.

This literature review was conducted in an exploratory context as these methods are at an elementary stage of research and few pilot experiments, field or laboratory studies directly evaluated the effect of metals or mining activities on the parameters examined. The assessment of the utility of benthic macroinvertebrate population level fitness parameters remains incomplete. **AETE Benthos Task Group members do not recommend these methods to be included as tools for monitoring effects for the mining industry.**

For more information on the monitoring techniques, the results from their field application and the final recommendations from the program, please consult the AETE Synthesis Report to be published in the spring of 1999.

Any comments concerning the content of this report should be directed to:

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PROGRAMME D'ÉVALUATION DES TECHNIQUES DE MESURE D'IMPACTS EN MILIEU AQUATIQUE

Avis aux lecteurs

Détermination des impacts de l'exploitation minière à l'aide de paramètres de l'état des populations de macro-invertébrés benthiques

Le Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ÉTIMA) visait à évaluer les différentes méthodes de surveillance des effets des effluents miniers sur les écosystèmes aquatiques. Il est le fruit d'une collaboration entre l'industrie minière du Canada, plusieurs ministères fédéraux et un certain nombre de ministères provinciaux. Sa coordination relève du Centre canadien de la technologie des minéraux et de l'énergie (CANMET). Le programme était conçu pour bénéficier directement aux entreprises minières ainsi qu'aux gouvernements. Par des évaluations techniques et des études de terrain, il a permis d'évaluer et de déterminer, dans une perspective coût-efficacité, les techniques qui permettent de respecter les exigences en matière de surveillance de l'environnement. Le programme comportait les trois grands volets suivants : évaluation de la toxicité aiguë et sublétale, surveillance des effets biologiques des effluents miniers en eaux réceptrices, et surveillance de la qualité de l'eau et des sédiments.

Les évaluations techniques ont été menées dans le but de documenter certains outils de surveillance sélectionnés par les membres d'ÉTIMA et de fournir une justification pour l'évaluation sur le terrain de ces outils ou de fournir des lignes directrices quant à leur application sur le terrain. Dans certains cas, les évaluations techniques pourraient inclure des recommandations relatives à la pertinence d'effectuer une évaluation de terrain que les membres d'ÉTIMA prennent en considération.

Les évaluations techniques sont publiées bien qu'elles ne reflètent pas nécessairement toujours l'opinion des membres d'ÉTIMA. Les évaluations techniques devraient être considérées comme des documents de travail plutôt que des revues de littérature complètes. Les évaluations techniques visent à documenter des outils particuliers de surveillance. Toutefois, les membres d'ÉTIMA tiennent à souligner que tout outil devrait être utilisé conjointement avec d'autres pour permettre d'obtenir l'information requise pour la compréhension intégrale des impacts environnementaux en milieu aquatique.

Cette étude documentaire a été réalisée dans un contexte exploratoire car ces méthodes sont à un stade initial de recherche et peu d'expériences pilotes et d'études sur le terrain ou de laboratoire ont porté directement sur l'évaluation des effets des métaux et des activités minières sur ces paramètres. L'évaluation de l'utilité des paramètres de l'état des populations de macro-invertébrés benthiques demeure partielle. **Les membres du Groupe de travail sur les benthos d'ÉTIMA ne recommandent pas que ces méthodes soient incluses en tant qu'outils de surveillance des effets pour l'industrie minière.**

Pour des renseignements sur l'ensemble des outils de surveillance, les résultats de leur application sur le terrain et les recommandations finales du programme, veuillez consulter le Rapport de synthèse ÉTIMA qui sera publié au printemps 1999.

Les personnes intéressées à faire des commentaires concernant le contenu de ce rapport sont invitées à communiquer avec M^{me} Geneviève Béchard à l'adresse suivante:

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EXECUTIVE SUMMARY

Under the auspices of the Aquatic Effects Technology Evaluation (AETE) Program, a literature review was undertaken to assess eight population level, benthic macroinvertebrate fitness parameters as potential biomonitors for the Canadian mining industry. The parameters were reviewed primarily from the perspectives of ecological significance, technical ease/constraints of execution, and business considerations such as labour requirements and general costs of application. The macroinvertebrate fitness parameters reviewed were:

1. density (no. individuals/species/area)
2. size (head-width, total body length)
3. condition (weight/head-width)
4. fecundity (no. eggs/female)
5. adult emergence (no. individuals, timing of emergence)
6. distributional changes (micro-distributional changes on or within substrate)
7. morphological deformities (irregularities in structure of menta and antennae)
8. fluctuating asymmetry (FA) (irregularities in bilateral symmetry)

These eight parameters were chosen because the first six have a history of use over the last 25 years in assessing natural and anthropogenically induced stress (i.e., impairment to Darwinian fitness) within both lotic and lentic ecosystems. Parameters 7 and 8 have been utilized to measure impacts within aquatic systems primarily since 1990 onward. Of the eight parameters listed, variability in benthic macroinvertebrate density, at both the population and community levels, has been a tool used by the mining industry to assess potential impacts within aquatic systems. Parameters 2-8 are discussed within an "exploratory context" for the mining industry, relative to the utility they may offer to assess potential impacts within aquatic ecosystems.

The linkage of benthic macroinvertebrate species to one another and other components within food webs/ecosystems is illustrated based on research documenting the complex structure and dynamic nature of food webs. These illustrations demonstrate that factors impacting one component of the food web can potentially have a cascading impact upon others. These linkages indicate that by biomonitoring the fitness of benthic macroinvertebrates, extrapolations may be made regarding the health of the food web and the overall potential impacts of mining activities.

Several advantages associated with the use of macroinvertebrates (e.g., general ubiquity, relatively high abundance, short life cycles, general sedentary nature, well defined taxonomy, relative ease of collection) to evaluate the potential impact of stress within aquatic ecosystems are outlined. The utility and advantages of population level rather than community level measures are discussed. Literature suggests that an examination of four species of intermediate density per site is the optimal choice to assess impacts, between control and reference sites, in a practical, meaningful and cost-effective manner.

While each of the eight fitness parameters discussed may be useful biomonitors from a scientific or ecological perspective, not all would be appropriate for use in evaluating environmental impacts from a technical and cost perspective. Based on an extensive literature review and discussions with Beak Environmental Consultants Ltd. and EBA Engineering Consultants Ltd., technical aspects of application and associate costs suggest that density, size, condition, morphological deformities and fluctuating asymmetry may be most useful to assess the effects of mining within aquatic ecosystems. Although analyses of fecundity, adult emergence and distributional changes offer meaningful ecological insight, consultants suggest these parameters may be prohibitively time consuming and costly to apply.

The literature reviewed in this study should provide a foundation upon which pilot experiments may be conducted to ultimately determine whether, and which, population level macroinvertebrate fitness parameters are practical, meaningful and cost-effective as biomonitoring tools for the mining industry. As the study illustrates, the application of these eight parameters to mining is at an elementary stage (with the exception of density,) thus pilot experiments to explore the utility of these parameters appear to be a "logical next step".

SOMMAIRE

Dans le cadre du Programme d'évaluation des techniques de mesure d'impacts en milieu aquatique (ETIMA), une étude documentaire visant à évaluer l'utilité de huit paramètres de l'état des populations de macro-invertébrés benthiques susceptibles d'être utilisés à des fins de surveillance biologique par l'industrie minière canadienne a été réalisée. L'évaluation des paramètres a été effectuée principalement en fonction des critères suivants : importance écologique, aspects techniques facilitant ou restreignant l'application sur le terrain et en laboratoire, et considérations commerciales telles que les besoins en main-d'oeuvre et les coûts généraux d'application. Les huit paramètres évalués sont les suivants :

1. Densité (n^{bre} d'organismes/espèce/unité de surface);
2. Taille (largeur de la tête, longueur totale du corps);
3. Condition (poids/largeur de la tête);
4. Fécondité (n^{bre} d'oeufs/femelle);
5. Émergence des adultes (n^{bre} d'organismes; moment des émergences);
6. Modification de la distribution (micro-distribution sur ou dans le substrat);
7. Malformations (irrégularités dans la structure du mentum et des antennes);
8. Variabilité de l'asymétrie (irrégularités intéressant la symétrie bilatérale).

Les six premiers paramètres énumérés ci-haut ont été choisis parce qu'ils ont été largement utilisés au cours des 25 dernières années pour évaluer l'incidence de divers facteurs de stress naturels ou anthropiques (p.ex. réduction du degré d'aptitude à la survie selon le concept darwinien) dans les écosystèmes lotiques et lénitiques. Les deux derniers paramètres sont utilisés surtout depuis 1990 pour mesurer des effets dans les écosystèmes aquatiques. L'industrie minière se fonde depuis de nombreuses années sur les fluctuations de la densité des macro-invertébrés benthiques (paramètre 1), tant à l'échelle des populations que des communautés, pour évaluer les effets potentiels de ses activités sur les systèmes aquatiques. L'évaluation des paramètres 2 à 8 s'inscrit dans un contexte exploratoire et vise à déterminer dans quelle mesure ces paramètres peuvent aider l'industrie minière à évaluer l'incidence potentielle de ses activités sur les écosystèmes aquatiques.

Les interactions entre les espèces de macro-invertébrés benthiques et entre ces mêmes espèces et les autres composantes des chaînes trophiques/écosystèmes sont illustrées à l'aide d'exemples tirés de recherches attestant de la structure complexe et de la nature dynamique des chaînes trophiques. Ces exemples démontrent que les facteurs qui agissent sur une composante de la chaîne trophique peuvent entraîner une série d'effets en cascades sur les autres composantes. Il est donc possible, en établissant par surveillance biologique l'état des populations/communautés de macro-invertébrés benthiques, de faire des extrapolations concernant la santé de l'ensemble de la chaîne trophique et les effets globaux potentiels des activités minières sur les écosystèmes aquatiques.

L’utilisation des macro-invertébrés benthiques pour évaluer l’incidence potentielle du stress lié aux activités minières dans les écosystèmes aquatiques présentent plusieurs avantages : bonne répartition générale, abondance relativement élevée, cycles de vie courts, mode de vie généralement sédentaire, statut taxonomique bien défini, facilité de capture, etc. Ces avantages sont passés en revue, de même que les raisons qui expliquent pourquoi il est préférable d’étudier les macro-invertébrés à l’échelle des populations plutôt qu’à l’échelle des communautés. L’analyse de la documentation existante révèle que sur le plan pratique et économique et en considération de la valeur des résultats obtenus, l’examen de quatre espèces de densité moyenne par site constitue la meilleure méthode pour évaluer les impacts de l’activité minière en procédant à des comparaisons entre des sites témoins et des sites de référence.

Bien que dans une perspective scientifique ou écologique, tous les paramètres étudiés constituent de bons indicateurs biologiques, certains se prêtent mal à l’évaluation des impacts de l’exploitation minière sur l’environnement en raison de diverses contraintes techniques et économiques. À la lumière des résultats de l’importante étude documentaire consacrée aux aspects techniques de l’application des paramètres et aux coûts associés et de discussions tenues avec des représentants de Beak Environmental Consultants Ltd. et de EBA Engineering Consultants Ltd., il semble que la densité, la taille, la condition, les malformations et la variabilité de l’asymétrie soient les meilleurs paramètres pour évaluer l’incidence des activités minières sur les écosystèmes aquatiques. En revanche, bien qu’elle livre des informations utiles au plan écologique, l’étude de la fécondité, de l’émergence des adultes et de la modification de la distribution se révèle à la fois fastidieuse et onéreuse.

Les sources documentaires examinées dans le cadre de cette étude devraient pouvoir servir de fondement à la tenue d’expériences pilotes visant à déterminer dans quelle mesure les paramètres de l’état des populations de macro-invertébrés peuvent se révéler des outils de surveillance biologique pratiques, fiables et économiques pour l’industrie minière et, le cas échéant, permettre de cerner les paramètres les plus efficaces. Comme l’a révélé cette étude, l’application des paramètres évalués (à l’exception de la densité) au secteur minier revêt encore un caractère exploratoire. La tenue d’expériences pilotes visant à évaluer l’utilité relative de ces paramètres apparaît donc comme une suite logique aux travaux menés à ce jour.

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The report was initiated on the authority of the Aquatic Effects Technology Evaluation (AETE) Program Technical Committee.

The report followed, in part, Environmental Effects Monitoring work performed as a cooperative research project between Falconbridge Ltd. and SSAL. Contributors to the EEM work included Mr. Michael Sudbury and Mr. Bob Michelutti (Falconbridge Ltd., Sudbury, Ontario) and Prof. D. Dudley Williams, University of Toronto (Toronto, Ontario).

Dr. Ian D. Hogg, University of Waicato (Waicato, New Zealand), provided useful comments pertaining to the discussion of Fluctuating Asymmetry (FA).

Ms. Danuta Zaranko of Zaranko Environmental Assessment Services (ZEAS), Mr. Dennis Farara of Beak Consultants Ltd. (Brampton, Ontario), and Mr. Darryl Arsenault, Mr. Tim Bekhuys and Mr. Ted Lederer of EBA Engineering Consultants Ltd. (Vancouver, British Columbia), were instrumental in providing insights pertaining to ecological, technical and business considerations of the eight benthic macroinvertebrate fitness parameters reviewed in this report.

Input from all individuals and firms named above was greatly appreciated.

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1.0 INTRODUCTION

The Canadian Government, mining companies and a diverse array of stakeholders are devoting increasing resources to monitoring and maintaining the integrity of ecosystems. Reflecting this trend is the Aquatic Effects Technology Evaluation (AETE) Program, a cooperative venture between the Canadian mining industry and several Federal and Provincial government departments coordinated through the Canada Centre for Mineral and Energy Technology (CANMET). AETE's mandate is to review appropriate technologies for assessing the impacts of mine activities/effluents on the aquatic environment. To date, this review has included a wide range of field and technical evaluations including, for example, the examination of sample collection methods (e.g., Golder Associates Ltd. 1995), the applied evaluation of aquatic effects monitoring methods (e.g., Beak Consultants Ltd. 1996), and the assessment of potential biomarkers (Couillard and St-Cyr 1997). Reflecting AETE's mandate, the present report focuses on the utility of benthic macroinvertebrate population level fitness parameters to measure the impact(s) of mining activity/effluents within aquatic ecosystems. The macroinvertebrate fitness parameters reviewed are:

1. density (no. individuals/species/area)
2. size (head-width, total body length)
3. condition (weight/head-width)
4. fecundity (no. eggs/female)
5. adult emergence (no. individuals, timing of emergence)
6. distributional changes (micro-distributional changes on or within substrate)
7. morphological deformities (irregularities in structure of menta and antennae)
8. fluctuating asymmetry (FA) (irregularities in bilateral symmetry)

Of these parameters, recommendations are made regarding those most appropriate for use in determining potential mining related impacts.

Consistent with AETE guidelines, and admonitions by Oliver and Beattie (1996), the fitness parameters are evaluated according to the criteria presented in Table 1.

Table 1. Examples of criteria for evaluating the utility of benthic macroinvertebrate fitness parameters.

General Criteria	Specific Criteria
Ecological	• correlation of parameter with other potentially impacted components within an ecosystem • geographic applicability (e.g., predisposition to use within oligotrophic vs. eutrophic systems, or to differing conditions of substrate composition, current speed, water depth, etc.) • potential impact response time
Technical	• simplicity of experimental design • simplicity of parameter measurement • simplicity of statistical analyses • interpretability of results to specialists and non-specialists • general accessibility of experimental equipment
Business	• labour requirements • cost effectiveness

In general, the eight fitness parameters reviewed in this study meet the ecological considerations outlined in Table 1. Limitations are realized more frequently in reference to technical and business considerations.

This report presents a discussion of data characteristics that should be taken into account to determine when parametric versus non-parametric data analyses are appropriate to assess mining related impacts. The discussion references mistakes common to analyses of field data (*sensu* Hurlbert 1984, Peterman 1990a,b). For detailed discussion pertaining to the application of parametric and non-parametric statistical tests, the reader is referred to Sokal and Rohlf (1981) and Zar (1984). Discussion of field sampling protocol and sampling equipment is presented.

Prior to evaluating each benthic macroinvertebrate fitness parameter, macroinvertebrates will first be reviewed, as a group, within the context of their utility as indicators of ecosystem health (*sensu* Rapport 1995).

2.0 BENTHIC MACROINVERTEBRATES AS INDICATORS OF ECOSYSTEM HEALTH

2.1 Historical Perspectives

The use of macroinvertebrates to indicate the condition of aquatic environments likely originated in Europe with the development of the Saprobien system (Kolkwitz and Marsson 1908, 1909). This classification scheme grouped rivers according to the degree of organic contamination and was based on the observation that certain macroinvertebrate taxa (so-called indicator taxa) were restricted in occurrence to aquatic environments with particular environmental conditions. In Europe, the Saprobien tradition has been revised and expanded many times since its inception (e.g., Sladecek 1965, Bick 1971, Foissner 1988) but has not found wide acceptance in North America (Cairns and Pratt 1993).

The North American experience has stressed the importance of chemical measures of water quality, although the indicator species concept was investigated as far back as the 1870's with F.A. Forbes' work on the Illinois River. More modern discussions concerning the use of benthic macroinvertebrates to assess ecosystem health in North America can be traced back to the 1940's and 1950's to the work of Patrick (e.g., Patrick 1949). She and her colleagues stressed the canonical distribution of species and applied this to their work on biological measures of stream condition.

The last two decades have seen the convergence of ideas, regarding the use of benthic macroinvertebrates as monitoring tools, between European and North American scientists, and these ideas have grown to include a variety of novel ways of measuring ecosystem health (e.g., Rapid Assessment Biomonitoring - see review by Resh and Jackson 1993). With few exceptions, macroinvertebrate collection and subsequent data interpretation has become an integral component of most environmental assessment programs.

2.2 Advantages of Benthic Macroinvertebrates as an Indicator Group

Benthic macroinvertebrates offer many advantages as indicators/biomonitors of ecosystem health. Rosenberg and Resh (1993) and Tavares-Cromar and Williams (1996) have summarized some of the advantages:

1. Macroinvertebrates are linked to one another, and by extrapolation to the rest of the food web, in any aquatic system. An example of the complexity of interdependencies within "typical" lotic (Tavares-Cromar and Williams 1996) and lentic (Warren 1989) food webs are illustrated in Figures 1 and 2, respectively. As is evident, impacts affecting one or more species would tend to be manifested by way of cascading impacts throughout the food web. In the event that an impacted species was also a keystone species (Paine 1969), the resulting "domino" effect could be substantial;
2. Macroinvertebrates are generally ubiquitous and found in sufficiently high densities and diversity for statistical analyses across wide-ranging habitats (Lenet *et al.* 1980; Williams and Feltmate 1994). Thus, mining activities would usually occur in localities where numbers of macroinvertebrates would be sufficient for statistical analyses;
3. Macroinvertebrate diversity is generally high within most aquatic ecosystems. High diversity increases the probability of detecting potential impacts. For example, if diversity was low, the few species in question might not be affected by an impact, whereas if diversity was high, the probability of detecting an impact would be greater (based on the chance of encountering sensitive species). High diversity, coupled with

multiple measurable fitness parameters, renders macroinvertebrates suitable to detecting potential stress (Hellawell 1986; Abel 1989);

4. The relative sedentary nature of macroinvertebrates predisposes them to reflect site-specific impacts (Slack *et al.* 1973; Hawkes 1979; Penny 1985; Hellawell 1986; Abel 1989);
5. The life-cycles of macroinvertebrates are typically univoltine, bivoltine or semi-voltine. Short generation times predispose macroinvertebrates to respond to impacts within an ecosystem more quickly than species with long generation times (Lenet *et al.* 1980; Penny 1985; Abel 1989);
6. Impacts to macroinvertebrates can result from physiological or ecological synergism. From a physiological perspective, individual chemical loadings to an ecosystem might not affect a species, however multiple loadings could act additively or multiplicatively resulting in chronic or acute impacts. From an ecological perspective, impacts within an ecosystem could affect changes in predator/prey dynamics, competitive interactions, mating behaviour or immigration/emigration patterns (Hawkes 1979; Wiederholm 1980; Williams and Feltmate 1994). For example, Clements *et al.* (1989) found that two species of net-spinning caddisflies (*Chimarra* sp. and *Hydropsyche morosa*) were more vulnerable to the predaceous stonefly *Paragnetina media* under conditions of elevated copper loading. They suggested that:

   > "the structure of benthic communities may be indirectly affected by metals",

   and that monitoring of aquatic ecosystems must be sensitive to such stress;

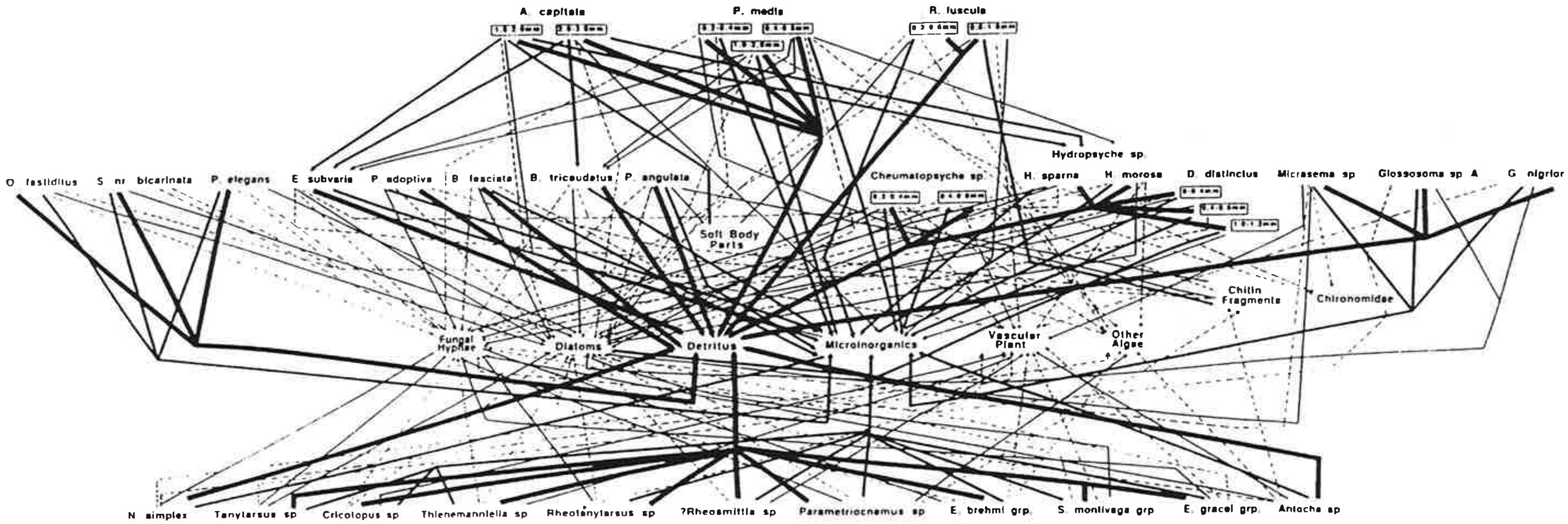

Figure 1. Summary food web of the Duffin Creek, Ontario, riffle insect fauna (the thickness of lines represents the relative importance of the resource; different size classes of some of the more prominent species are indicated in boxes). (redrawn after Tavares-Cromar and Williams 1996).

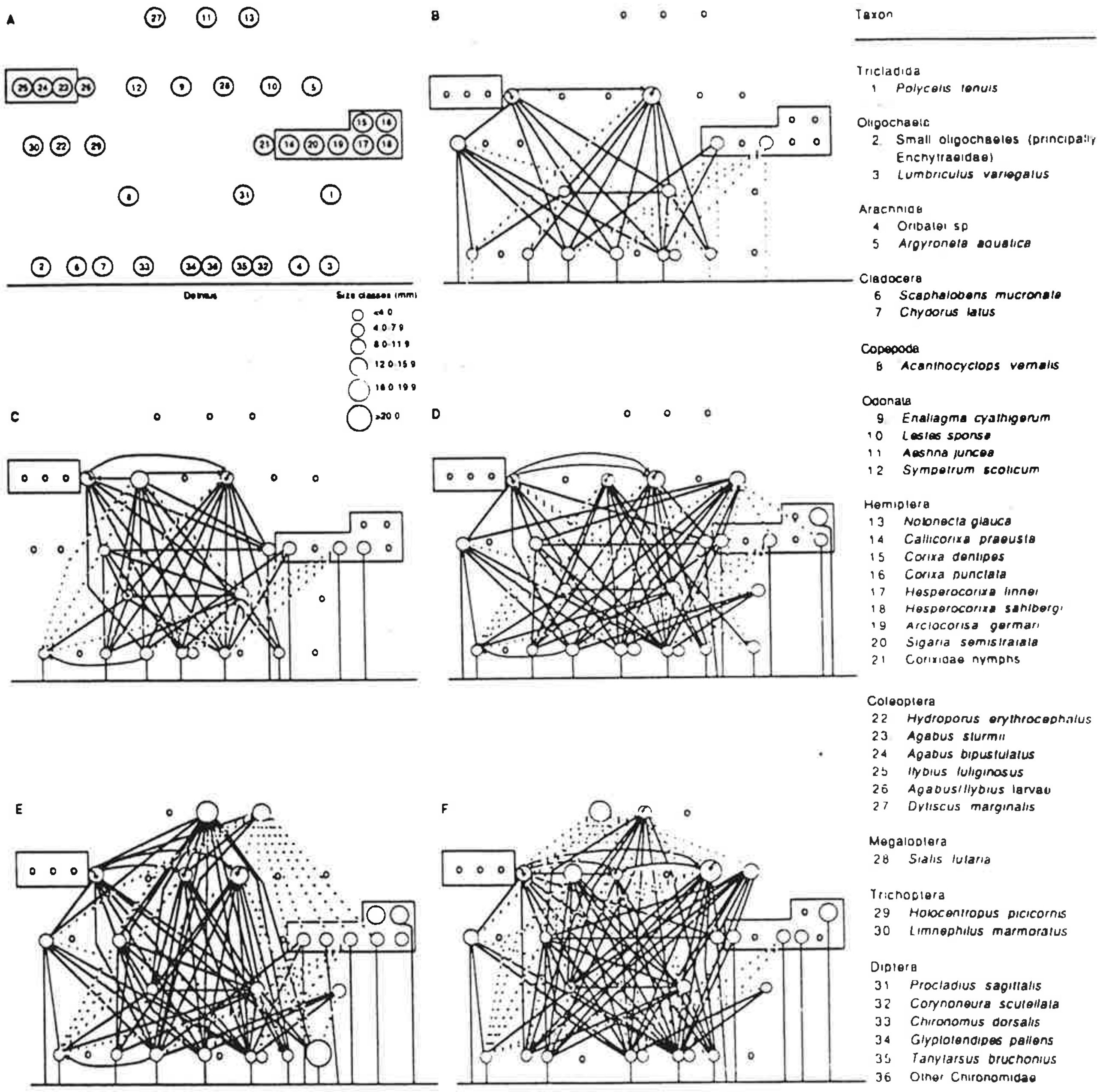

Figure 2. Food web for selected date/habitat combinations in a pond in Yorkshire, England. Species are represented by circles and links are directed downwards from consumer to resource unless otherwise indicated. Links within circles denote cannibalism. Very small circles (with no links) represent the positions (on Web Template A) of species which were not found in that particular sample. The six sizes of largest circles indicate the mean sizes of species. Boxes enclosing species indicate that the adult and larval stages of those species occurred in the web. A = Web Template: B = Open Water, March: C = Open Water, June: D = Open Water, October: E = Margin, March: F = Margin, June. (redrawn after Warren 1989).

7. Qualitative and quantitative sampling of macroinvertebrates can be performed using inexpensive equipment (Hellawell 1986; Williams and Feltmate 1994);
8. The taxonomy of nearly all orders of macroinvertebrates is well illustrated with keys (Abel 1989);
9. The responses of certain groups (e.g., Ephemeroptera, Plecoptera, Trichoptera - EPT) to physical/chemical impacts has been reasonably well established (Hilsenhoff 1987; Williams and Feltmate 1994);
10. Macroinvertebrates generally survive well under laboratory conditions, and are predisposed to laboratory experimentation. In the laboratory, factors affecting impacts observed in the field can often be isolated (Rosenberg *et al.* 1986); and
11. Many analyses involving macroinvertebrates are non-labour-intensive, and in general are cost effective.

2.3 Disadvantages of Benthic Macroinvertebrates as an Indicator Group

Although benthic macroinvertebrates convey advantages as biomonitors, they also present limitations that can make it difficult to determine whether mining or naturally occurring factors affect change at either the population or community level. Rosenberg and Resh (1993) discuss these limitations:

1. The distribution and abundance of macroinvertebrates within ecosystems can be affected by many abiotic and biotic factors other than water quality, such as current speed (Erman and Erman 1984; Feltmate *et al.* 1986), substrate composition (Allen 1975; Reice 1980; Minshall 1984; Erman and Erman 1984), distribution of prey (Sweeney 1984; Feltmate and Williams 1989), and the presence of predators (Stein

and Magnuson 1976; Brusven and Rose 1981; Feltmate and Williams 1991);

2. Benthic macroinvertebrates do not respond to all impacts. For example, Hawkes (1979) reported minimal effects of a herbicide at low concentrations on the invertebrate fauna of a river, yet angiosperms downstream were adversely affected;
3. Variation in distribution and abundance of macroinvertebrates occurs naturally due to ontogenetically driven change (Butler 1984) and to the physico-chemical dynamics of ecosystems; and
4. Macroinvertebrates, although relatively sedentary, can drift/crawl from one location to another (Brittain and Eikeland 1988; Anholt 1995).

In each case, the difficulties associated with the use of benthic macroinvertebrates as monitoring tools can be minimized by incorporating previously determined ecological knowledge into either the experimental design or the interpretation of results specific to the species under investigation. For example, life-history knowledge of the species will help to offset the potential confounding effects of the seasonal distribution of many taxa. Similarly, knowledge of habitat preferences and drifting behavior is fundamental to adequately assess the localized effects of point-source pollution (i.e., is the presence/absence of potential indicator species a result of the localized pollution impact, or is it the result of natural life-history variation?).

2.4 Population Versus Community Level Analyses

Historically, impact assessment field studies have primarily focused on benthic invertebrate community level measures of the absolute or relative density of all species present (e.g., Pulp and Paper EEM field component, Environment Canada), whereas population level analyses have mostly been restricted to laboratory toxicity testing (Rosenberg and Resh 1993). Several advantages may be realized if population level analyses complement community surveys, or where appropriate, are conducted in their absence. Conversely, population level studies are not without disadvantages.

Advantages of population level investigations generally relate to the execution of studies as well as to data analysis. For example, as only a select number of taxa need to be identified in a population level study (i.e., four species are recommended as a generally applicable number; see Section 4.3 for further discussion), as opposed to all macroinvertebrates in a community survey, sample handling time (e.g., taxonomic identification and sorting) would be reduced. As Allen (1975) suggests, this may be particularly advantageous when it is determined that many samples are required to reach desired statistical power.

Additionally, analysis of population level data is simpler and less time consuming -- but not necessarily less rigorous -- than multivariate tests often used to analyze community level data (Gerritsen 1995). Population level data need only be investigated using univariate tests (e.g., T-test, ANOVA), the results of which are usually more easily interpreted than multivariate analyses (Sokal and Rohlf 1981). As suggested by Gerritsen (1995), procedures for operational bioassessment should be simple and understandable, and analytical complexity should be kept to a minimum when possible.

Further benefits regarding population versus community level assessments pertain to the monetary costs associated with field and laboratory work. In systems where macroinvertebrate diversity is low, the costs of population and community analyses will obviously approximate one another. Alternatively, in highly diverse systems, population level studies may be cost effective relative to community assessments.

Assigning specific cost savings to population versus community level studies is difficult, because most studies tend to focus on populations or communities (thus making direct cost comparisons difficult), and the monetary cost of studies is seldom available. To assess approximate costs associated with various population level metrics analyses, pilot studies which monitor the comparative costs of population and community level analyses would be instructive (for further discussion see Section 5.0).

The disadvantages associated with population level studies are two-fold. First, when focusing on populations, historical knowledge of the systems under study can be instructive in choosing representative taxa (for further discussion see Section 4.1). Unfortunately, historical data are often not available. Second, an extensive literature review (see Section 3.0) reveals that there has been limited application of population level measures of size, condition, fecundity, adult emergence, distributional changes, morphological deformities and fluctuating asymmetries to mining impact studies. Hence, direct evidence regarding the utility of population level measures to assess the potential stress of mining does not currently exist. The potential of these measures can be only be inferred based upon the application of these measures within other industry sectors and pure and applied academic research. Thus, as suggested previously, pilot studies would prove useful in identifying the potential utility of population versus community level measures to assess the potential impact of mining within aquatic systems.

3.0 BENTHIC MACROINVERTEBRATE FITNESS PARAMETERS

3.1 Measuring Impacts Using Benthic Macroinvertebrates

Potential impacts (i.e., "change" relative to ecosystem conditions that would characterize an area naturally) to benthic macroinvertebrates resulting from ecosystem stress can be measured using various response variables. Impacts can be manifested biochemically and physiologically through reductions in fitness parameters and/or morphological abnormalities at the level of populations, or through changes in community composition and dynamics. In certain instances, change can occur in a direction that, for example, might increase species diversity and abundance relative to natural conditions. This change would also be an impact, because the ecosystem no longer resembled its natural state. A fundamental principle of mining should therefore be that the "environmental footprint" of operations be minimized vis-à-vis natural, background conditions.

This review focuses on the potential utility of eight population level benthic macroinvertebrate fitness parameters -- density, size, condition, fecundity, adult emergence, distributional changes, morphological deformities, and fluctuating asymmetry -- as means to assess impact/change within aquatic environments. Each parameter is described and details are provided regarding its use in previous impact assessment related studies.

A search of over 600 articles/reports representing the refereed and gray (i.e., non-published reports prepared by consulting firms) literature was conducted, to identify studies where one or more of the eight fitness parameters under review had been used to assess the impact of various forms of stress within aquatic ecosystems. The studies reviewed focused on organic and non-organic forms of stress originating from both anthropogenic and non-anthropogenic sources. Although relatively few in number, studies that reviewed ecosystem impacts resulting from mining activities or metalliferous waste were identified.

In the following discussion, an "impact site" refers to an aquatic habitat potentially disturbed by mining, and a "reference site" refers to its undisturbed, natural analogue. Impact and reference sites are usually in close geographic proximity.

3.2.1 Density

Field measurements of the effects of heavy metals on aquatic environments using benthic invertebrates are most frequently accomplished by comparing the population density of several species between reference versus impact sites. Where differences in density occur, and the reference and impact sites are statistically identical relative to physical characteristics with the exception of metal contamination, the effect is assumed to result from the contaminant. Examples where this type of strategy has been employed are illustrated in Table 2.

Table 2. Examples of selected studies linking changes in benthic macroinvertebrate density to metalliferous waste.

Reference	Comments
Winner *et al.* 1980	• data collected from two streams: one experimentally augmented with metals and the other receives effluent from a small metal plating industry • significant differences between density of chironomids, caddisflies and mayflies at sites classified as unpolluted, moderately polluted or heavily polluted • chironomids numerically dominant at heavily polluted sites, chironomids

	+ caddisflies at intermediately polluted sites and mayflies at unpolluted sites • metal concentrations (µg/l) range from: Cu, 28-1250; Cr <1-910; Zn, 16-508
Leland *et al.* 1989	• experimental dosing of an oligotrophic stream with Cu (2.5-15 µg/l) • noted decline in density of all major orders (Ephemeroptera, Plecoptera, Trichoptera, Diptera) with herbivores and detritivores more sensitive (i.e., responded to lower Cu doses) than predators
Clements *et al.* 1990	• response by invertebrates to Cu (11.3 µg/l) in laboratory and field experimental streams • following 10 days of exposure density of several numerically dominant taxa (e.g., net-spinning hydropsychid caddisflies) was reduced up to 75% and 44% in the laboratory and field streams respectively • concluded laboratory toxicity tests may overestimate metal toxicity and recommended that field studies be used in their place
Dickman *et al.* 1990	• comparison of density of several invertebrate taxa of the Welland River near the Atlas Specialty Steel Co. • 0-10 m downstream of the point source discharge, benthic invertebrates were absent; 10-15 m downstream only oligochaetes found; 15-120 m downstream appearance of pollution tolerant chironomids; 120-180 m downstream isopods, snails and leeches established and overall benthic invertebrate density highest • source vs. reference concentrations of metals: 4,900 vs. 10 ppm Ni, 890 vs. 5 ppm Pb, 1050 vs. 30 ppm Zn, 5120 vs. 10 ppm Cr
Moore *et al.* 1991	• comparison of benthic invertebrate densities for several taxa over a 215 km course of the Blackfoot River, Montana, whose headwaters receive mine effluent • significant difference between densities of Trichoptera, Ephemeroptera, and Plecoptera at most vs. least contaminated sites • Cd, Zn, and Cu the most toxic and bioavailable metals to the fauna with metal concentrations from 1 to 3 orders of magnitude higher at contaminated vs. uncontaminated sites
Munkittrick *et al.* 1991	• comparison of benthic fauna at contaminated (from a mixed metal mine) vs. reference sites in lakes in northern Ontario • density ranges from 30 to 100% greater at reference vs. contaminated sites for several chironomid taxa (e.g., *Polypedilum simulans*, *Tanytarsus*, *Parachironomus*) • absence of several invertebrate orders (e.g., Plecoptera, Ephemeroptera, Odonata, Amphipoda) at contaminated vs. reference sites • Diptera account for 78-96% vs. 40-75% of total individuals at contaminated vs. reference sites
Clements *et al.* 1992	• benthic taxa compared upstream and downstream from a coal-fired power plant • reference stations dominated by several species of Ephemeroptera and Tanytarsini chironomids whereas impacted sites were dominated by Orthocladiini chironomids and net-spinning caddisflies • total invertebrate density 1 to 2 orders of magnitude less at impacted vs.

	reference sites • Cu and Zn concentrations an order of magnitude higher at impacted vs. reference sites
Clements 1994	• upstream/downstream comparison of benthic fauna along Arkansas River, Colorado • reference sites were dominated by Ephemeroptera (Baetidae, Heptageniidae, Ephemerellidae), whereas moderately and highly contaminated sites were dominated by Trichoptera and Orthocladiinae chironomids • density of dominant taxa was 2 to 3 times greater at reference vs. impacted sites • Zn concentrations ranged from ~250 ppb at reference sites to > 3000 ppb at impacted sites
Gower *et al.* 1994	• benthic macroinvertebrate data collected from 46 sites on 12 Cornish streams affected to varying extents by past metalliferous mining • at sites with the highest Cu and Al concentrations (up to 1271 ppb and 1671 ppb, respectively), Ephemeroptera were absent but the Orthocladiinae chironomids, flatworms and net-spinning Trichoptera were abundant
Kiffney and Clements 1996a,b	• experimental exposure of macroinvertebrates to metals in stream microcosms and compared the response to those measured at reference and metal polluted sites • total abundance, abundance of Ephemeroptera, abundance of Plecoptera and abundance of non-net-spinning Trichoptera were all higher at reference vs. treated microcosm and impacted sites • taxa in higher elevation streams were more sensitive than higher order and low altitude streams

3.2.2 Use as a Tool for the Mining Industry

The evidence presented suggests a link between benthic macroinvertebrate density at the population level and metalliferous waste, and both field and experimental studies have shown that several taxa from different invertebrate orders respond to elevated metal concentrations. However, it is not suggested that density be used as a "stand alone" measure for assessing the potential impacts of mining effluent on aquatic environments. Although it is an indicator of an effect, benthic macroinvertebrate density can be influenced by numerous other variables. For example, Clements (1994) noted density differences in caddisflies and blackflies between reference and impacted sites in the Upper

Arkansas River, but suggested that their distribution was influenced more by factors such as food availability rather than metal concentrations. Moreover, macroinvertebrate responses were complicated by natural seasonal and longitudinal changes (*sensu* the River Continuum Concept) in community structure and he suggested that determination of the effects of heavy metals on benthic taxa requires careful evaluation of natural changes in community composition. Similarly, Feltmate *et al.* (1997) found significant statistical differences in density for three target species between reference and potentially impacted sites along Coniston Creek, Sudbury. Measures of other macroinvertebrate fitness parameters (e.g., chironomid mouth part deformities, size and condition) suggested that the differences observed may have resulted from changes in stream morphology. Both of these studies indicate that cautious interpretation of data is required in order to not overestimate the relationship between metal loading and faunal density at the expense of other possible explanations.

From a technical perspective, density is a simple fitness parameter to measure. Quantitative samples can be collected using Hess, Surber, Wilding, Ekman or other readily available samplers (Williams and Feltmate 1994). The taxonomical expertise required to identify the fauna is also readily available. The costs associated with the measurement of density are significantly lower than a typical community survey impact assessment study (see Table 4), as only a predetermined number of species need to be identified and subsequently counted (i.e., four species are recommended as a generally applicable number; see Section 4.3 for further discussion). The selection of appropriate species would be greatly enhanced if historical benthic survey data were available for reference and potentially impacted sites.

3.3.1 Size

The size (i.e., head capsule width or body length) of benthic macroinvertebrates can be affected if organisms, under conditions of stress, change feeding habits, suffer reductions in food availability, alter physiological resource allocation or generally succumb to what Sih (1987) referred to as a “slow life-style”. Theoretically, as energy is diverted from “growth” to “maintenance to cope with stress”, body size should decrease (Odum 1985). Many studies corroborate this theory.

For example, Hogg and Williams (1996) found that elevated temperature which mimicked global warming resulted in reductions in size at maturity for *Nemoura trispinosa* (Trichoptera) and *Hyallela azteca* (Amphipoda). Similarly, Rempel and Carter (1987) observed that higher temperatures resulted in smaller body size of stream Diptera in outdoor experimental channels. This trend was consistent for both predator and detritivore groups. Baker and Feltmate (1987) found that a higher availability of food to newly moulted *Ischnura verticalis* (Odonata) increased the relative size of nymphs between moults. Feltmate and Williams (1991) showed significant reductions in the size of nymphs of *Paragnetina media* and *Alloperla mediana* (Plecoptera) between sections of stream where predator stress was high versus low. Palmer (1995) found that *Baetis bicaudatis* and *Epeorus deceptivus* (Ephemeroptera) grew larger in environments where resources were distributed homogeneously rather than in patches.

Evidence from a limited number of both laboratory and field studies demonstrates similar responses to metalliferous contamination. In laboratory tests, Biesinger and Christensen (1972) reported decreases in body size of > 20% for *Daphnia magna* following incubation at metal concentrations 16% of the reported 3 week LC_{50}. Wentsel *et* *al.* (1977) showed that the growth of the chironomid *Chironomus tentans* was severely impaired in sediment with 1,030 ppm Cd, 17,300 ppm Zn and 1,640 ppm Cr. In the absence of any other explanation, they attributed this to the toxicity of the metals. More recently, Hatakeyama (1987) investigated the chronic effects of Cd on growth of the chironomid *Polypedilum nubifer* in a flow-through aquarium. At Cd concentrations of 20 and 40 ppb growth was reduced by 20 and 40%, respectively, relative to control.

Kosawalt and Knight (1987a, b) observed that growth of fourth instar larvae of the chironomid *Chironomus decorus* was inhibited by sediment bound copper at concentrations that were 6 times lower than the reported LC_{50} value. In laboratory experiments, larvae of the chironomid *Glyptotendipes pallens* were exposed to several sublethal concentrations of cadmium, resulting in lower biomass of exposed larvae compared to unexposed larvae (Heinis *et al.* 1990). More specifically, larvae exposed to 1.0 mg/l Cd weighed significantly less than unexposed larvae and larvae exposed to Cd concentrations of 2.5 mg/l and greater had reduced biomass compared to the larvae exposed to 1.0 mg/l Cd. Similarly, Simkiss *et al.* (1993) observed a significant decrease in both pupae size (as dry weight) and adult emergence size (as dry weight) for blowflies exposed to cadmium concentrations of 2.0 mg/l and higher in laboratory microcosms.

3.3.2 Use as a Tool for the Mining Industry

Direct evidence of the usefulness of size as a parameter to measure stress within a mining context is presently lacking. However, in a few studies (e.g. Biesinger and Christensen 1972, Kosawalt and Knight 1987a, 1987b), reductions in size have been observed at metal contaminant concentration levels that indicated no impacts based on

LC_{50} analyses. These observations appear to indicate that invertebrate size is a useful "early warning" indicator of stress in aquatic environments. The fact that size has been used successfully to measure stress resulting from various impacts supports the notion that it has the potential to be useful to measure mining impacts, but it must still be substantiated with complementary laboratory and field testing.

From a technical standpoint, the measurement of size requires no special expertise nor equipment. Size can be measured as total body length or head capsule width. To make such measures, an ocular micrometer would be used in conjunction with the dissecting microscope used for taxonomical identification. An ocular micrometer is a device that places a ruler in the field of vision of the microscope user and is standard equipment in most laboratories. Alternatively, size can be measured as biomass. Dry weight, freeze dry weight and wet weight are all commonly used measures (Williams and Feltmate 1994). Mass can be measured with either a six or eight place mechanical or electronic balance.

Comparison of the size of individuals from control versus impact sites is most appropriately done for individuals of the same instar. If comparison of individuals at different developmental stages is made, differences in size cannot be reliably attributed to the potential stress. Readily available taxonomic keys provide details regarding the characteristics that help to identify the different developmental stages, or instars, of larval aquatic insects.

If the size measurement to be used is biomass it is important that samples are collected, processed and stored identically to avoid differences measured as the result of weight loss to the storage medium (Wen 1992). This likely precludes the use of biomass comparison of individuals that are presently being maintained in archive collections, especially if comparisons of inter-annual specimens are required. If dry-weight measures are used, the drying process generally destroys animals for future use.

Performed as part of a benthic invertebrate community survey study, measures of species' size would not add significantly to project costs, as measurements could be made for target species during the invertebrate identification stage of analysis (see Table 4).

3.4.1 Condition

Condition is a direct measure of the fitness or "health" of an individual in a population. For benthic invertebrates, condition is the ratio of individual weight per unit head width (e.g., mg/mm, µg/mm). A higher condition value indicates a more "fit" macroinvertebrate. The greater weight a macroinvertebrate possesses per unit head-width is a reflection of more resources that can be channeled into growth and reproduction. In the event that an animal is stressed, energy (i.e., weight) is diverted to the "maintenance" of life systems, thus affecting a reduction in condition (Odum 1985). The discussion of causal factors affecting stress in reference to size would also apply to condition. Changes in condition resulting from stress have been documented, though not explicitly in reference to mining effluent or metalliferous waste.

For example, Baker and Feltmate (1987) found that higher availability of food to newly moulted *I. verticalis* increased the relative size of larvae between moults, and their condition. Baker (1989) tested the effects of food shortage on condition of larval *Ischnura verticalis* (Odonata) under laboratory conditions. He noted statistically significant differences in the condition of larvae fed on diets varying in quality. Feltmate and Williams (1991) showed significant impacts on the condition of nymphs of *Paragnetina media* and *Alloperla mediana* (stoneflies) as the result of predator stress. Nymphal condition decreased by as much as 50% in sections of a stream where trout were added versus sections in which trout were excluded. In addition, adult condition was reduced for emergent *P. media* and *A. mediana* from predator stressed sections of stream. Hogg and Williams (1996) noted a decrease in condition for amphipod, stonefly and caddisfly species following an ecosystem level manipulation of the thermal regime of a stream. Condition differences were only observed between the control and experimental channels in the post manipulation phase of the study. Prior to the temperature manipulation, no differences were detected between the sections of the stream designated control and experimental.

In sum, these studies indicate that condition varies in response to stress within aquatic ecosystems.

3.4.2 Use as a Tool for the Mining Industry

Macroinvertebrate condition has only seen limited use as a tool in assessing impacts, and it has received virtually no attention as a measure of mining related stress within aquatic ecosystems. Nevertheless, as with measures of size, where condition has been used it has been an effective parameter for measuring the effects of agents of stress. Thus, evidence suggests that it may offer a practical tool for measuring mining related impacts.

From a practical perspective, labour requirements and overall costs associated with measures of condition would parallel those required to assess macroinvertebrate density and size (see Table 4). Because the determination of condition requires measuring biomass, specimens will be destroyed when the dry weight measure is used. As with comparisons of size, it is important to compare the condition of specimens from control and impact sites that are within the same instar.

3.5.1 Fecundity

Stress within ecosystems can affect reductions in benthic macroinvertebrate fecundity (e.g., number of eggs/female). Various studies have documented reductions in fecundity resulting from impacts.

Hogg and Williams (1996) found evidence of reductions in fecundity of immature *Hyallela azteca* (Amphipoda) and *Nemoura trispinosa* (Plecoptera) in sections of stream heated to mimic global warming. Similarly, Feltmate and Williams (1991) observed lowered fecundity in nymphal *Paragnetina media* and *Alloperla mediana* (Plecoptera) collected from predator stressed sections of stream.

Studies linking the effects of contaminant related stress on fecundity are often similar in design to common toxicity tests. For example, Sibley *et al.* (1997) observed significantly decreased reproductive output of *Daphnia magna* (Branchiopoda) and *Tubifex tubifex* (Oligochaeta) for individuals cultured with sediment collected up to 400 m downstream from bleached kraft pulp mill outflow. Green and Chandler (1996) conducted an experiment to assess chronic effects of a sediment containing organophosphate pesticide on a population of benthic copepods. They observed a chronic toxicity response, including reduced fecundity, with significant population effects resulting from all pesticide treatments (7-32% of the 96 hr LC_{50}) versus the control.

Some research has focused on the effects of metal toxicity on the fecundity of invertebrates. Koivisto and Ketola (1995) measured the effect of copper (10-30 ppb) on the fecundity of two benthic zooplankton species, *Daphnia pulex* and *Bosmina longirostris*. Of the two, only *Bosmina* showed reduced fecundity (>40%) and the effect was strongly correlated with increasing copper concentrations. Wiederholm *et al.* (1987) and Wiederholm and Dave (1989) studied the influence of trace metal pollutants on a number of life-history traits of 5 oligochaete species. They found reproductive capacity was the most sensitive measure of sediment toxicity and that fecundity was reduced by as much as 100% (generally 50-60%) after 270 days of incubation in bulk sediment microcosms where metal levels were greater than twice background levels.

Reductions in fecundity can be directly attributable to smaller body size (Clifford and Boerger 1974). Clifford and Boerger (1974) observed an upward curvilinear relationship between total body length and egg production in the mayflies *Leptophlebia cupida* and *Hexagenia limabata*. These effects were dramatic as, for example in *L. cupida*, differences of 6 mm in total body length (an approximate 40% reduction) lowered fecundity from 5,600 to 1,900 eggs. Such changes in fecundity per unit of body length occur because abdominal volume changes as a cubic function of length. It is this allometric relationship that likely makes fecundity a sensitive indicator of stress within ecosystems. As has been demonstrated in the preceding sections, decreased size can be a consequence of stress inducing agents, including metals, therefore the likelihood that the same agent will cause a decrease in fecundity is strong.

3.5.2 Use as a Tool for the Mining Industry

While the effects of metals on benthic macroinvertebrate fecundity have only been documented to a limited extent, the available evidence suggests that it is a sensitive measure of stress within aquatic systems.

Measuring mining-related impacts vis-à-vis fecundity of immature larvae and nymphs would present technical difficulties. Fecundity measures would be restricted to reproductively mature, late instar specimens, sufficiently large to dissect and enable an egg count. From a consultant's perspective, "fecundity measurements seem more appropriate for fish than invertebrates. Almost all aquatic invertebrates are at the larval stage of their life-history. Consequently, gonadal structures are not developed well enough to accurately identify fecundity levels" (*personal communication*, Darryl Arsenault, EBA Engineering Consultants Ltd.). Discussions with Danuta Zaranko (*personal communication*, ZEAS) regarding the utility of fecundity measures parallel comments by D. Arsenault. Both consultants suggest that fecundity measures could be cost prohibitive (see Table 4). The usefulness of fecundity as a practical measure of mining related stress ultimately requires a pilot study.

3.6.1 Adult Emergence

A large proportion of benthic macroinvertebrate insects emerge from aquatic to terrestrial environments when making the transition from immature to adult. Previous work has demonstrated that if stressed, emergence by macroinvertebrates (e.g. the number of animals that successfully moult to adulthood, the timing of emergence) can be affected.

For example, Schulz and Liess (1995) observed that the addition of insecticides in experimental streams resulted in reduced emergence of *Limnephilus lunatus* (caddisfly) and *L. bipunctatus*. Notably, the concentration of the insecticide that demonstrated stress on these species was approximately five orders of magnitude below the 96 hr LC_{50}. They concluded that chronic insecticide exposure can be hazardous to freshwater macroinvertebrates at unexpectedly low concentrations, and that these effects are not reliably predicted by standard toxicity tests.

In an extensive review of literature on the impacts of water warmed by power stations, Langford (1975) documented how even slight elevations in temperature could lead to early emergence of macroinvertebrates into cold air that may be lethal to mating. Similarly, Hogg and Williams (1996) found that the artificial warming of a stream caused the early onset of adult insect emergence. Altered adult emergence may isolate the affected population reproductively, as unaffected conspecifics follow an unaltered timing of emergence. Breeding restricted to the same population will almost certainly reduce the genetic and overall fitness of populations over the long term (Hogg *et al.* 1996).

Few field or laboratory studies have directly assessed the impact of metals on aquatic insect emergence. In classic work performed by Hall *et al.* (1980) in the Hubbard Brook Experimental Forest, New Hampshire, dilute concentrations of sulfuric acid were added to a stream until a pH of 4 was reached. This condition was maintained for a 4 month period, April to July. The experimental addition of sulfuric acid caused aluminum and magnesium to be mobilized in the stream, eventually reaching concentrations more than double background levels (> 0.2 mg/l and > 0.5 mg/l respectively). As a result of the combined effects of lowered pH and elevated metals, total insect emergence decreased by 37%. The decrease was mostly confined to three insect orders: mayflies (Ephemeroptera), stoneflies (Plecoptera), and true flies (Diptera). Larger scale detrimental effects of the acid-metals stress were also observed. For example, as overall species diversity decreased, there was an increase in the representation of community dominants and a decrease in the complexity of the food web.

Pascoe *et al.* (1989) and McCahon and Pascoe (1991) studied the effects of low doses of cadmium on *Chironomus riparius* larvae in the laboratory. In both cases, significantly more animals survived from the control conditions than from any of the test solutions, and emergence was delayed by up to 50% for concentrations as low as 0.15 μg/l. In laboratory experiments with the same species, Williams *et al.* (1989) noted effects on emergence at concentrations 5000x lower than the 48 hr LC_{50} for fourth instar larvae. They suggested that chronic metal exposure was likely to have a much greater impact on aquatic insect taxa at lower concentrations than predicted by toxicological tests.

3.6.2 Use as a Tool for the Mining Industry

The above studies demonstrate a link between both reduced and delayed macroinvertebrate emergence and various environmental disturbances, including metal loading to aquatic ecosystems resulting in concentrations higher than background levels. Unfortunately, the data from which these conclusions are drawn are not extensive.

The probable results of impacted macroinvertebrate emergence on the next larval generation are reduced density, reduced species diversity, and increased representation of tolerant taxa. Historically, studies have focused on these latter measures, rather than emergence, to assess potential impacts. While it may be desirable to examine emergence as a biomonitoring tool, there are practical limitations.

Collecting adult macroinvertebrates requires placing emergence traps (e.g., Mundie Pyramid Samplers, Hamilton Samplers, Edmunds Samplers, Aquatic Light Traps) in field studies for extended periods of time (i.e., 1-2 months) (Williams and Feltmate 1994). The traps, once placed, must be monitored at least every 3-4 days to remove adults, and subsequently reset the traps. Although this exercise is very simple, it can elevate project costs significantly by increasing billable labour costs, especially where the work requires extensive travel (see Table 4).

Another difficulty with emergence traps is ensuring that they are not destroyed by "passers-by", or lost during floods. This problem is not insoluble, but it calls for vigilance and care in anchoring equipment which, again, is likely to affect labour costs.

3.7.1 Distributional Changes

Distributional changes refer to changes in habitat location of benthic macroinvertebrates on a micro scale. That is, these changes occur on the scale that would be equivalent, for example, to changes in substrate size and type selection (e.g., rock vs. sand vs. plant), or changing exposure to current speed, water depth or shading. Micro distributional changes are distinct from macro distributional changes, as larger scale changes (e.g., exclusion of certain taxa from a river reach due to toxic conditions) would be manifested and measured as changes in other fitness parameters such as density (see Section 3.2).

Many species of benthic macroinvertebrates have the behavioural capacity to select favourable habitats. For example, in a laboratory setting the distribution of the stonefly nymph *Paragnetina media* was significantly influenced by the independent and interactive effects of current speed and substrate size as well as the presence of a potential predator (Feltmate *et al.* 1986).

Similarly, animals may alter their distribution in response to a perceived environmental stress (Clements 1994). Pennuto and deNoyelles (1993) examined changes in micro habitat selection of *Drunella coloradensis* (Ephemeroptera) nymphs in experimental channels following pH reductions of 1 and 2 pH units below ambient. At an intermediate decline in pH, nymphs were more active and burrowed more frequently than in control channels (micro level distributional change). Following a larger decline in pH, burrowing activity approximated normality, however drifting and crawling increased (macro level distributional change). They suggested that changes in distribution through micro habitat selection might offer useful tools in toxicological research, testing for potential hazards by yielding more realistic estimates of "first-effects" concentrations of stressors.

No studies have explicitly tested hypotheses relating to the effects of metals on distribution and micro habitat selection, as characterized here. Most often, micro-location changes are manifested and subsequently measured as macro location changes.

3.7.2 Use as a Tool for the Mining Industry

Although measuring changes in the distribution of species to detect impacts can provide useful ecological insights, logistically this parameter presents many challenges. It can be extremely labour intensive (i.e., weeks of sampling; see Table 4) to measure changes in the distribution of only one species, at one location, and is not well suited for measurement outside the laboratory. Thus, relative to detecting mining impacts this measure is of little practical value. Only under exceptional circumstances would this measure prove useful. In addition, at present, there is little direct evidence available that links predictable changes in micro distribution to metalliferous or mining effluent. As indicated previously, changes in distribution have been, historically, measured at the macro scale and are likely to continue as such.

3.8.1 Morphological Deformities (Menta and Antennae)

Under natural conditions, aberrant forms may spontaneously occur within different invertebrate groups (Milbrink 1983). Most often, however, deformities occur concomitantly with some form of stress in the environment. Deformities in benthic macroinvertebrates can be manifested in several ways, but most occur in the mouth parts (e.g., missing/extra teeth, highly misshapen teeth), chaeta (e.g., irregular teeth and prongs), and/or antennae (e.g., loss of genuine segments, reduction in antennal length).

The greatest volume of research performed in this area has focused on, though has not been limited to, chironomids and the causal link between contaminants and morphological deformities in larval menta and antennal structures. Numerous causal agents have been implicated including herbicides, chlorine, industrial effluents (including heavy metals), petrochemicals, pesticides and municipal waste. Below, examples are provided where heavy metal contamination was thought to be the primary or a significant causative agent. Table 3 summarizes studies in which deformities were used to assess ecosystem impacts, but not necessarily as the result of metalliferous effluent.

Warwick *et al.* (1987) collected larvae of the chironomid *Chironomus* in the harbour of Port Hope, Ontario, where sediments were known to be contaminated by both radionuclides (^{238}U and ^{232}Th) and various heavy metals at concentrations that far exceeded reported values for similar habitats in surveys around the Great Lakes. In total, 83% of specimens collected from the inner harbour had deformed mouth parts, while only 14% were found to be deformed in outer, less contaminated harbour sediments. They suggested that the data implied a direct relationship between the degree of sediment contamination and the incidence of deformities in the *Chironomus* populations studied. They were unable, however, to account for the relative causative nature of the deformities as both radionuclides and heavy metals are known to have teratogenic affects.

Warwick (1990) examined morphological deformities in several chironomid taxa in the Lac St. Louis and Laprairie basin section of the St. Lawrence River near Montreal, Quebec. The most severely deformed larvae in Lac St. Louis were found in close proximity to an industrial complex where heavy metal contamination was known to be severe. In areas shielded from the main flow of the St. Lawrence River, and in areas farther from industry, the incidence and severity of deformities decreased. Fossil chironomid data indicated extensive changes in the benthic community over the last 100 years. Warwick suggested that the presence of deformities in the contemporary chironomid communities was likely the result of chemical contaminants.

Van Urk *et al.* (1992) documented mouth part deformities in the chironomid genus *Chironomus* over a contaminated sediment gradient in the Rhine River. Sediments at the six contaminated study sites had trace metal concentrations an order of magnitude higher than those observed at the reference site, but also had high levels of persistent organic pollutants (e.g., PCB's). No attempt was made to account for the relative contributions of the likely causative agents. The frequency of deformities was greater at sites heavily and moderately contaminated when compared to reference and mildly contaminated sites. They also found that prior life-history knowledge of the target species was of great importance as the frequency of deformities was highest within the over-wintering generation, and that this cohort was most suitable for assessing ecosystem impacts using this approach.

Feltmate *et al.* (1997) compared a variety of benthic invertebrate fitness measures, including the incidence of chironomid mouth part deformities, at potentially impacted and reference sites along Coniston Creek, near Sudbury, Ontario. The head waters of Coniston Creek are located in close proximity to a smelter waste depository. No significant differences were detected in the frequency of deformities for the two target species at sites close to the head waters versus downstream locations, although trace metal concentrations were marginally higher at the former. As a positive correlation between the incidence of deformities and environmental stress (including heavy metal contamination) has been shown elsewhere (e.g., Kosawalt and Knight 1987a, Van De Guchte and Van Urk 1989, Jannssens *et al.* 1992), it is possible (but untested) that the metal concentrations at upstream locations were below the threshold at which abnormal levels of deformities would be induced.

Table 3 provides examples of studies describing morphological deformities where various other causative agents were implicated.

Table 3. Examples of selected studies linking morphological deformities in benthic macroinvertebrates to environmental stress.

Reference	Taxa	Deformity	Comments
Hamilton and Saether 1971	*Chironomus, Procladius, Protanypus*	mouth and body	< 1% of specimens collected from L. Erie and Okanagan lakes, BC, deformed; industrial and agricultural pollutants
Hare and Carter 1976	*Chironomus cucini*	mouth	0-78% of specimens from 9 sites affected; the most contaminated site yielded most deformities; industrial effluents
Tooby and Macey 1977	Hemiptera: Corixidae *Corixa punctata, Sigara dorsalis*	lack of pigmentation	only presence, not relative incidence of deformity, provided; herbicide
Donald 1980	Plecoptera: Capniidae *Isocapnia integra, Utacapnia columbiana*	mouth, antenna	11-14% and 15-80% of *U. columbiana* and *I. Integra* deformed at affected stations, respectively (<3% at control sites); domestic and industrial pollutants
Milbrink 1983	Oligochaeta: Tubificidae *Potamothrix hammoniensis*	chaeta	16-78% of specimens deformed in areas of heavily contaminated sediments vs. < 10% in unaffected areas; industrial and municipal waste
Cushman 1984	*Chironomus decorus*	mouth	4.6, 2.7 and 3.0% of specimens deformed at 75, 45 and 15 ml oil/m^3 respectively; coal liquid
Warwick 1985	*Chironomus tentans, Chironomus spp.*	antenna	incidence of deformity increased with relative amount of pollutedness; industrial and agricultural pollutants including DDE
Percy *et al.* 1986	*Chironomus samoensis*	abdomen	higher incidence of deformities at higher doses of UV radiation
Warwick and Tisdale 1988	*Chironomus spp., Procladius, Cryptochironomus*	mouth, antenna	2x incidence of deformities for each taxa at more polluted sites; industrial and agricultural pollutants

Dickman *et al.* 1990	Chironomidae, various genera	mouth	9% deformed at control site vs. 47% deformed at site downstream from a tire manufacturing company; industrial pollutants
Camargo 1991	Trichoptera *Cheumatopsyche spp.*, *Hydropsyche pellucidula*	gills, anal papillae	62-83% of specimens deformed at affected sites; specimens at control sites normal; similar laboratory results; chlorine
Madden *et al.* 1992	*Chironomus spp.*, *Procladius paludicola*, *Dicrotendipes conjunctus*	mouth, antenna	laboratory and field components show higher incidence of deformities with exposure to high levels of DDT and Dacthal
Diggins and Stewart 1993	*Chironomus spp.*	mouth	high incidence of mouth part deformities likely the result of prolonged exposure to industrial pollutants in the heavily polluted Buffalo River
Hudson and Ciborowski 1996	*Chironomus*, *Cryptochironomus*, *Polypedilum*, *Stictochironomus*, *Phaenopsectra*	mouth	incidence of deformities varied significantly among genera; deformities more common in sites with intermediate contamination vs. heavily polluted sites; industrial and municipal pollutants

3.8.2 Use as a Tool for the Mining Industry

Few studies have attempted to link metal levels in invertebrate larvae tissue to the incidence of deformities. Janssens *et al.* (1992) compared Cd, Pb, Cu, and Zn concentrations in field populations of *Chironomus thummi* with and without deformed mouth parts. They found significantly greater overall metal concentrations in the tissue of deformed specimens. Similarly, Kosawalt and Knight (1987b) and Van De Guchte and Van Urk (1989) found a linear relationship between percentage of deformities in the epipharyngeal pecta of larval chironomids, and substrate and food bound copper concentrations and sediments spiked with Cd, Cu and Zn, respectively. These types of studies, though not abundant, are important because they not only establish the correlative nature of the relationship between metal contamination and the incidence of morphological deformities, but they also suggest a causal linkage.

Some limitations pertaining to the utility of deformities as indicators of stress include the following: (1) although the direction of response (i.e., incidence of deformity) to stress is predictable, the magnitude is not; and (2) at present the full extent of deformities in benthic invertebrates has not been indexed, though indices are available for particular taxa (Warwick 1985, 1991). More field studies and the initiation of intensive laboratory experiments are needed to redress these deficiencies.

At present it is likely that consultants are not well familiar with the techniques required to apply morphological deformities as a biomonitoring tool (*personal communication*, Danuta Zaranko, ZEAS). This situation exists because to date the need for this specialization has not existed (*personal communication*, Dr. Ian D. Hogg, University of Waicato, New Zealand, and Dr. D. Dudley Williams, University of Toronto, Canada). Active researchers in this field are mostly found in universities. In the event that researchers skilled in the science of morphological identification and quantification become accessible, the application of morphological deformities as a biomonitoring tool would not be prohibitive from a labour or cost perspective (see Table 4). The process of taxonomical identification of taxa that are commonly used in deformity studies (e.g., chironomids, oligochaetes) requires a compound microscope. The presence/absence of deformities can be assessed during identification (*personal communication*, Darryl Arsenault, EBA Engineering Consultants Ltd.).

3.9.1 Fluctuating Asymmetry (FA)

Fluctuating asymmetry (FA) is defined as random differences which occur between the left and right sides of normally bilaterally symmetrical organisms (Van Valen 1962; Clarke 1993). The underlying theory of FA analysis proposes that the presence of environmental stress during development may disrupt normal developmental processes, thus affecting an increase in FA (Clarke *et al.* 1986). In practice, FA is simply measured as the absolute difference between morphological traits (e.g., antennae segment length) on the right versus left side of the body. FA differs from morphological deformities (which include only structural abnormalities) and tends to focus on "easily" measured physical characteristics.

Positive correlations between FA and numerous environmental stress types have been noted in a variety of species. For example:

- FA of stenopleural chaeta in *Drosophila melanogaster* and abnormal rearing temperature (Parson 1962);
- FA of pectoral fins in populations of grunion and DDT concentrations (Valentine *et al.* 1973);
- FA in skulls of the Baltic seal and pollution level (Zakharov and Yablokov 1990);
- FA in shrew skulls and heavy metal concentrations (Pankakoski *et al.* 1992); and
- FA in fins of rainbow trout and water temperature (Leary *et al.* 1992).

These studies indicate that FA as a direct measure of environmental stress is applicable across a variety of species. However, few studies have directly assessed the impacts of environmental perturbations (including mining effluent) on benthic populations. In fact, although a recent review documented an increased popularity in studies attempting to link phenotypic/morphologic variability in benthic invertebrates to environmental perturbations (see Hogg *et al.* 1996), only three examples of FA were cited.

Hogg *et al.* (1996) examined seven readily measurable morphological features (e.g., length of various antennal segments, the number of dorsal spines, and the length of leg structures) of the amphipod *Gammarus fasciatus* at seven sites along the St Lawrence River, between Cornwall, Ontario, and Quebec City, Quebec. As predicted, FA scores differed among the seven sites, with the three control sites scoring lowest (i.e., least asymmetrical), followed by three agricultural/municipal sites, and finally the highest scores (i.e., most asymmetrical) were found at a site known to be PCB contaminated.

In a separate study, Hogg *et al.* (*personal communication*, unpublished data; I.D. Hogg, University of Waicato, New Zealand) found significantly greater FA (leg segment and antenna measurements) in stoneflies collected from an experimentally heated spring-brook channel than from an adjacent reference site.

Clarke (1993) measured FA in populations of the chironomid *Chironomus salinarius*, to assess the impact of a fertilizer manufacturing facility on surrounding freshwater ecosystems. Specimens from the "waste-water site" (a pond receiving facility waste after biological treatment) were significantly more asymmetric than those at the "site pond" (pond on facility property but not directly receiving facility waste), or the "control site" (control pond approximately 15 km from the facility). Further, the asymmetry value for the site pond population was greater than that of the control pond, although the difference was not significant. The high FA levels were attributed to developmental stress caused by high levels of methanol, nitrates, phosphates and ammonia generated by the fertilizing manufacturing processes, and subsequent release (directly or indirectly) into the surrounding ecosystems.

3.9.2 Use as a Tool for the Mining Industry

The potential of FA to assess the impacts of mining activities on freshwater ecosystems is presently untested. Evidence suggests that FA is widespread among invertebrate and vertebrate taxa, and that it provides a direct biological measure of environmental stress. Clarke (1993) suggests other relevant scientific advantages to the FA approach, as follows: (1) FA is readily adaptable to the laboratory and can be used to assess the response of asymmetry to known concentrations of stressors to develop dose-response curves; (2) when control populations are difficult to identify, museum or preserved reference specimens can be assessed to determine baseline FA levels for comparison with potentially impacted populations; and (3) FA can be used following remedial action and thus provide a means for evaluating its utility as an indicator of stress.

From a technical and business standpoint, FA presents few, if any, logistical difficulties. Both Hogg *et al.* (1996) and Clarke (1993) suggest that such metrics would fall easily within the skill sets of most practitioners and that FA analyses would require only readily accessible sampling equipment. Overall projects costs would be minimal relative to community surveys (*personal communication*, Darryl Arsenault, EBA Engineering Consultants Ltd.).

Presently, however, there is little baseline data on the use of FA applied to benthic macroinvertebrates, especially regarding the effects of metalliferous waste. Consequently, questions remain regarding which species may be most suitable to this analysis, and what body parts should be compared. Field and laboratory pilot studies are required to evaluate the significance of the FA approach for assessing the impacts of mining effluents in freshwater ecosystems.

4.0 TECHNICAL CONSIDERATIONS

4.1 Benthic Macroinvertebrate Field Collection

Although field sampling procedures and collection techniques for benthic macroinvertebrates are fully described in Golder Associates Ltd. 1995, Beak Consultants Ltd. 1996 and Taylor 1997 (the reader is encouraged to review these studies), a brief review of sampling considerations and precautionary measures is appropriate.

A common goal in benthic macroinvertebrate studies pertaining to mining activity is to estimate the abundance of one or more species within a defined locality. Descriptive or survey sampling is used to estimate parameters such as means, standard errors, or the total number of species per unit area, using a variety of techniques.

The most popular technique used in descriptive field sampling is the simple random sample. For example, if the goal is to assess the mean density for one or more species within a reach of stream below a tailings area, an artificial grid can be placed over the stream (a physical grid is not necessary, it can simply be estimated), and sampling locations may be determined by consulting a random numbers table. Any one of a number of schemes can be used to ensure that there is a one-to-one correspondence between a randomly-selected number and grid location. As a general rule, a specific sampling location is usually not sampled twice, as many sampling methods are destructive and subsequent sampling would yield biased estimates.

When dealing with large populations in most aquatic systems, it is generally not important to distinguish between sampling with and without replacement (i.e., returning the test "unit" or specimen, with minimal disturbance, to its place of origin) when calculating confidence limits. However, as Eberhardt and Thomas (1991) suggest, sampling with replacement should be considered if the sampling procedure is prolonged and can actually affect density over time.

For convenience, researchers often sample using a systematic protocol. However, there is inherent danger in doing so if, by chance, periodicity in the sampling regime coincides with some natural repetition (thus biasing estimates). To limit potential error, the individual conducting the field sampling should be sufficiently familiar with the system to ensure that the sampling regime does not coincide with any natural or artificial periodicity patterns (Hansen *et al.* 1953).

Benthic macroinvertebrates, once collected and stored in alcohol, can be kept indefinitely. However, as discussed previously, comparative analyses of size and condition of specimens should be limited to samples collected and stored during the same time period, thus limiting the likelihood of potential differences in biomass loss to the storage medium.

4.2 Species Selection

Benthic macroinvertebrate species appropriate for inclusion in population level studies share common features. Foremost, individual species used to discern potential impacts must be sufficiently widespread to facilitate comparisons between both impacted and control sites (Johnson *et al.* 1993).

Johnson *et al.* (1993) suggest that the choice of species appropriate for detecting impacts should include those that are high or intermediate in abundance, and that rare species should be avoided. Where possible, species should be chosen from the Ephemeroptera, Plecoptera and Trichoptera, as mayflies, stoneflies and caddisflies are typically sensitive to environmental impacts.

Alternatively, Pearson *et al.* (1983) argues that taxa from intermediate abundance classes are most appropriate to assess impacts. Rare species are not preferred as they may be rare for reasons other than the effects of the impact. Furthermore, time and money constraints preclude rare species for inclusion in studies in sufficient number to perform meaningful statistics. According to Pearson *et al.*, numerically dominant taxa should also be excluded from analyses, because they may have opportunistic characteristics, such as high reproductive capacity and strong dispersal mechanisms, that allow for high numbers to exist within systems that are "stressed" environments.

Johnson *et al.* (1993) suggest that in the future, regional or national surveys may be used to classify sites based on macroinvertebrate assemblages that should be present in the absence of environmental stress (*sensu* Furse *et al.* 1984). For example, the work of Johnson (1989) on 45 Swedish lakes enabled the classification of lake type based on chironomid assemblages. However, Johnson recognizes that the classification of systems will remain an objective that will not be achieved for decades on any large geographical basis.

In summary, the selection of benthic macroinvertebrate species for use in detecting mining related impacts should, where possible, be chosen from the Ephemeroptera, Plecoptera, and Trichoptera. If these Orders are not represented, selection of more common taxa, such as chironomids and amphipods, is perfectly acceptable. Consensus favours selection of species that are intermediate in abundance within the control site. Pilot studies which focus on population level multiple response variables could prove instructive in helping to direct the selection of organisms most appropriate for inclusion in mining impact studies, at either the genus or species level of classification.

4.3 Number of Species Tested

As discussed in reference to trophic relationships, macroinvertebrates are linked to one another within food webs (see Figures 1 and 2). Thus, direct impacts to specific organisms in a food web would probably be evident as secondary impacts to otherwise non-impacted species (Cain *et al.* 1995; Tavares-Cromar and Williams 1996). For both scientific and practical reasons (i.e., cost and labour), it is important to determine the number of species (or lowest taxonomic level practically achievable -- *personal communication* Danuta Zaranko, ZEAS) that must be examined to detect potential impacts within an ecosystem.

Although essentially no research has focused on the number of benthic macroinvertebrates that must be examined to assess impacts related to metalliferous mining, the topic has been addressed within the broader context of toxicity testing. Birge and Black (1982), Mount (1982), Mayer and Ellersieck (1986) and Blanck *et al.* (1984) suggest that from three to five species (incorporating two to three orders) should be used to establish a biological response range. Specifically, Blanck *et al.* (1984) noted only a marginal increase (< 4%) in the range of sensitivity to toxins when more than five species were included in an impact study. Similarly, Mayer and Ellersieck (1986) found that adding additional species, beyond five, only increased the probability of obtaining a lower toxicity value by 2.5% per organism. Their review indicates that the cost involved in adding another test species would not substantially improve the database/impact assessment.

Relative to the above studies, it would seem reasonable that four or five species of benthic macroinvertebrate per site evaluation would likely be effective for population level field surveys. For example, Feltmate *et al.* (1997) used four fitness measures (density, size, condition, morphological deformities) applied to each of four species to assess the potential impacts of mining effluent within Coniston Creek near Sudbury, Ontario. Although they observed density differences between upstream and downstream sampling locations, no differences were noted in reference to size, condition or morphological deformities. They suggested that given the lack of any more plausible explanations, that density differences were probably not causally linked to discharges from a tailings area, but rather to natural morphological variation within Coniston Creek.

Pilot studies which focus on the utilization of three to six species of benthic macroinvertebrate to detect impacts of mining related impacts would help to determine the appropriate number of species for use in assessing potential mining related impacts. At present, and until further studies indicate otherwise, consensus favours focusing on four or five species, representing several orders where possible, per location to assess potential impacts.

4.4 Precautionary Statistical Procedures

A review of all statistical procedures (e.g., parametric tests such as t-tests/ANOVA, or non-parametric tests such as Mann-Whitney U-Test/Kruskal-Wallis) used in impact assessment studies is beyond the scope of this review. Accordingly, the reader is referred to any one of numerous texts on the subject (e.g., Sokal and Rohlf 1981, Zar 1984, Gilbert 1989).

However, despite extensive literature on the subject, statistical tests used to assess impacts within aquatic systems are often misapplied (Peterman 1990a, Eberhardt and Thomas 1991). Therefore, it is appropriate to briefly review issues that may help practitioners to avoid mistakes that may be committed when assessing mining related impacts within aquatic systems.

Peterman (1990a) suggests that the strongest inferences between cause and effect relationships in aquatic systems can be determined using randomized, replicated experimental approaches. However, because there is generally an inverse relationship between replicate number and the size of sampling units, *a priori* power analyses should be used to determine the number of replicates necessary to detect treatment effects between experimental and control sections of stream or lake. Failure to do so increases the probability of committing Type II errors (i.e., accepting false "null" hypotheses), a reality largely ignored in aquatic studies. To illustrate, Peterman (1990b) found that 98% of surveyed papers in the fisheries and aquatic literature that did not reject the null hypothesis (*Ho*), failed to report *b* (the probability of committing a Type II error), and of those papers 52% drew conclusions as if *Ho* were true. As a precaution, power calculations (*b*) should at least be used in impact studies where the probability outcome of tests is close to the $\propto = 0.05$ level. Failure to do so could result in an incorrect belief that an activity was not impacting an ecosystem.

Closer adherence to the underlying assumptions of parametric versus non-parametric analyses would also help to decrease the probability of falsely accepting or rejecting hypotheses in aquatic studies. Three assumptions which apply to parametric tests that are commonly ignored by practitioners include (Zar 1984): (1) The need for true random sampling of individuals from a population. Non-random sampling can result in non-normal distributions, lack of homogeneity of variances, or non-independence of sample species, items, etc. (Sokal and Rohlf 1981). Precautionary measures and procedures to ensure that sampling is random are essential (see Section 4.1). It is, in some instances, possible to address a lack of independence in data using tests designed for such circumstances (e.g., Repeated Measures Analysis of Variance, RMANOVA). (2) Samples should be drawn from populations that are normally distributed. Either a simple graphical analysis or Goodness of Fit check (e.g., Kolmogorov-Smirnov) may be used to test for normality. To correct for, or to "normalize" data, one of several transformations may be applied (e.g., square root, logarithmic [x+1], arcsin $\sqrt{x}$). (3) Sample variance between groups should be homogeneous within the bounds of random variation. The equality of variances is referred to as the condition of *homoscedasticity*, and a check for this condition can be made using an F-max test. The test relies on the cumulative probability distribution of a statistic that is the ratio of the largest to the smallest of several variances.

When making multiple comparisons of means using pair-wise comparisons (as is often the case when testing for potential aquatic impacts), an experiment-wise or Bonferroni error adjustment of the alpha should be made. This adjustment essentially splits the level of significance evenly among all comparisons, thus lowering the probability of committing a Type I error (i.e., when performing multiple comparisons the probability of finding a significant difference, due to chance, increases with the number of comparisons).

When parametric criteria are not met, non-parametric analogues which are distribution neutral and relatively quick and easy to perform may be used (Lehner 1979).

However, because there are fewer constraints on using non-parametric tests, they tend to be less powerful than their parametric analogues in detecting treatment effects. Thus, where possible, transformations to meet assumptions of normality and homoscedasticity should be applied. Some argue (e.g., Kirk 1968) that parametric tests are sufficiently robust that assumptions can sometimes be violated without affecting the validity of tests. However, such violations, without clearly defined limits, enter into a nebulous realm of statistics that can make parametric analyses questionable (i.e., at what point does the violation of test criteria become unacceptable?). Thus, it is prudent to adhere to the axioms of parametric analyses when testing for potential impacts resulting from mining.

The degree to which experimental criteria are violated in field and laboratory studies was discussed by Hurlbert (1984) vis-à-vis pseudoreplication, defined as "the use of inferential statistics to test treatment effects with data from experiments where either treatments are not replicated (although samples may be) or replicates are not statistically independent". He found that 48% of experimental studies which used inferential statistics, published between 1960-1984, involved pseudoreplication. Although the incidence of pseudoreplication is greatest in studies of marine benthos and small mammals, it was found to be common in studies on benthic macroinvertebrates. Thus, caution should be used to ensure that *perceived* replicates are *true* replicates when assessing mining related activities within aquatic systems (see Section 4.1).

5.0 COSTS OF APPLICATION

Biomonitoring tools must offer cost effective insights into impacts that mining activities may cause within aquatic systems (Brinkhurst 1993). Relative to the eight population level parameters reviewed in Section 3.0 -- i.e., density, size, condition, fecundity, adult emergence, distributional changes, morphological deformities, fluctuating asymmetry -- the consulting firms Beak Consultants Ltd. (Brampton, Ontario), and EBA Engineering Consultants Ltd. (Vancouver, British Columbia), were asked to provide estimates and/or comment on costs associated with field collection, laboratory and statistical analyses, and to relate those costs to comparable community level assessments of macroinvertebrate density and richness.

Both firms agreed that absolute monetary estimates were difficult to establish, for two reasons. First, of the eight fitness parameters reviewed in Section 3.0, density is the only measure that has been commonly applied to assess mining impacts within aquatic systems. The other seven parameters are relatively unknown, thus without a reference point, assigning costs proves difficult and is subject to error. Second, the variability encountered between sites (e.g., size of systems, depth of water, substrate composition, species density, diversity, etc.) would affect costs. Accordingly, Beak Consultants Ltd. and EBA Engineering Consultants Ltd. agreed that pilot studies (perhaps two in each of eastern and western Canada) would be instrumental in identifying costs associated with the fitness parameters under review, and to relate those costs to community level assessments.

Although pilot studies offer the optimal means to benchmark costs associated with each of the eight population level fitness measures, general estimates are possible.

The results of estimated costs associated with the eight population level fitness parameters, and community level assessments of density and richness, are presented in Table 4, based on data supplied by EBA Engineering Consultants Ltd. Discussions with ZEAS and Beak Consultants Ltd. determined that their estimate of costs paralleled those presented in Table 4 (*personal communication*, Danuta Zaranko and Dennis Farara). Both firms agreed that the equipment costs associated with population and community level analyses were negligible relative to labour costs.

Table 4. Costs incurred for field collection, laboratory and statistical analyses of benthic macroinvertebrates, to assess impacts between a control and reference site. Population analyses focus on the eight fitness parameters listed, and community analyses focus on density and richness. The reference system is a 3rd Order stream, mixed substrate composition, with a benthic invertebrate diversity of 40-50 species.

Benthic Macroinvertebrate Fitness Parameter	Costing ($ Can.)[1] Field Collection	Laboratory Analysis	Statistical Analysis	Total
Population Level				
1) Density	1,000	240	300	1,540
2) Size	760	260	300	1,320
3) Condition	760	260	300	1,320
4) Fecundity	760	500	300	1,560
5) Adult Emergence	2,000	90	300	2,390
6) Distributional Changes	3,000	400	600	4,000
7) Morphological Deformities	760	180	300	1,240
8) Fluctuating Asymmetry	760	180	300	1,240
Community Level	2,000	300	1,200	3,500
Combined Fitness Parameters[2]				
2,3,7 and 8	760	300	600	1,660
1,2,3,7 and 8	1,000	340	750	2,090

[1] Costing is based on two test sites (one upstream and one downstream of a point source) and a field crew of two persons. Population analyses include four invertebrate species x 50 individuals. Costs do not include chemical analyses or other site characterization measures (e.g., substrate composition, plant species assemblages, etc.) which would apply equally to population and community assessments.

[2] Costing is reduced by collecting a single sample, sorting a single sample, analyzing invertebrates for all features simultaneously, and performing the same set of univariate statistics on each set of parameters.

(Table 4 data supplied by D. Arsenault, EBA Engineering Consultants Ltd.).

As Table 4 indicates, costs associated with the two fitness parameters -- adult emergence and distributional changes -- are high relative to those of the other six parameters. Accordingly, Beak Consultants Ltd. and EBA Environmental Consultants Ltd. suggest that adult emergence and distributional changes would probably not be appropriate for assessing mining related impacts. Measures of fecundity were also thought to be cost prohibitive by both consultants.

Consultants recognized that if density was the focus of a study, that the additional costs associated with assessments of size, condition, morphological deformities and fluctuating asymmetry, if all parameters were treated simultaneously, would be minimal (see Table 4, Combined Fitness Parameters). Relative to this point, D. Arsenault (*personal communication*, EBA Engineering Consultants Ltd.) suggested that "combinations of fitness parameters should be considered, as it is very little additional effort to assess several measurements while invertebrates are under the microscope. Consequently, if these variables were analyzed simultaneously, a substantial cost savings would be realized."

Within the larger context of the use of parametric analyses versus community level assessment, D. Arsenault suggested that "the use of fitness parameters is, in my opinion, more defensible and more easily interpretable by review agencies and the general public than community ordination procedures." Discussion with D. Zaranko (*personal communication*, ZEAS) also reflects this point.

Relative to cost resulting from a community level impact evaluation (i.e., $3,500.00, see Table 4), the additional costs resulting from assessments of size, condition, morphological deformity and/or fluctuating asymmetry would not be excessive (i.e.,

$1,660.00 ÷ 4 = $415.00/parameter). From a cost perspective, it may be judicious to consider these four parameters as a complement to a community level survey in a pilot study. Again, it should be noted that the cost estimates presented in Table 4 are approximate, and that a pilot study will be the ultimate cost arbiter.

6.0 SUMMARY

The suitability of the eight population level benthic macroinvertebrate fitness parameters -- i.e., density, size, condition, fecundity, adult emergence, distributional changes, morphological deformities, fluctuating asymmetry -- for use in detecting mining related impacts is contingent upon ecological (see Section 3.0), technical (see Section 4.0) and business (see Section 5.0) considerations, initially identified in Table 1. As discussed throughout this report, the application of these fitness parameters to assess potential mining impacts within aquatic systems has been limited, and largely restricted to measures of density. Pilot studies would therefore be useful to elucidate strengths and weaknesses associated with these parameters, and their overall utility as biomonitors.

In the absence of pilot studies, only general conclusions can be drawn regarding the practicality of population level macroinvertebrate response variables to detect mining related impacts within aquatic ecosystems. For example, a literature review suggests that fitness parameters pertaining to fecundity (Sections 3.5.1. 3.5.2), adult emergence (Sections 3.6.1, 3.6.2) and the micro distribution of species (Sections 3.7.1 and 3.7.2) could be challenging from a technical perspective, and estimates/comments by Beak Consultants Ltd. and EBA Engineering Consultants Ltd. suggest that the relative costs associated with these parameters would be high (Section 5.0 and Table 4). Thus, these measures of fitness may be inappropriate for discerning mining related impacts from both technical and business perspectives.

Other fitness measures -- i.e., density, size, condition, morphological deformities, fluctuating asymmetry -- appear to be non-prohibitive from a technical perspective.

Although in the absence of pilot studies cost factors are difficult to project, Table 4 suggests that none of these factors would be excessively costly for inclusion in studies, especially if they are assessed simultaneously relative to field collection, laboratory and statistical analyses (Table 4).

Relative to ecological, technical and business considerations discussed in Sections 3.0, 4.0 and 5.0 respectively, Table 5 provides a general summary of parameters as being *appropriate*, *inappropriate* or *marginal* for use as biomonitors to detect the potential impact of mining activities within aquatic ecosystems. Again, in the absence of pilot studies, and recognizing a paucity of studies which have applied population level fitness parameters to assess mining related impacts, a more definitive assessment pertaining to the acceptability or not of the eight fitness parameters to assess mining related impacts is speculative.

Table 5. Evaluation of eight population level fitness parameters as biomonitors to assess mining related impacts within aquatic ecosystems. An (A) *appropriate* evaluation indicates no significant limitation, an (IA) *inappropriate* evaluation indicates a definite limitation, and a (M) *marginal* evaluation suggests a possible limitation. Evaluations are based on reviews presented in Sections 3.0, 4.0 and 5.0 of this report.

Population Fitness Parameter	Ecological Considerations	Technical Considerations	Business Considerations
Density	A	A	A
Size	A	A	A
Condition	A	A	A
Fecundity	A	M	M
Adult Emergence	A	IA	IA
Distribution	A	IA	IA
Morphological Deformities	A	A	A
Fluctuating Asymmetry	A	A	A

As Table 5 indicates, and based upon an extensive literature review and discussions with Beak Consultants Ltd. and EBA Engineering Consultants Ltd., benthic macroinvertebrate density, size, condition, morphological deformities and fluctuating asymmetry appear to offer utility -- from ecological, technical and business perspectives -- as means to assess potential mining related impacts within aquatic ecosystems. From a technical and cost perspective, it is appropriate to consider the five population response variables simultaneously (see Table 4). Measures regarding fecundity, adult emergence and distributional changes convey technical and/or business limitations, as discussed.

Pilot studies offer the next logical step to assess the utility of population fitness parameters reviewed in this study, as means to determine potential mining related impacts within aquatic ecosystems. The parameters may potentially act as a complement to community level assessments of mining impacts, or where appropriate, they may provide an independent evaluative tool.

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