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SUMMARY AND OVERVIEW OF ENVIRONMENTAL EFFECTS OF THE ANACONDA BRITANNIA MINE ON JUVENILE SALMONIDS AND THE MARINE ENVIRONMENT IN HOWE SOUND, BC

PREPARED FOR

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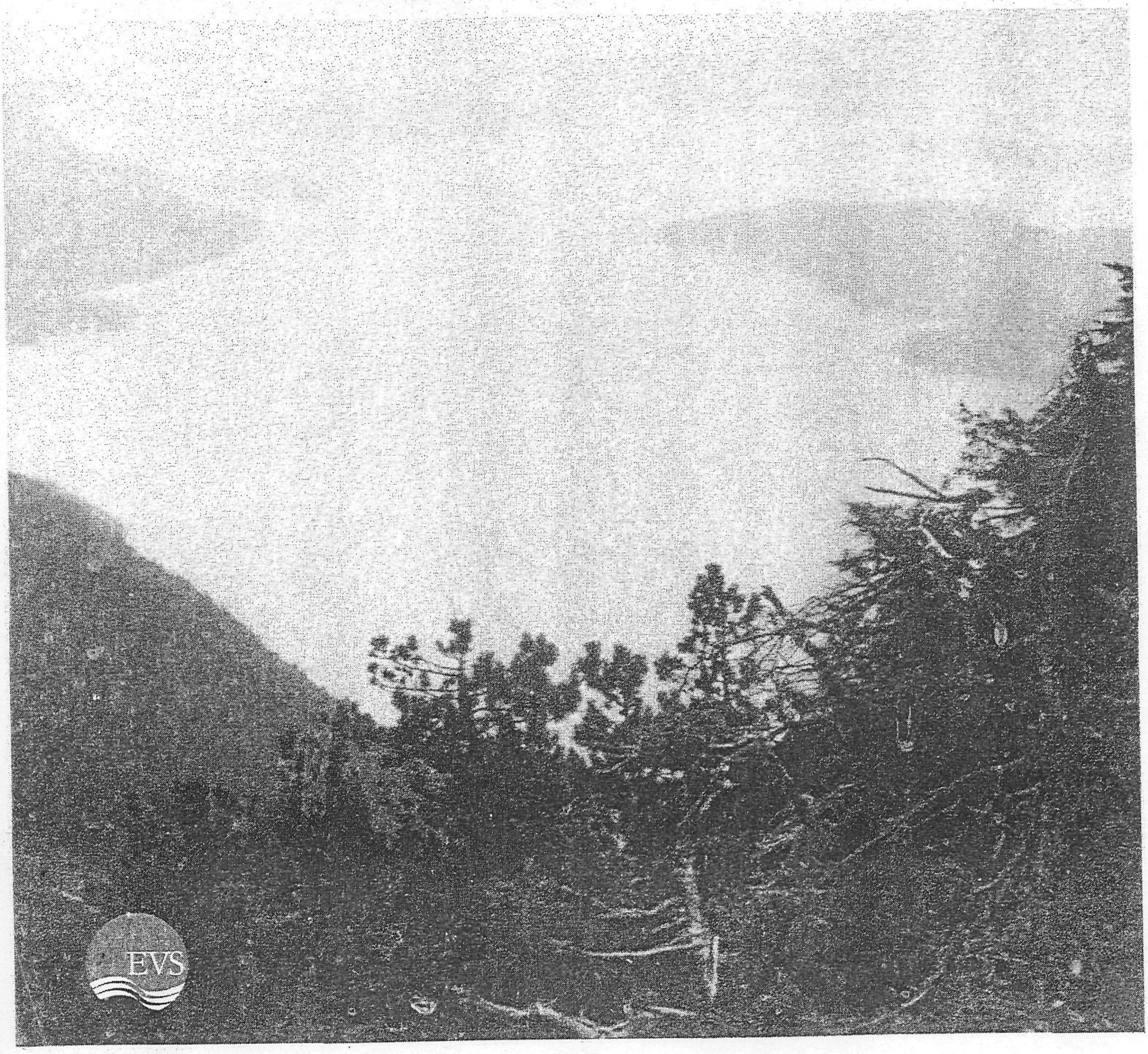

Summary and Overview of Environmental Effects of the Anaconda Britannia Mine on Juvenile Salmonids and the Marine Environment in Howe Sound

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1.0 INTRODUCTION

1.1 BACKGROUND

Copper mining and milling operations at the Anaconda Britannia Mine complex, Howe Sound, B.C. began in 1905 and ended in 1974. Acid mine drainage from the Anaconda Britannia complex was carried for more than 70 years in Britannia Creek, until a pipeline was constructed to divert drainage water directly into Howe Sound through a submerged marine outfall. Recent investigations, however, have indicated that contaminated water is again draining to the Sound via Britannia Creek (Broughton, 1992). The Anaconda Britannia Mine drainage is currently the single largest source of dissolved metals to the Howe Sound marine system with loadings of copper and zinc approaching five tonnes per day.

Much of the foreshore around Howe Sound and in the Squamish River Estuary has been identified as important fish habitat (Levings and Riddell, 1992; Nassichuk, 1992). Although less fish habitat is found along the east side of Howe Sound compared to the west side, salmon and herring frequent the area extending south of Britannia Beach to Lions Bay (Hatfield, 1994). In addition, the Squamish River Estuary is used extensively as rearing habitat for juvenile salmonids and the abundance, timing and growth patterns of salmon fry using the estuary have been shown to be closely related to the patterns of aquatic plant and benthic invertebrate production (Levings and Riddell, 1992). Elevated concentrations of copper and zinc measured in water, sediment and biota near Britannia Beach (Dunn et al., 1992; Harding, 1992) as a result of acid mine drainage have the potential to adversely affect juvenile salmonids and other components of the Howe Sound marine environment (Van Aggelen and Moore, 1986; Goyette and Ferguson, 1985).

Environment Canada (DOE) and Fisheries and Oceans Canada (DFO), with the cooperation of the B.C. Ministry of Environment, Lands, and Parks (BCMELP) and Employment and Investment (MEI), wish to end the acid mine drainage from the Anaconda Britannia Mine complex. To support the evaluation of alternative treatment methods as well as to justify the expenditures required to build a treatment plant, it is essential for DOE and DFO that the spatial extent and magnitude of environmental effects be quantified.

1.2 PURPOSE AND OBJECTIVES

The purpose of this study is to provide a basis for assessing whether the extent and magnitude of environmental effects related to drainage from the Anaconda Britannia Mine can be quantified using available data from reported and unpublished studies or whether supplementary data are necessary to make a defensible determination. Specific objectives are to:

- Provide a list of all publications, unpublished reports, and field data on environmental effects of the Anaconda Britannia Mine drainage in Howe Sound.

- If supplementary data are required, identify appropriate assessment and measurement endpoints and recommend a generic field assessment program to quantify the effects of acid mine drainage on juvenile salmonids and other components of the Howe Sound marine environment.

- Provide a detailed summary of potential mine drainage effects on juvenile salmonids and other components of the Howe Sound marine environment.

1.3 APPROACH

To address the above purpose and objectives, four tasks were identified. With the exception of the workshop for which minutes are provided in appendices, the three phases are presented as separate sections (i.e., Sections 2, 3 and 4 respectively) which are complementary:

- Phase I - Compile available information by contacting scientists at DOE, DFO, BCMELP, universities, and private practices as well as by conducting a literature search through scientific databases. Specifically, compile information in a Data Matrix format allowing direct identification of data gaps requiring further investigation.

- Conduct a workshop with representatives from DOE, DFO, BCMELP, Simon Fraser University (SFU) and the University of British Columbia (UBC) to: (1) review the data gaps identified in the Data Matrix; (2) prioritize the data gaps requiring further assessment in the field; and (3) discuss alternative study designs and technical approaches.

- Phase II - Building on findings of the Data Matrix and discussions held at the workshop, develop recommendations for a focused, powerful and cost-effective field assessment.

- Phase III - Perform a macro-level review of mine drainage effects to juvenile salmonids and the Howe Sound marine environment. The review will: (1) provide an overview of transport and late characteristics of heavy metals, particularly copper (Cu) and zinc (Zn), in the marine environment; (2) provide an overview of heavy metals toxicity (i.e., copper and zinc) to juvenile salmonids and other marine organisms; and (3) summarize environmental effects which have been associated with the Anaconda Britannia Mine discharge.

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2.0
PHASE I - DATA COMPILATION

2.1 SUMMARY OF METHODS

An extensive literature search was conducted to obtain information on the environmental effects of the Anaconda Britannia Mine on the marine environment in Howe Sound. The literature search was conducted using over 17 scientific DIALOG databases (Appendix A) and focussed on the Anaconda Britannia Mine and the adjacent Howe Sound receiving environment. In addition, scientists at DOE, DFO, BCMELP, universities, and private practices were contacted (Appendix B) to acquire further unpublished information about the mine site.

The data obtained were compiled into a Data Matrix format, thereby allowing direct identification of significant data gaps which require further investigation. A list of references is included with the Data Matrix. Information included in the Data Matrix was reviewed to ensure data acceptability. Acceptability criteria focussed on data quality and included:

- Description of methods within the report (including general quality assurance/quality control information).
- Data source (e.g., unpublished data and reports, published reports, and peer-reviewed papers).
- Sources of variability within the study (i.e., other natural and/or anthropogenic factors which may have confounded findings).

There are nine references that EVS was made aware of but which could not be obtained in time for inclusion in the Data Matrix (See Section 2.3). These unobtained references, however, do not significantly affect the assessment of data gaps presented in the Data Matrix. The missing documents referred to: data collected prior to the mine closure in 1974; data which are not specific to the Anaconda Britannia Mine site; historical perspectives on the development of the mine; or information on hypotheses that have been assessed as having no data gaps and low associated uncertainty.

The components for preparation of the matrix are described below:

Assessment Objectives and Null Hypotheses — Table 1 provides a list of assessment objectives, null hypotheses, and required background information to support the compilation of available data on the environmental effects of the Anaconda Britannia Mine.

These were developed to: (1) classify available data according to testable hypotheses; and (2) specify relevant assessment and measurement endpoints which are considered for the field assessment recommendations. The objectives and associated hypotheses are meant to be comprehensive and address most environmental issues which are likely to be related to potential effects of discharge from the Anaconda Britannia Mine.

Hypothesis Testing — The null hypotheses (Table 1) were either rejected or accepted based on a qualitative evaluation of conclusions from relevant data. A weight-of-evidence approach, using best professional judgement was adopted. Each hypothesis was accepted or rejected according to the amount and quality of supporting data (see acceptability criteria above). Conclusions and rationale was provided for each decision.

Uncertainty Assessment — The level of uncertainty (i.e., low, moderate, or high) associated with the result of hypothesis testing (i.e., acceptance or rejection) was determined based on factors such as: the amount of available information; the extent of data collected (e.g., the number of samples collected); conflicting results between reports (e.g., two reports in support of the null hypothesis and one report rejecting the null hypothesis); and the data source (i.e., unpublished data and reports, published reports, and peer-reviewed papers).

Data Gaps — Hypotheses with high or moderate uncertainty levels were identified as areas with data gaps.

Recommendations for Field Assessment — To ensure that the approach and scope of the field assessment are consistent with the needs of government agencies, as well as to ensure the development of a focussed, powerful, and cost-effective program, the data gaps were prioritized during a workshop attended by representatives from DOE, DFO, BCMELP, SFU and UBC (Appendix C). Study components and assessment endpoints were also discussed and recommendations to address priority data gaps are provided in Section 3. Note that measurement of additional physical, chemical, and biological parameters which were not specifically identified as data gaps was also recommended to provide recent exposure data and integrate other components of the Howe Sound marine environment.

Table 1. Data Matrix.

KEY OBJECTIVES AND NULL HYPOTHESES		HYPOTHESIS TESTING: ACCEPTED/ REJECTED	RATIONALE	UNCERTAINTY ASSESSMENT	DATA GAPS	RECOMMENDED FOR FIELD ASSESSMENT
JUVENILE SALMONIDS						
Objective 1 Assess Lethal and Sublethal Toxicity of the Anaconda Britannia Mine (ABM) Drainage to Juvenile Salmonids.						
Ha:	*Survival and growth of juvenile salmonids are not adversely affected by the mine drainage.*	Rejected	Chum fry suffered mortality in 30 day toxicity tests.[2]	**High:** Limited data. Lack of additional supporting studies.	Yes	✓
Hb:	*Swimming, respiratory, and cardiovascular performance of juvenile salmonids are not adversely affected by the mine drainage.*	nd	nt	**High:** No data.	Yes	✓
Hc:	*Biochemical (e.g., saltwater acclimation) and immune systems of juvenile salmonids are not adversely affected by the mine drainage.*	Rejected	2/10 juvenile chum died in 1:350 diluted effluent. Possibly due to impairment in ability to acclimate to seawater. [2]	**High:**Limited data. Possible misinterpretation of data. Lack of additional supporting studies.	Yes	
Hd:	*Behaviour of juvenile salmonids is not affected by the mine drainage.*	nd	nt	**High:** No data.	Yes	✓

* The reference(s) supporting the rationale are listed numerically in Section 2.2.
na Not applicable
nd No data
nt Not tested

Table 1. (continued)

Key Objectives and Null Hypotheses		Hypothesis Testing: Accepted/ Rejected	Rationale	Uncertainty Assessment	Data Gaps	Recommended for Field Assessment
Objective 2 Characterize Metals Concentrations in Juvenile Salmonid Tissues near the ABM Site.						
Ha:	*Metals concentrations in juvenile salmonid tissues (e.g., liver, gills, muscle, whole-body) near the ABM site are not elevated as a result of exposure to mine drainage and/or are not higher than literature-based concentrations associated with adverse effects.*	Accepted	Liver metalothionein analysis of coho salmon suggests copper levels are not biochemically harmful,[2] but juvenile coho were found to accumulate copper in a laboratory 0.3% effluent tank population.[1]	**High:** Limited data. Lack of additional supporting studies.	Yes	✓
Objective 3 Determine Life History Characteristics of Juvenile Salmonids near the ABM Site.						
Ha:	*Condition, liver weight and age at maturity of juvenile salmonids near the ABM site are not affected by exposure to the mine discharge.*	nd	nt	**High:** No data.	Yes	✓

* The reference(s) supporting the rationale are listed numerically in Section 2.2.
na Not applicable
nd No data
nt Not tested

Table 1. (continued)

		HYPOTHESIS TESTING			
KEY OBJECTIVES AND NULL HYPOTHESES	ACCEPTED/ REJECTED	RATIONALE	UNCERTAINTY ASSESSMENT	DATA GAPS	RECOMMENDED FOR FIELD ASSESSMENT
Objective 4 Characterize Adult and Juvenile Salmonid Populations Near the ABM site.					
Ha: *Characteristics of salmonid fisheries (e.g., populations, residence time, escapement, staging locations and timing, foraging efficiency) are not affected by exposure to the mine drainage near the ABM site.*	Rejected	Field observations suggest that juvenile salmonids are not currently present near and/or in Britannia Creek.[3,4,7,8,36]	**High:** Considerable data for Howe Sound but limited information pertaining to the ABM site.	Yes	✓
HOWE SOUND MARINE ENVIRONMENT					
Objective 5 Assess Water and Sediment Quality near the ABM site.					
Ha: *Metals concentrations in water near the ABM site are not elevated as a result of exposure to mine drainage and/or are not higher than relevant water quality criteria.*	Rejected	Four sources conclude that copper and zinc concentrations in water near Britannia beach are elevated.[1,2,10,41]	**Low:** Supporting studies. Volume and quality of data.	No	✓

* The reference(s) supporting the rationale are listed numerically in Section 2.2.
na Not applicable
nd No data
nt Not tested

Table 1. (continued)

	Key Objectives and Null Hypotheses	Hypothesis Testing: Accepted/ Rejected	Hypothesis Testing: Rationale	Uncertainty Assessment	Data Gaps	Recommended for Field Assessment
Hb:	*During flushing (i.e., worst-case) into receiving waters, the survival, growth, and reproduction of fish, invertebrates, and algae are not adversely affected by the mine drainage.*	nd	nt	**High:** No Data.	Yes	✓
Hc:	*Metals concentrations in sediments near the ABM site are not elevated as a result of exposure to mine drainage and/or are not higher than relevant sediment quality values.*	Rejected	Five sources conclude that copper and zinc concentrations in sediment near the ABM site are elevated.[10,12,13,14,20]	**Low:** Supporting studies. Volume and quality of data.	No	
Hd:	*Survival, growth, and abnormality of benthic invertebrates are not adversely affected by sediments near the ABM site.*	nd	nt	**High:** No data.	Yes	
He:	*Bioaccumulation of metals from sediments to benthic organisms does not occur as result of exposure to the mine drainage*	nd	nt	**High:** No data.	Yes	

* The reference(s) supporting the rationale are listed numerically in Section 2.2.
na Not applicable
nd No data
nt Not tested

Table 1. (continued)

KEY OBJECTIVES AND NULL HYPOTHESES		HYPOTHESIS TESTING ACCEPTED/ REJECTED	RATIONALE	UNCERTAINTY ASSESSMENT	DATA GAPS	RECOMMENDED FOR FIELD ASSESSMENT
Objective 6 Characterize Benthic, Pelagic and Littoral Communities near the ABM Site.						
Ha:	*Benthic abundance, biomass, diversity, and community structure near the ABM site are not affected by exposure to the mine drainage.*	Rejected	ABM site is impoverished with respect to the number of benthic taxa.[11,15,16,17,18]	**Moderate:** [15] Data are not referenced; [16]Confounding factors (i.e gravel concentrating facility); [17]Data prior to closure of the mine; [18]Small number of replicates.	Yes	✓
Hb:	*Phyto- and zooplankton abundance, biomass and diversity near the ABM site are not affected by exposure to the mine drainage.*	Rejected	May be limited impact on plankton communities. Britannia beach is one of the least productive regions for phytoplankton in the Sound. Plankton fecal pellet abundance rated as poor.[2,13,37]	**Moderate:**Limited data.	Yes	✓
Hc:	*Macrophyte abundance, biomass and diversity near the ABM site are not affected by exposure to the mine drainage.*	Rejected	Macrophyte community abundance and diversity severely limited.[10]	**Moderate:** Limited data. Lack of additional supporting studies.	Yes	

* The reference(s) supporting the rationale are listed numerically in Section 2.2.
na Not applicable
nd No data
nt Not tested

Table 1. (continued)

	Key Objectives and Null Hypotheses	Hypothesis Testing: Accepted/ Rejected	Rationale	Uncertainty Assessment	Data Gaps	Recommended for Field Assessment
Hd:	*Metals concentrations in plankton, invertebrate and macrophyte tissues near the ABM site are not affected by exposure to the mine drainage and/or are not higher than literature-based concentrations associated with adverse effects.*	Rejected	Elevated concentrations of copper and zinc found in seaweed, plankton, mussels, shrimp and marine bacterium.[1,10,11,20,38,39]	**Low**: Supporting studies. Volume and quality of data.	No	✓
Objective 7 Characterize Pelagic and Demersal Non-Salmonid Fishes Residing near the ABM Site.						
Ha:	*Metals concentrations in tissues of pelagic and demersal fishes (e.g., liver, gills, muscle, whole-body) residing near the ABM site are not elevated as a result of exposure to mine drainage and/or are not higher than literature-based concentrations associated with adverse effects.*	Rejected	Metal concentrations in viscera and gill tissue of pelagic and demersal fishes are elevated.[20] Metal concentrations are not elevated but sample size is small (n=5).[19]	**High**: Conflicting results.	Yes	
Hb:	*Community structure of pelagic and demersal fishes residing near the ABM site is not affected by exposure to the mine drainage.*	Rejected	Absence of expected fish species. [11,20]	**Moderate:** Qualitative studies.	Yes	

* The reference(s) supporting the rationale are listed numerically in Section 2.2.
na Not applicable
nd No data
nt Not tested

Table 1. (continued)

		Hypothesis Testing			
Key Objectives and Null Hypotheses	**Accepted/ Rejected**	**Rationale**	**Uncertainty Assessment**	**Data Gaps**	**Recommended for Field Assessment**
Hc: *Life-history characteristics (e.g., length, weight, age, sex, gonad weight, fecundity) of pelagic and demersal fishes residing near the ABM site are not affected by exposure to the mine drainage.*	nd	nt	**High**: No data.	Yes	
Supporting Background Information (S1) *General water quality parameters (e.g., salinity, temperature, dissolved oxygen, pH) and physical and chemical oceanography of Britannia Bay (e.g., water circulation, sedimentation patterns, metals loadings)*	na	21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,40	Low	No	✓

* The reference(s) supporting the rationale are listed numerically in Section 2.2.
na Not applicable
nd No data
nt Not tested

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3.0
PHASE II - STUDY DESIGN

3.1 OVERVIEW

3.1.1 Basis for Design

The overall purpose of this section is to provide a generic study design focused on endpoints recommended to monitor water quality and biological characteristics in the Howe Sound receiving environment exposed to drainage from the Anaconda Britannia Mine. General guidance on technical approaches, samples sizes, timing of field studies and other logistical considerations are provided rather than detailed methodologies.

As indicated by assessment objectives and null hypotheses prioritized in Section 2 (see Table 1), the proposed field program focuses on assessing risks to juvenile salmonids. Specifically, information is required to characterize the distribution of juvenile salmonids in proximity of the mine discharge. To support findings of a juvenile salmonid survey, additional data are necessary to assess: metals exposure, metals bioavailability, direct toxicity (i.e, lethal and sublethal effects), behavioural responses (e.g., metals avoidance), indirect effects on fish habitat and effects to other resident marine organisms. Exposure to metals in the water column (i.e., total and dissolved metals from the mine discharge) is of primary concern and is therefore addressed in the proposed study design. Potential precipitation and flux of metals to littoral and offshore sediments may also lead to increased metals exposure for aquatic organisms (see Appendix C). However, exposure to metals in sediment is currently considered of secondary concern and field and/or laboratory studies are not recommended at this time.

The specific objectives of the present section are to provide:

1. A generic study design for testing all of the priority hypotheses. Once details of a field program have been finalized, a specific sampling and analysis plan (SAP) and quality assurance project plan (QAPP) should be prepared prior to initiating field studies.

2. Overall guidance on statistical design, planning of field and laboratory studies, and quality assurance/quality control (QA/QC).

3. Options for prioritizing components of the study design based on environmental concerns, timing for initiation of field studies, and program funding.

4. Preliminary cost estimates for the field program options.

3.1.2 Guidance Questions

The study design is also based on the following four guidance questions:

1. Are contaminants entering the system?

This question deals solely with exposure, i.e., the presence and concentrations of contaminants, particularly of metals. If contaminants are not present, there is no likelihood of adverse effects on the biota related to contaminants. However, biota may respond to other environmental factors, for instance natural variations in habitat characteristics. Answering this question requires measurements of contaminant concentrations (e.g., metals) in receiving water and also requires measurements of other factors which may influence biota (e.g., salinity, temperature, dissolved oxygen [DO], pH). Both types of measurements are included in the proposed study design.

2. Are contaminants bioavailable?

This is a key question, since only if contaminants are bioavailable can they possibly produce an adverse effect on the biota. If contaminants are not bioavailable, they are not of concern. Information to answer this question will be obtained by analyses of biota (e.g., metals concentrations in liver of juvenile salmonids and soft tissues of mussels) following *in situ* exposure and by laboratory toxicity testing (e.g., Britannia Creek discharge water).

3. Is there a measurable biological response?

This question applies if both the previous two questions (exposure and bioavailability) are answered in the affirmative. Answering this question will require both field studies (e.g., juvenile salmonid survey, *in situ* caged-fish and caged-mussel studies, fish habitat assessment) and laboratory studies (e.g., toxicity testing, swimming challenges). These are included in the proposed study design.

4. Are the contaminants causing this response?

This question applies if all previous questions are answered in the affirmative. The presence of confounding factors (e.g., salinity and temperature gradient coincident with metal exposure gradient) may, however, complicate the evaluation of contaminant-related effects. To resolve this issue, the use of an integrated approach is proposed: the various study components will be considered separately and relative to each other; a weight-of-evidence approach will then be used to draw overall conclusions.

3.2 GENERIC STUDY DESIGN

3.2.1 Hypotheses

The assessment objectives and null hypotheses provided in Table 1 were prepared to support the compilation of available data on the environmental effects of the Anaconda Britannia Mine. To reflect the more specific objectives of the field program, priority hypotheses to be tested were revisited and are presented in Table 2 along with recommended study components.

3.2.2 Overview of Statistical Design

3.2.2.1 General Considerations

The units of replication necessary for monitoring various study components differ among hypotheses presented in Table 2 (e.g., juvenile salmonid survey [H4] vs. caged-fish [H5a]). Two different basic designs are therefore proposed for hypothesis testing: a control vs impact (CI) design, in which an exposed area is compared with a reference area; and a gradient design, in which stations are distributed along a strong exposure gradient. In this study, the CI design is primarily useful for testing hypotheses related to components which require temporal replication (e.g., several fishing days at one location) or which require sampling at different spatial scales (e.g., fish habitat assessment); the gradient design is primarily useful for assessing relationships among variables which can be sampled coincidentally (synoptically) on a similar spatial scale (e.g., station).

Figure 1 indicates the extent of the metals exposure gradient (in the water column) generally attributed to the Anaconda Britannia Mine discharge (Moore, 1985; Dunn et al., 1992; Swain and Moore, 1992). Figure 1 also provides a preliminary study design indicating sampling allocations and sample sizes for the various study components based on the two design types (see Section 3.2.3 for more details). The study hypotheses can be grouped into the above designs as follows:

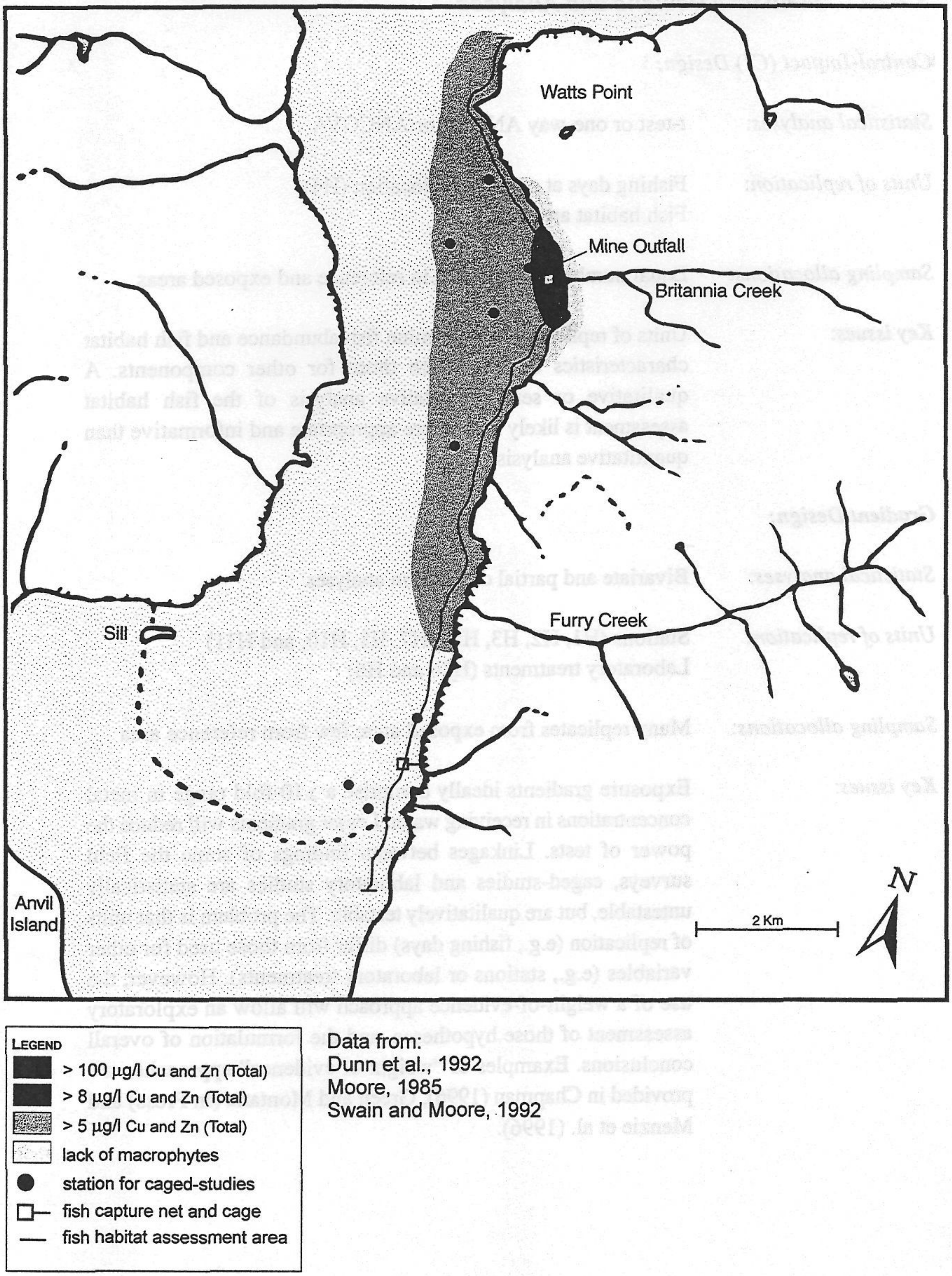
- CI design: H4 and H9.

- Gradient design: H1, H2, H3, H5a, H5b (laboratory), H6 (laboratory), H7, H8, H10 and H11.

Table 2. Hypotheses to be tested and recommended study components for the Anaconda Britannia Mine field assessment.

HYPOTHESES	RECOMMENDED STUDY COMPONENTS
EXPOSURE	
1. Metals concentrations in the water column: *H: There is no relationship between metals concentrations in the water column and proximity to the Anaconda Britannia Mine (ABM) discharge.*	- Measurements of total and dissolved metals
BIOAVAILABILITY	
2. Metals concentrations in liver of juvenile salmonids: *H: There is no relationship between metals concentrations in liver of juvenile salmonids and exposure to the ABM discharge.*	- *In situ* caged-juvenile salmonids (concentrations of metals in liver)
3. Metals concentrations in mussel tissue: *H: There is no relationship between metals concentrations in mussel soft tissues and exposure to the ABM discharge.*	- *In situ* caged-mussels (concentrations of metals in soft tissues)
BIOLOGICAL RESPONSES	
4. Presence and distribution of juvenile salmonids: *H: There is no relationship between juvenile salmonid species composition and abundance and exposure to the ABM discharge.*	- Juvenile salmonid survey (species composition and abundance)
5. Survival and growth of juvenile salmonids: *H: There is no relationship between survival and growth of juvenile salmonids and exposure to the ABM discharge.*	- *In situ* caged-juvenile salmonids (survival and growth) (H5a) - Laboratory toxicity tests (survival) (H5b)
6. Swimming performance of juvenile salmonids: *H: There is no relationship between swimming performance of juvenile salmonids and exposure to the ABM discharge.*	- Laboratory swimming challenges
7. Life-history characteristics of juvenile salmonids: *H: There is no relationship between life-history characteristics of juvenile salmonids and exposure to the ABM discharge.*	- *In situ* caged-juvenile salmonids (condition and liver weight)
8. Behaviour (avoidance) of juvenile salmonids: *H: There is no relationship between behaviour (avoidance) of juvenile salmonids and exposure to the ABM discharge.*	- *In situ* caged-juvenile salmonids (distribution within vertically stratified cages)
9. Fish habitat: *H: There is no relationship between fish habitat characteristics and exposure to the ABM discharge.*	- Fish habitat assessment
10. Survival and growth of mussels: *H: There is no relationship between survival and growth of mussels and exposure to the ABM discharge.*	- *In situ* caged-mussels (survival and growth)
SUPPORTING BACKGROUND INFORMATION	
11. General water quality parameters: *H: There is no relationship between general water quality parameters and biological responses.*	- Measurements of salinity and temperature (continuous measurements using HOBO), DO, pH

Figure 1. Water chemistry and suggested sampling sites near the Anaconda Britannia Mine site. Note that an appropriate reference area remains to be identified.



Data from:
Dunn et al., 1992
Moore, 1985
Swain and Moore, 1992

3.2.2.2 Statistical Designs and Analyses

Control-Impact (CI) Design:

Statistical analyses:	*t*-test or one-way ANOVA or ANCOVA
Units of replication:	Fishing days at one fishing location (H4) Fish habitat area (H9)
Sampling allocations:	Equal number of replicates in reference and exposed areas
Key issues:	Units of replication to determine fish abundance and fish habitat characteristics do not match those for other components. A qualitative or semi-quantitative analysis of the fish habitat assessment is likely to be more appropriate and informative than quantitative analysis

Gradient Design:

Statistical analyses:	Bivariate and partial correlation analyses
Units of replication:	Stations (H1, H2, H3, H5a, H7, H8, H10, and H11) Laboratory treatments (H5b and H6)
Sampling allocations:	Many replicates from exposed area; few from reference area
Key issues:	Exposure gradients ideally comprise a ≥10-fold range in metal concentrations in receiving water. Lesser gradients will reduce the power of tests. Linkages between findings of some the field surveys, caged-studies and laboratory studies are statistically untestable, but are qualitatively testable. The problem is that units of replication (e.g., fishing days) differ from those used for other variables (e.g., stations or laboratory treatments). However, the use of a weight-of-evidence approach will allow an exploratory assessment of those hypotheses, and the formulation of overall conclusions. Examples of "weight of evidence" approaches are provided in Chapman (1996), Green and Montana (In Press) and Menzie et al. (1996).

3.2.2.3 *Statistical Power and Sample Sizes*

Most statistical analyses are not robust unless there are ≥10 error degrees of freedom (df) (Paine, pers. comm. 1997). For a comparison of *a* areas, with *n* stations per area, error df = *a* (*n*-1). A study comparing two areas (e.g., exposed vs reference), with six stations per area, will have 10 error df. For a correlation along an exposure gradient, with *n* stations, error df = (*n*-2). A study using 12 stations will have 10 error df. Beyond the general requirement for ≥10 error df, the number of replicates required depends on the effect size (ES; usually a difference between areas, but could also be a correlation) which is to be detected, the variance among replicates (SD), and the power (probability of Type II error) which is required. Since it is difficult to agree on appropriate effect sizes, that there is little information available to assess variance (see Section 2), and that the required number of replicates is likely to differ among variables, conducting *a priori* power analyses may not be possible or useful. However, discussions of power *a posteriori* can provide much insight into results of hypothesis testing (Paine et al., 1996).

Given limited costs, the important issues for finalization of the Anaconda Britannia Mine field assessment plans and sample sizes will be:

- The total budget available.

- Assuming limited budgets, the importance of each hypothesis relative to the costs of testing that hypothesis (= cost allocation among hypotheses) (see Section 3.3 for recommendations on prioritization of hypotheses and design options).

Some assumptions about cost allocation have been made in this report, but the ultimate decisions must be made by the client. However, we agree with Green et al. (1993):

> *When cost limits the number of variables and/or replicates which can be used, and compromises must be made, the number of variables (which could mean the number of hypotheses in this study) should be reduced rather than the number of replicates.*

Regardless of the sample sizes finally selected, power can be increased by manipulating the other variables which influence power (i.e., α and SD). Major recommendations made in this section include:

- Add more stations rather than replicates within stations.

- Use one-tail rather than two-tail tests when appropriate.

- Composite water, and tissue samples within stations whenever the composites can be analyzed for the same cost as single samples.

- Use composite variables (e.g., community metrics based on many species; principal components based on concentrations of all metals) when possible.

3.2.3 Guidance on Planning of Field and Laboratory Studies, and QA/QC

General guidance on methods for sampling, testing and analysis of each study parameter or component is provided in this section. Further details and information will be required once the scope of the field program has been finalized (i.e., selection of hypotheses, timing for initiation of field studies, and program funding).

At the present time, the following study components (associated hypotheses are in parentheses) are recommended for the Anaconda Britannia Mine field assessment (see Section 3.3 for prioritization):

- Juvenile salmonid survey (H4)
- Caged-juvenile salmonid study (H2, H5a, H7, and H8)
- Laboratory toxicity testing (H5b) and swimming challenges (H6)
- Fish habitat assessment (H9)
- Caged-mussels study (H3 and H10)
- Water quality survey (metals and other parameters) (H1 and H11)

3.2.3.1 Field and Laboratory Studies

For each of the following study components, general guidance is provided on technical approach, sample size, timing of study and additional logistical considerations.

Juvenile Salmonid Survey — At one location within the exposed area and at one location within the reference area, a trap-net should be set with a net running perpendicular to shore for a distance of approximately 100-200m (Figure 1). Note that an appropriate reference area remains to be determined based on natural habitat characteristics similar to the exposed area. Each day for seven days ($n = 7$) the two traps should be emptied and juvenile salmonids identified and enumerated to estimate catch-per-day. The juvenile salmonid survey should preferably be conducted between March-April. We also recommend sampling during a period of neap tide to minimize likelihood of physical damage to the trap-net structures.

Caged-juvenile Salmonid Study — Using standard net cages (approximately $1m^3$), 10 juvenile salmon should be placed sub-surface at four near-field, four far-field, and four reference stations for a period ranging from a minimum of 10 days to a maximum of 28 days (Figure 1). The length of exposure period will depend on potential fouling of the cages (a longer exposure period is preferable). Note that species selection should be based on availability and size of juvenile salmonids. Fish should be screened for narrow size range to maximize power in detection of growth effects. The cages should be monitored daily to clear floating debris and ensure appropriate deployment. It is not recommended that the fish be fed during the exposure period. This will allow for an integrated assessment of potential direct toxic effects (e.g., lethal and sublethal toxicity of metals to fish) as well as potential indirect effects (e.g., metals toxicity to food organisms). Prior to deployment, the fish should be measured and weighed to allow for assessment of growth and condition. Following the exposure period, surviving fish should be enumerated, re-measured and re-weighed. The fish should then be sacrificed to collect the liver which should be weighed and processed for metals analysis. The study should be conducted during both fall ("best-case scenario") and spring ("worst-case scenario") periods. In addition to this caged-study aimed at assessing survival, growth and metals bioaccumulation, a parallel caged-study should be aimed at assessing fish behaviour (i.e., avoidance of the freshwater plume potentially containing elevated metals concentrations). The latter should use vertically stratified cages (approximately 2 m. X 1 m. X 5 m. deep with several compartments). The cages should be deployed for a period of approximately 10 days at a subset of stations (four near-field and four references). The depth of the cage would determine whether the fish were in the upper freshwater vs. lower seawater. Continuous water chemistry measurements (see below) will indicate dilution relative to the plume. Fish should be introduced to the cages and allowed to freely migrate vertically between compartments. At the end of the exposure period the compartments should be closed, trapping fish in whichever compartment they preferred. The distribution of fish can then be related to the water chemistry. The station locations could determine the effect of the plume. For example, comparing two cages, one in the plume and one out of the plume but at similar distances from the shore and set at similar depths might reveal that exposed fish preferred to be in the lower compartments vs the upper compartments, or that dead fish were found in upper compartments and not lower compartments.

Toxicity Testing — Tailored toxicity tests should be performed using discharge water collected from Britannia Creek during a period of high flow (flow data are available from BCMELP). The same juvenile salmonid species as per the caged-studies should be used for consistency. The fish should be exposed 96-h to a brine-adjusted discharge dilution series (e.g., 100%, 50%, 25%, 12.5%, 6.25%, and control) tested at various salinities consistent with the variability observed in the receiving environment. LC50s can later be extrapolated to predict toxicity under various field conditions. The main advantage of the laboratory testing is that it can done at any time of the year. Therefore, once the timing for initiation of field studies has been established, laboratory studies can be conducted.

Swimming Challenges — Using surviving juvenile salmonids from the toxicity tests to assess sublethal effects, swimming performance should be measured in flow through chambers under exposure conditions consistent with toxicity testing (i.e., discharge dilution series and various salinities).

Fish Habitat Assessment — Using guidance from Williams (1989a,b), fish habitat characteristics should be determined for the exposure area and reference area (Figure 1). Given that the habitat assessment is an integral part of larger field assessment, its focus should be on coastal/estuarine fish habitat description. This step is comprised of the following tasks: summary of project data; data sources identification; determination of ecosystem type; compilation of oceanographic data; site sketch and shore units determination; complete shore unit descriptions; and, complete site summary.

Caged-mussels Study — Using small wire cages, 50 - 100 mussels (*Mytulis edulis*) should be placed sub-surface at four near-field, four far-field, and four reference stations for a period of 28 days (Figure 1). The final number of organisms should be based on requirements for tissue analysis. The stations should be the same as for the caged-juvenile salmonid study. The cages should be monitored daily to clear floating debris and ensure appropriate deployment. Prior to deployment, the mussels should be measured to allow for assessment of growth. Following the exposure period, the surviving mussels should be enumerated and re-measured. Soft tissues should then be collected and processed for metals analysis. The timing of the caged-mussel study should be consistent with that of the caged-juvenile salmonid study (i.e, both fall and spring).

Water Quality Survey (metals and other parameters) — Water quality measurements should be made at four near-field, four far-field, and four reference stations (Figure 1). The stations should be the same as for the caged-studies. Samples for metals analysis (total and dissolved metals) should be collected sub-surface and below the pycnocline using a Van Dorn-type sampler. Samples should be collected at Day 0 and Day 28 of the exposure period for the caged-studies. Continuous measurements of salinity and temperature should be made using "Hobo" recording device attached to the cages; measurements of DO and pH should be made using a Hydrolab at Day 0 and Day 28. The timing of water quality measurements should be coincident with the caged-studies.

3.2.3.2 QA/QC

As indicated in Section 3.1.1, a detailed QAPP should be prepared once the scope of the field assessment has been finalized and prior to initiation of field and laboratory studies. Appropriate QA/QC protocols are essential to ensure that environmental data achieve a high level of quality commensurate with the intended use of the data. The purpose of this section is to outline the key components of the QAPP. These include:

- Identification of data quality objectives (DQOs) which are qualitative and quantitative statements of the level of uncertainty that a decision maker is willing to accept in decisions made with environmental data. DQOs typically include references to method, method detection limit, sample volume, precision, accuracy, completeness, container type, holding time, and sample preservation method.

- Description of sampling procedures with reference to sample acceptability and field quality control criteria (e.g., cross contamination blanks, filter blanks, field duplicate samples).

- Description of sample handling and custody procedures.

- Identification of quality control procedures for chemical, toxicological and biological analyses/testing.

- Reporting of procedures for data validation.

3.3 OPTIONS FOR FIELD ASSESSMENT

At the present time, estimates of program funding are not available to finalize the scope of the Anaconda Britannia Mine field assessment. However, the various hypotheses and study components can be prioritized to allow for cost allocation once a budget has been determined. Prioritization was based on the following rationales:

- Program funding may not allow for testing of all hypotheses.

- It is essential that the spatial extent and magnitude of effects be quantified. The use of study components using a gradient design is more powerful to address this objective.

- Risks to juvenile salmonids are a first order priority.

- Assessment of effects should, at a minimum, be conducted during "worst-case" conditions.

- Other marine organisms are likely to show a response to mine discharge and logistical considerations may support their use for future monitoring efforts.

To assist in cost allocation, preliminary estimates were derived for each of the study components. These are provided along with prioritized study components (with associated hypotheses in parentheses):

1. Caged-juvenile salmonid study (H2, H5a, H7, and H8); spring study (fall study is not a priority)
   Preliminary cost estimate: $50,000 - 60,000

2. Water quality survey (metals and other parameters) (H1 and H11); spring study (fall study is not a priority)
   Preliminary cost estimate: $15,000 - 20,000

3. Caged-mussels study (H3 and H11); spring study (fall study is not a priority)
   Preliminary cost estimate: $15,000 - 25,000

4. Laboratory toxicity testing (H5b) and swimming challenges (H6)
   Preliminary cost estimate: $30,000 - 40,000

5. Juvenile salmonid survey (H4)
   Preliminary cost estimate: $30,000 - 40,000

6. Fish habitat assessment
   Preliminary cost estimate: $20,000 - 30,000

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4.0
PHASE III - REVIEW OF EFFECTS ON JUVENILE SALMONIDS AND THE MARINE ENVIRONMENT IN HOWE SOUND

4.1 OBJECTIVES AND APPROACH

The purpose of this section is to provide a macro-level review of heavy metals effects on juvenile salmonids and other components of the Howe Sound marine environment. Emphasis has been placed on heavy metals which are of particular concern for the Anaconda Britannia Mine discharge, specifically copper and zinc. Three objectives were identified:

- Provide an overview of transport and fate characteristics (e.g., persistence and bioaccumulation) of copper (Cu) and zinc (Zn) in the marine environment.
- Provide an overview of copper and zinc toxicity to juvenile salmonids and other marine organisms.
- Summarize environmental effects which have been associated with the Anaconda Britannia Mine discharge.

The approach taken to address the above objectives was to:

- Review literature on transport, fate and effects of heavy metals in the marine environment.
- Summarize findings of environmental studies reviewed in Phase I (see Section 2).
- Provide an overall assessment regarding the risk posed by heavy metals exposure to juvenile salmonids and other marine organisms in Howe Sound, particularly in proximity of the Anaconda Britannia Mine site.

4.2 OVERVIEW OF HEAVY METALS TRANSPORT AND FATE CHARACTERISTICS IN THE MARINE ENVIRONMENT

All elements have a known natural abundance in the environment, as geochemical components of sediments, soils and rocks. Natural weathering processes mobilize these compounds which are transported into streams, rivers and eventually, the oceans. In the marine environment, most metals will partition directly into sediments, while some may enter other cycles through the aquatic environment. Fates are typically influenced by factors such as pH, solubility, salinity, redox potential (Eh), presence of organic matter, and presence of competing compounds. These factors are dependent on the element, the chemical species, and any associated complexing compounds. The concentration of free metal ions (commonly thought to be the most bioavailable form [Campbell, 1995]) and the relationship between these various forms is dictated by a complex interaction of many physiochemical processes. Each of the following factors may influence the form, and hence bioavailability, of metals (e.g., Cu, Zn) in the marine/aquatic environment (Campbell et al., 1988):

Organic Content — Organic compounds (dissolved, suspended or deposited) have a high capacity for complexation with metals. Bioavailability will tend to decrease as the organic content of a system increases (Meador, 1991; Welsh et al, 1993).

Complexation — The quantity of a free hydrated metal ion is a function of the total metal ion in solution, modified by its complexation to various materials. Several types of complexing materials bind metal ions: inorganic ions (such as carbonates and hydroxides); organic compounds (such as humics); biological surfaces (such as algae); and biological membranes (such as gills). Each of these complexing materials compete for metal ions and ultimately reduce the quantity of free hydrated ion in solution.

Metal-Metal Interactions — Organisms are often exposed to more than one metal in the aquatic environment. Complex interactions between groups of metals will affect: metal speciation; uptake and fate within the organism; and, toxicity.

pH — pH can affect the mobilization of certain metals; generally as pH decreases (becomes more acidic), bioavailability increases (Campbell and Stokes, 1985). However, the buffering capacity of salt water generally ameliorates the influence of pH on metal availability.

Salinity — Concentrations of major ions will influence both metal speciation and the permeability of organisms to metals (Forstner and Wittman, 1979). Generally, as salinity increases, metal bioavailability decreases.

Temperature — Temperature may affect the rate at which metals are accumulated and metabolized by an organism. Temperature also appears to affect the rate of metal equilibration, i.e., the equilibrium between influx and efflux.

Once metals are in the marine environment, they partition between the water column, suspended particulate matter, and sediments. Sediments may bind metals but, as such, may continue to act as a source of contaminants to biota long after the original source has subsided (Eisler, 1988). Adsorption onto suspended particles and bed sediments is probably the most important process determining the fate of metals in the aquatic environment. Sorption is strongest onto organic materials. Most metals will bioaccumulate in biota, although, this may not be an accurate indicator of adverse biotic effects.

Several metals are essential or beneficial for life (e.g., copper, zinc) and must accumulate in various tissues and organs to ensure proper biological function. The requirements of different organisms for essential metals vary substantially, but optimal concentration ranges are often narrow and under homeostatic control (Chapman et al., 1996). Beyond optimal ranges toxic effects may occur. For, example, zinc which is one of the most ubiquitous of the trace metals, is an essential micronutrient known to play a role in the synthesis of DNA and RNA, hormone metabolism, the immune response and the stabilization of ribosomal and cell membranes (Leland and Kuwabara, 1985). To meet their metabolic requirements, organisms have evolved efficient means for accumulating zinc. In the marine environment, zinc is not limiting and tends to be accumulated far in excess of that which is immediately required by the organism (Eisler, 1980). When essential metals (e.g., copper, zinc) are present at low levels such that organisms are unable to extract sufficient levels to meet their metabolic needs, metal deficiency may result (Fry, 1971). However, when present at high enough concentrations (i.e., exceeding optimal concentrations for life-processes), these metals may be detrimental.

4.2.1 Transport and Fate of Copper

Anthropogenic sources of copper in the aquatic environment include municipal and industrial effluents, particularly from smelting, refining, and metal plating industries (EPA, 1985). Speciation of copper can vary according to complexation, adsorption, precipitation constituents, and pH. Copper forms many compounds and complexes that are readily soluble and highly mobile in soil and surface water. Sorption onto clay materials, hydrous iron, manganese oxides, and organic material is a key fate process (Clement Associates, 1985). Various sorption processes tend to limit the concentration of dissolved copper complexes, especially as pH increases. A high percentage of copper is removed from the water column at pH greater than 6. In organically rich sediments, sorbed and precipitated copper may become redissolved through complexation and may persist in the water column for extended periods (EPA, 1985).

Copper toxicity is governed by hardness, alkalinity, and total organic carbon concentration as well as its ability to complex with other ions or compounds (Clement Associates, 1985). The toxicity of copper to aquatic life has been shown to be related primarily to activity of the cupric (Cu^{2+}) ion, and possibly to some of the hydroxy complexes. The cupric ion is highly reactive and forms moderate to strong complexes. It precipitates with many inorganic and organic constituents of natural waters. Most organic and inorganic copper complexes and precipitates appear to be much less toxic than free cupric ion and tend to reduce toxicity attributable to total copper (Spear and Pierce, 1979).

4.2.2 Transport and Fate of Zinc

Anthropogenic sources of zinc include fossil fuel combustion, smelting, domestic wastes, mining, fertilizers and pesticides (Kierkens, 1995). The predominant fate of zinc in aerobic systems is sorption by hydrous iron and manganese oxides, clay minerals, and organic matter. The efficiency of these materials in removing zinc from solution varies according to their compositions and concentrations, the pH and salinity of the water, the concentrations of complexing ligands, and the concentration of zinc. Zinc tends to be more readily sorbed at higher pH and tends to desorb from sediments as salinity increases. In reducing environments, precipitation of zinc sulphide limits its mobility. However, under aerobic conditions, precipitation of zinc compounds is probably important only where zinc is present at high concentrations. Concentrations of zinc in suspended and bed sediments always exceed concentrations in ambient water. Zinc compounds are soluble in most neutral and acidic solutions, so that zinc is readily transported in most unpolluted, relatively organic-free waters (Clement Associates, 1985).

Zinc interacts with other metals including calcium, cadmium, copper, lead, magnesium and nickel. These interactions can affect the patterns of accumulation and toxicity of zinc. Zinc has also demonstrated a synergistic relationship with phosphorus (Eisler, 1993; Pascoe and Blanchet, 1993). Because zinc is an essential nutrient, it is strongly bioaccumulated in all organisms even in the absence of unnaturally high ambient concentrations. However, biota appear to represent a relatively minor sink compared to sediment (Clement Associates, 1985).

The major routes of zinc uptake include ingestion via the food, across the gills, and directly through the body integument. As might be expected, filter feeding organisms (e.g., the blue mussel, *Mytilus edulis*) accumulate zinc at rates higher than other invertebrates. It is thought that zinc is stored to fulfil the organism's physiological needs for this essential trace metal. For example, both decapods and molluscs typically accumulate zinc at higher levels in the liver/kidney (Eisler, 1980). Several marine species of oysters, gastropods and some crustaceans are known to concentrate zinc.

Marine organisms all contain a background zinc level, generally in excess of seawater or sediment concentrations. Changes in accumulation rates of zinc by aquatic organisms may reflect complex interactions between water temperature, ambient zinc concentrations, duration and season of exposure, physiological saturation and detoxification mechanisms (Eisler, 1980).

4.3 OVERVIEW OF HEAVY METALS TOXICITY TO JUVENILE SALMONIDS

4.3.1 Toxic Effects of Copper on Salmonid Fishes

4.3.1.1 Overview

Spear and Pierce (1979) provide an excellent review of copper in the aquatic environment and provide coverage of the literature on toxic effects of copper to fishes in general, including salmonids. The majority of literature at that time pertained to the effects of copper at circumneutral pH. Figure 2 is a summary graph provided by Spear and Pierce (1979) showing the range of total copper concentration known to elicit various effects in freshwater fish. Salmonid species are among the more sensitive species of these groupings. Therefore, the No Observed Effect Concentration (NOEC) for salmonids will be near or at the lowest value in each range (5-40 μg/L Cu).

It is reasonably clear that most of the toxic action of copper is best explained by the concentration of dissolved copper ion (Andrew, 1976) and perhaps also of copper hydroxide, but not copper carbonate. A recent review by Taylor et al. (1996) covers new information on the effects of copper at low pH. At pH 5.0, copper exists mostly as dissolved copper and therefore the toxicity of copper is often greatest around this pH. Recent work has demonstrated that the range for sublethal NOEC at circumneutral pH (5-40 μg/L Cu) will be lower at pH 5.0. In contrast, copper complexation and adsorption, along with other water quality factors such as water hardness, can reduce the toxicity of copper-containing water to salmonids. The above concentrations refer to total copper concentrations in water that are relatively free of agents that may complex or adsorb dissolved copper.

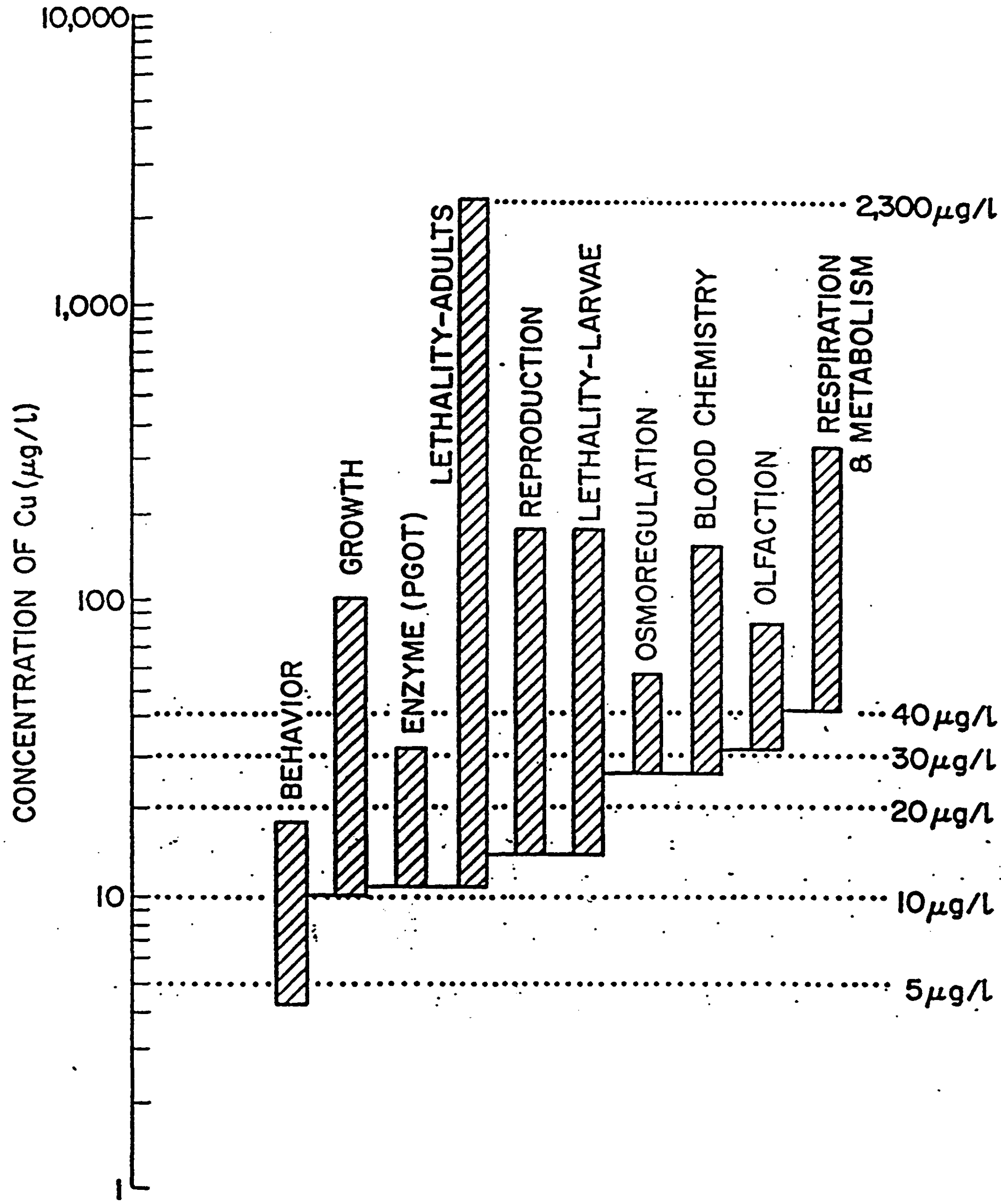
4.3.1.2 Lethality

Acute Lethality

Acute lethality of copper to fishes cannot be considered without also considering water quality. Water quality has marked effects on acute copper toxicity. Water hardness is the most important modifier of copper toxicity. Copper is more toxic in soft water (Figure 3). The 96-h LC50 values for salmonids in circumneutral freshwater range from 10 μg/L Cu to 1,100 μg/L Cu for water hardness values ranging in from 13 to 360 mg/L $CaCO_3$. For trout (*Salmo sp.*), Atlantic salmon (*Salmo salar*) and two species of Pacific salmon (*Oncorhynchus nerka and Oncorhynchus gorbuscha*), Spear and Pierce (1979) report the following relationship between the 96-h LC50 and water hardness (H):

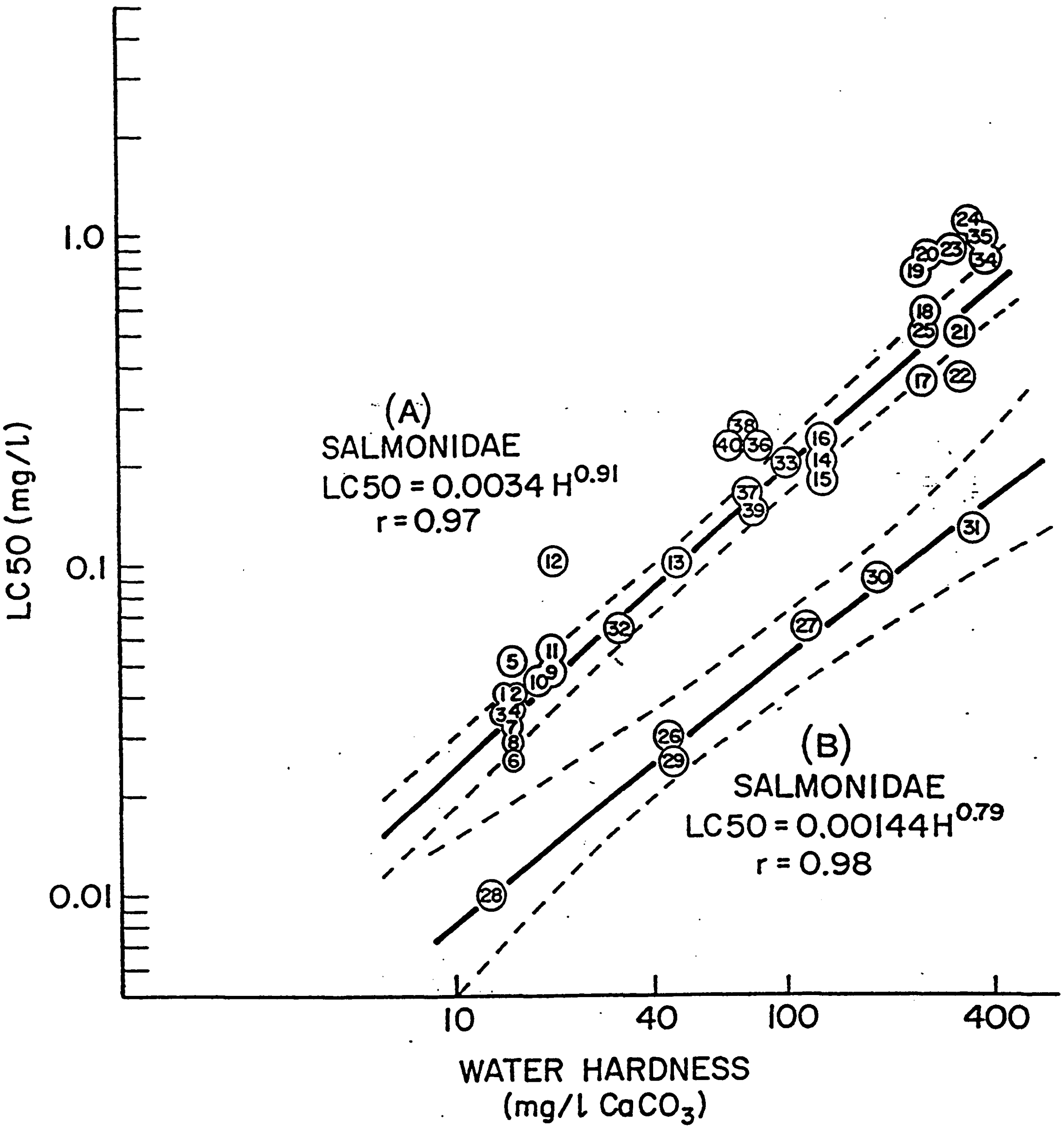
$$\text{96-h LC50 (mg/L Cu)} = 0.0034\ H^{0.91}\ (\text{mg/L } CaCO_3)$$

Figure 2. Total copper concentration known to elicit various effects in freshwater fish (Spear & Pierce, 1979)



[a] Concentrations pertain to waters that are relatively free of agents which may complex or adsorb dissolved copper.

Figure 3. Short term lethal tolerance of Clupeiformes to copper (Spear & Pierce, 1979)



(A) FAMILY SALMONIDAE (salmon-like fishes) including trout, Atlantic salmon and two species of Pacific salmon (*O. nerka* and *O. gorbuscha*).

(B) FAMILY SALMONIDAE (salmon-like fishes) two species of Pacific salmon (*O. kisutch* and *O. tshawytscha*).

However, Pacific salmon were more sensitive to copper as shown by the following relationship:

$$96\text{-h LC50 (mg/L Cu)} = 0.0014\ H^{0.79}\ (\text{mg/L } CaCO_3)$$

Consequently, Pacific salmon tend to be more vulnerable than other salmonids to the acute toxic effects of copper because of: (1) an inherent species sensitivity; and (2) the soft freshwater generally found on the west coast of Canada. In view of this finding, it is important to note that the database on copper toxicity to fishes is dominated by tests performed in hard water, and so the relevance of the database as a whole to the B.C. coastal environment should therefore be treated with some caution.

The influence of water hardness on copper toxicity is thought to be related to the formation of the relatively non-toxic $CuCO_3$. Several studies reported by Spear and Pierce (1979) support the suggestion of Andrew (1976) that "... toxicity is shown to be directly related to the ionic activity of the cupric-ion." This means that comparisons between acute toxicity of differing water quality could be misleading if only the total copper concentration is presented. Further, it is clear that components of water that can either adsorb (e.g., sediments) or complex (e.g., humic acids) copper will reduce the toxicity as a function of the total copper concentration, but not as a function of the dissolved copper concentration (Spear and Pierce, 1979). The concentration of copper hydroxide is also thought to be important in copper toxicity (Taylor et al., 1996). This means that in the marine environment, copper toxicity toward salmonids is as much as 100-fold lower, being in the 1-10 mg/L Cu range rather than the 10-100 μg/L Cu range (e.g, Eisler and Gardner, 1973). Some of this difference may also be related to the mechanism of action of copper on fish.

Low pH increases the acute toxicity of copper. For example Beaumont et al. (1995a) reports 96-h LC50 values of brown trout (*Salmo trutta*) in 5°C soft water at pH 5.0 as 5 μg/L Cu. This value is 50% lower than the lowest salmonid LC50 at circumneutral pH. This effect is explained by the majority of the total copper being in the ionic form at this pH. In addition, temperature also affects acute toxicity at low pH. The same study reported a higher LC50 value (30 μg/L Cu) at 15°C compared with 5°C using the same species.

Chronic Lethality

LC50 values are time dependent and generally decrease with increasing exposure duration. With copper, time to lethality studies with fish suggest a threshold concentration on the order of 0.02 to 0.7 mg/L Cu, below which there is no lethality with exposure (Lloyd and Herbert, 1962; Sprague and Ramsey, 1965; Calamari and Marachetti, 1973). While the lowest of these threshold concentrations is for rainbow trout, these limited data do not account for important variables such as water hardness and pH. As with acute toxicity, low pH and soft water are anticipated to increase the chronic toxicity of copper to salmonids.

The reason for a threshold copper concentration may be partially related to the fact that salmon can acclimate to sublethal copper concentrations. Some studies (e.g., Rewholdt et al., 1971; Dixon, 1978; Waiwood and Beamish, 1978; Lauren and McDonald, 1985), but not all (Petersen, 1974) show an increase in lethal tolerance following pre-exposure to sublethal copper. In Dixon's study with rainbow trout (*Salmo gairdneri*), the 144-h LC50 value was almost doubled by pre-exposure to copper (see also effects on swimming performance in Section 4.3.1.3).

4.3.1.3 Sublethal Effects

Sublethal effects of copper on salmonids have varying degrees of relevance to the overall survival, growth, and reproduction of the fish. However, consistent patterns are beginning to emerge from sublethal effects studies, including target actions on locomotory performance, gill function, and neural function (including olfaction) at concentrations as low as 5-20 μg/L Cu. Again, these effects are exacerbated by low pH and ameliorated by hard water. Overall, sublethal effects of copper on salmonids all seem to occur at concentrations higher than 1% of the acute lethality concentration.

Sublethal Effects of Copper on Gill Structure and Function

A major target site for copper sublethal toxicity is the gills. This is clearly shown by the accumulation of copper in gill tissues, histological damage to the gills, and altered ionic and osmotic balance (as reflected in altered blood characteristics). In a freshwater environment, copper exposure results in hemodilution and increased erythropoiesis (Spear and Pierce, 1979). In general, the threshold concentration for these effects is around 25 μg/L Cu for freshwater fish in general and brook trout (*Salvelinus fontinalis*) specifically (McKim et al., 1970).

In rainbow trout exposed to 30 μg/L Cu for 48 hours in water of hardness 30 mg/L $CaCO_3$, hematocrit (Hct) and red blood cell count increased (Waiwood, 1977). Furthermore, the change in Hct was greater at low pH and water hardness.

Wilson and Taylor (1993a) exposed rainbow trout to 200 μg/L Cu for 24-h in circumneutral soft water. They observed declines in plasma sodium and chloride concentrations and arterial oxygen content. In addition, the Hct doubled within this time period. The decrease in arterial blood saturation began 1 hour into the exposure and was compensated for by the increase in Hct. These changes were correlated with severe gill histopathology including cell swelling and thickening and hematomas. These findings and other effects related to gill disturbances are reviewed in Taylor et al. (1996).

The mechanisms of action of copper in salmonid gills are on permeability and ion transport (Lauren and McDonald, 1985). At concentrations of around 10 μg/L Cu active uptake of ions is inhibited. Both the sodium and chloride transporters are thought to be impaired. At a slightly higher concentration (40 μg/L Cu) the permeability of the gill membrane to these ions increases as a result of weaker intracellular tight junctions. This causes a greater passive efflux of sodium and chloride from the fish. These mechanisms of action are similar to those of low pH (Lauren and McDonald, 1985). Whereas increased calcium and magnesium content in freshwater can compensate for the effects on gill permeability, they do not prevent the inhibition of ion transporters in rainbow trout (Lauren and McDonald, 1985).

Because the ambient concentration of calcium in seawater is more than 26-fold higher than in freshwater, the expectation is that the toxic actions of copper are ameliorated. Indeed, trout (*Oncorhynchus mykiss*) exposed to 300 μg/L Cu in seawater at pH 7.9 for 24 h showed no lethality, no respiratory problems, no ionic disturbance and no gill damage (Wilson and Taylor, 1993b), unlike the freshwater fish. The nature of the ionic transporters in the gill is also different in seawater compared with freshwater fish (see Taylor et al., 1996).

Sublethal Effects of Copper on Swimming Performance

Given the effects of copper on gill structure and blood oxygen saturation, it is not surprising that copper exposure has sublethal effects on swimming performance. Swimming performance is normally measured as the critical swimming speed (Ucrit). This is defined as the maximum prolonged swimming that a fish can maintain following a series of increases in water velocity of at least 15 minutes in duration. Fish can swim faster than their Ucrit but only for a period of seconds.

To date the most comprehensive analysis of the effect of copper exposure on swimming performance at pH 6 and pH 8 in juvenile rainbow trout is that performed by Waiwood and Beamish (1978). Their major findings are as follows. A five day exposure to copper at pH 8 resulted in decreased Ucrit at copper concentrations ranging from 15 to 200 μg/L Cu, depending upon the water hardness. The effects were greatest in soft water (30 mg/L $CaCO_3$), with a 25% reduction in Ucrit with 30 μg/L Cu. Water hardness ameliorated this effect such that a much smaller decrease in Ucrit was observed for 200 μg/L Cu with a water hardness of 360 mg/L $CaCO_3$. At pH 6, swimming performance was further debilitated, with significant effects at 10 μg/L Cu in soft water and with 50 μg/L Cu in hard water. Regardless of water hardness, pH or the severity of the impairment after 5 days of copper exposure, the trout had fully recovered by days 10 through 30 from the copper exposure. This recovery was metabolically costly as was indicated by elevated metabolic rates at rest and at any given swimming speed. Metabolic loading (Brett, 1958) as a result of copper exposure and recovery could have deleterious consequences for migratory salmon on fixed energy budgets.

Similar swimming performance experiments have been performed in England with rainbow trout and brown trout, providing support for the general findings of Waiwood and Beamish (1978) and adding additional detail (see Taylor et al., 1996). Of importance is the finding that

5 μg/L Cu at pH 5 in soft water reduced Ucrit by 50% at 5°C but by only 25% at 15°C (Beaumont et al., 1995b). Furthermore, at 25 μg/L Cu, trout could not swim in pH 5 soft water at 5°C. Thus, this impairment of swimming performance seems to be of greater concern at a lower water temperature. This is congruent with the effect on ionic regulation. In addition to elevated metabolic rates, Taylor et al. (1996) present a case for increased fish ammonia levels associated with copper exposure resulting in impairment in neural control.

Sublethal Effects of Copper on Behaviour

The most sensitive sublethal effect for salmonids exposed to copper is avoidance behaviour. Sprague (1964) found 100% of juvenile Atlantic salmon (*S. salar*) avoided 24 μg/L Cu and 50% avoided 4.4 μg/L Cu. Goldfish show a similar behavioural response at similar concentrations (Spear and Pierce, 1979). The additional effects of low pH have not been studied to our knowledge.

Even though some of the sublethal effects of copper on salmonids appear to be greater at lower temperature, Peterson (1974) found no effect of copper (8 - 30 μg/L Cu) on temperature selection.

Both feeding and exploratory behaviours are altered with short exposures (2-24 h) of copper (6-300 μg/L Cu). However, with continuous exposure of 6-15 days at low copper concentrations (6-12 μg/L Cu), these behaviours are restored. These data suggest low thresholds for detection of and impairment by copper, but with acclimation over time. However, the olfactory response of rainbow trout to L-serine is suppressed by 50-100% with a 4-h exposure to copper of 50-100 μg/L Cu in moderately hard water (90 mg/L $CaCO_3$) (Hara et al., 1976). Therefore, the behavioural acclimation mechanism could involve an inability to detect copper.

Sublethal Effects of Copper on Growth

Based on the effects of copper on metabolic rate, it is not surprising that McKim and Benoit (1975) found a concentration-dependent decrease in growth rate for 14 month old brook trout after a 26 week exposure to copper in water of hardness 45 mg/L $CaCO_3$. The threshold concentration for this effect was around 10 μg/L Cu. This suppression in growth is regarded as an initial temporary phenomenon with normal growth rates being restored despite continued sublethal exposure. A similar finding was made by Waiwood (1977) using rainbow trout, with the additional finding that the threshold for decreased growth varied from 10 μg/L Cu in soft water at pH 6 to 100 μg/L Cu in hard water at pH 8. Low pH, soft water is likely to exacerbate this effect.

In addition to elevated metabolic rate causing a depression in growth rate, there is a temporary loss of appetite in rainbow trout (Lett et al., 1976) that can take 1-15 days to recover at copper concentrations of 100-300 μg/L Cu.

In sea water, the growth of chum salmon (*Oncorhynchus keta*) was unaffected by exposure to 2.5 and 5.0 μg/L Cu (Thompson and Paton, 1976a).

Sublethal Effects of Copper on Migration

Copper exposure significantly retards salmonid migratory behaviours. Lorz and McPherson (1976) demonstrated a significantly smaller percentage of juvenile coho salmon (*Oncorhynchus kisutch*) migrating downstream after laboratory exposure to as low as 5-10 μg/L Cu. Similarly, Sprague and Ramsay (1965) reported that 17-21 μg/L Cu in a copper-zinc mixture delayed upstream migration of Atlantic salmon.

When moving from freshwater to sea water, salmonids must and do show a salinity tolerance. This salinity tolerance is impaired by copper exposure. Coho salmon exposed to 20 μg/L Cu in freshwater accrued a 50% mortality when challenged with seawater (Lorz and McPherson, 1976). This lowering of salinity tolerance with copper was associated with reduced ion transporter activity in the gills.

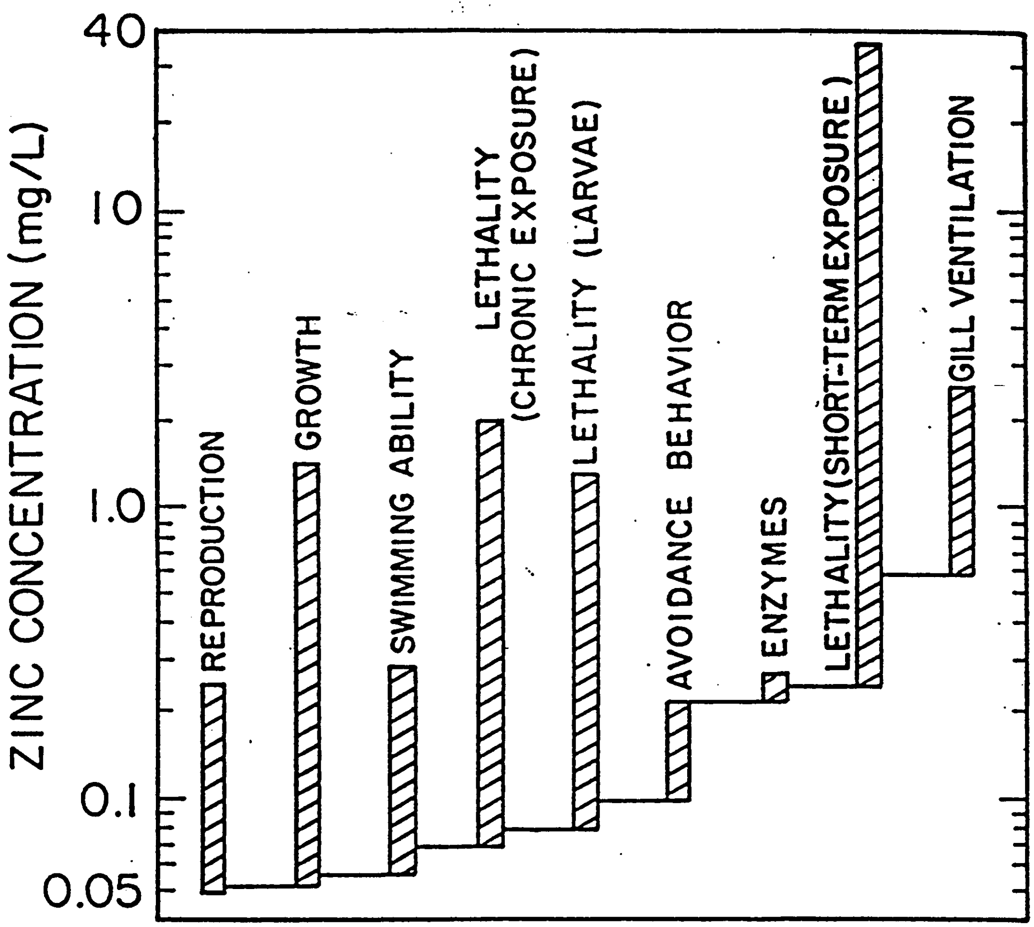
4.3.2 Toxic Effects of Zinc on Salmonid Fishes

4.3.2.1 Overview

The review of zinc in the aquatic environment by Spear (1981) provides excellent coverage of the literature on toxic effects of zinc to fishes in general, including salmonids. The majority of literature at that time pertained to the effects of zinc at circumneutral pH. Figure 4 is a summary graph provided by Spear (1979) showing the range of total zinc concentration known to elicit various effects in freshwater fish. Salmonid species are among the more sensitive species of these groupings. Therefore, the NOEC will be near or at the lowest value in each range (5-600 μg/L Zn).

More recent studies examined the influence of water hardness and pH on zinc toxicity to rainbow trout in some detail (Bradley et al., 1985). A recent review by Hogstrand and Wood (1996) considers findings with respect to zinc toxicity, as well as its effect on physiology since zinc is an important and essential trace metal for fish. Acute toxicity for salmonids, ranging from 300 to 8,000 μg/L Zn, is lower than for other fishes, and is linearly related to water hardness. The toxic action of zinc is best explained by the concentration of dissolved zinc ion and perhaps also of zinc carbonate. Zinc hydroxide is relatively non-toxic. Toxicity of zinc is greatest at low pH in soft water. In the marine environment, the proportion of

Figure 4. Total zinc concentration known to elicit various effects in freshwater fish (Spear, 1981)



dissolved zinc and zinc toxicity both decrease compared to fresh waters. Acute toxicity can occur at zinc concentrations expected to precipitate zinc carbonate.

There are two known mechanisms of toxic action in salmonids, and both can be fatal. At high concentrations, fish die from hypoxia, caused by zinc-induced gill damage. At low concentrations, zinc impairs branchial calcium uptake which leads to a potentially fatal hypocalcemic state.

4.3.2.2 Lethality

Acute Lethality

Acute lethality of zinc to fishes is modulated by water quality. Water hardness is the most important modifier of zinc toxicity (Figure 5). Zinc is more toxic in soft water. The 96-h LC50 values for salmonids in circumneutral freshwater range from 300 to 1,000 μg/L Zn for water hardness values ranging in from 10 to 40 mg/L $CaCO_3$ (Spear, 1981). The order Clupiformes, which include salmonids, shows the following relationship between the 96-h LC50 and water hardness (H):

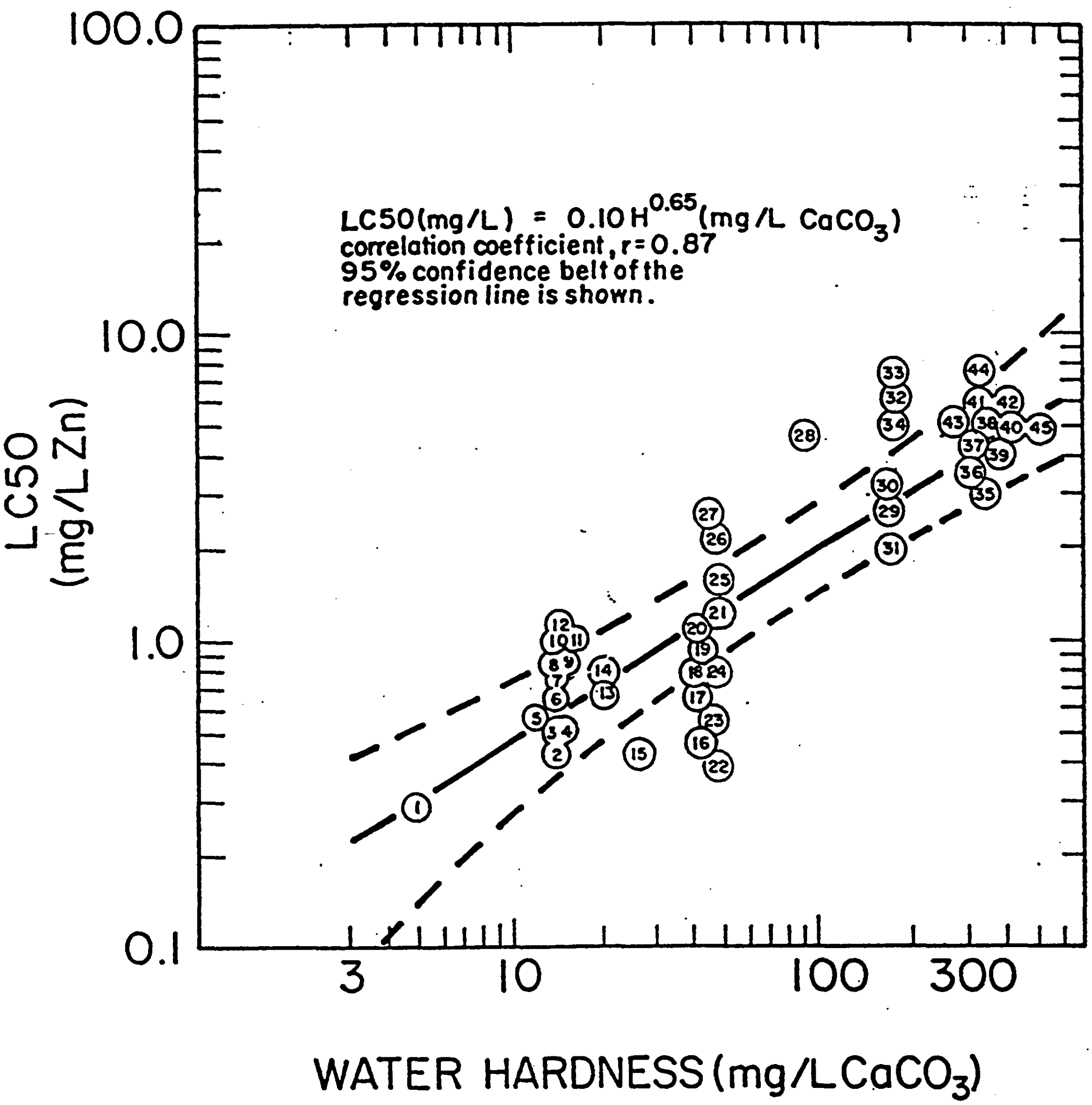
$$\text{96-h LC50 (mg/L Zn)} = 0.10\ H^{0.65}\ (\text{mg/L } CaCO_3)$$

At the highest water hardness tested, the acute toxicity was 7.2 mg/L Zn. Clupiformes are more sensitive to zinc than other fish orders over the whole range of water hardness. More recent tests with salmonid species in soft water show a somewhat higher acute toxicity. Cusimano and Blake (1986) reported a 96-h LC50 of 97 μg/L zinc for juvenile steelhead trout. Mayer and Ellersieck (1986) reported 96-h LC50 values from 61-600 μg/L zinc for cutthroat trout.

The influence of water hardness on zinc toxicity is thought to be related to the formation of the less toxic $ZnCO_3$. However, $ZnCO_3$ is still regarded as toxic (Spear, 1981) since the LC50 values for marine fish are often above the solubility limit for zinc, and since a flocculant precipitate, presumed to be $ZnCO_3$, was found to be toxic to marine fish (Herbert and Wakeford, 1964). The influence of agents that can adsorb (e.g., sediments) and complex (e.g., humic acids) zinc is poorly understood, even though it is expected that these agents should reduce toxicity as a function of the total zinc concentration (Spear, 1981).

Low pH decreases the acute toxicity of zinc to rainbow trout (Bradley et al., 1985). LC50 values for juvenile rainbow trout were 2 to 5 fold higher (depending on water hardness) at pH 5.5 compared with pH 7.0. For example, in hard water the LC50 was 4.4-5.1 mg/L Zn at pH 7.0 compared with 9.9-11.1 mg/L zinc at pH 5.5. In soft water the LC50 was 170-190 μg/L Zn at pH 7.0 compared with 880 μg/L Zn at pH 5.5. Therefore, low pH is protective.

Figure 5. Short term lethal tolerance of Clupeiformes to zinc (Spear, 1981)



At pH 9.0 LC50 values are higher than at pH 5.5, with a similar dependence on water hardness.

Development stages in the salmonid life cycle have important implications for zinc toxicity. Embryos are more tolerant than alevins, and tolerance increases with body mass over the size range 0.2 to 32 g. The exponent for the relationship between 96-h LC50 and body mass ranges from 0.19 to 0.40 (Spear, 1981). Alevins are therefore the most sensitive life stage as they make the transition to exogenous food sources.

Chronic Lethality

LC50 values are time dependent and generally decrease with increasing exposure duration. With zinc, chronic LC50s are 1/5th to 1/50th of acute LC50s. Caution should be used when interpreting the time-to-lethality curves for zinc because there are two mechanisms of action and both can be fatal. At high concentrations, fish die from hypoxia, caused by zinc-induced gill damage. At low concentrations, zinc impairs branchial calcium uptake which leads to a potentially fatal hypocalcemic state.

4.3.2.3 Sublethal Effects

A number of sublethal effects of zinc exposure are known. Threshold concentrations for effects on reproduction, growth and swimming ability are among the most sensitive for sublethal effects (5-6 μg/L Zn). It is possible that the sublethal effects on growth and swimming are through gill actions, but reproductive effects appear to be related to gametogenesis.

Zinc proteins are important enzymes (e.g., carbonic anhydrase) and several zinc proteins are important in replication and transcription of DNA (Hogstrand and Wood, 1996). Zinc is an essential trace metal in fish and, on average, fish contain about 10 - 40 mg/kg wet weight located mainly (50-60%) in the skin, bone and muscle (Hogstrand and Wood, 1996). Zinc can be taken up across the gill and intestinal epithelial and, once inside the blood and cells, it is associated with metallothionein binding proteins. In rainbow trout 99.8% of the plasma zinc is normally bound to plasma proteins, serves to reduce zinc toxicity (Bettger et al., 1987). Hogstrand and Wood (1996) suggest that a high zinc diet does not appear to be a concern toxicologically, and may in fact stimulate growth. However, Takeda and Shimuzu (1982) found that 0.4 mg zinc oxide per gram of food inhibited growth and decreased feeding efficiency in juvenile rainbow trout. The different conclusions may be related to the high dietary levels required to elicit this response and the fact that dietary calcium moderates the effect of dietary zinc. The main concern for zinc toxicity is via water exposure.

Sublethal Effects of Zinc on Gill Structure and Function

A major target site for zinc sublethal toxicity is the gills. Zinc can accumulate in gill tissues and cause gross histological damage (edema, cell sloughing and cell fusion) (Skidmore, 1970; Skidmore and Tovell, 1972; Spry and Wood, 1984). These sorts of histological changes are found with other metals. The concentrations required to elicit gross histological damage are high, e.g., 1 mg/L Zn (Tuurala, 1983); 40 mg/L Zn (Skidmore and Tovell ,1972), 4 mg/L Zn (Lappivaara et al., 1995). The consequence of these histological changes is hypoxia due to a greater diffusion distance between the water and blood (Tuurala, 1983; Tuurala and Soivo, 1982; Lappivaara et al., 1995) and, depending upon the level of exposure, a decrease in blood pH (Spry and Wood, 1984).

Zinc is a competitive inhibitor of calcium uptake (Hogstrand et al., 1994) and calcium can inhibit zinc uptake at the gills (Spry and Wood, 1989). This mechanism of toxic action is manifest at lower zinc concentrations when compared with gill damage. At 150 μg/L, zinc can inhibit calcium influx (Hogstrand et al., 1994, 1995) without any long-term (one month) effects on plasma calcium and growth, although plasma calcium level and growth rate are initially depressed. In the same study, 450 μg/L Zn continuously depressed plasma calcium. Hypocalcemia and acid-base disturbances are thought to be the major causes of toxicity in rainbow trout at sub-mg/L Zn concentrations (Spry and Wood, 1984, 1985)

There a number of reports that sublethal zinc exposure confers a resistance to subsequent lethal exposure (Spear, 1981). Collectively, these data suggest that the 96-h LC50 may increase as much as 2-fold. Resistance may reflect the induction of metallothioneins and may be enhanced with sub-lethal exposures occurring early in development (Sinley et al., 1974). Indeed, Hogstrand et al. (1995) suggested that the lack of increased tolerance in their experiments with juvenile rainbow trout may have been because the fish were too large (21 g) for the induction of plasma metallothioneins at that level of zinc exposure. Even gill damage observed at higher levels of zinc exposure (>1 mg/L Zn) showed some recovery after 24-h (Lappivaara et al., 1995). Roche and McCarter (1984) suggest a threshold of 40 μg/L Zn for the induction of liver metallothioneins in rainbow trout exposed to a Zn:Cu:Cd mixture of 400:20:1

Sublethal Effects of Zinc on Swimming Performance

Given the effects of zinc exposure on gill structure (Skidmore, 1970; Skidmore and Tovell, 1972; Spry and Wood, 1984) and blood oxygen saturation (Tuurala, 1983; Tuurala and Soivo, 1982; Lappivaara et al., 1995), the prediction is that sublethal effects on swimming performance will occur. While this idea has not been tested, acute toxicity to zinc is increased in rainbow trout exposed to hypoxic water (Lloyd and Herbert, 1962).

Sublethal Effects of Zinc on Behaviour

Atlantic salmon avoid 0.1 mg/L Zn and adults delay upstream migration at around 0.2 mg/L Zn in a zinc-copper mixture (Sprague et al., 1964).

Sublethal Effects of Zinc on Reproduction

Data on effects on reproduction of adult salmonids are not available. In other fish species, zinc has negative effects on reproductive performance at very low concentrations in soft water (Spear, 1981). It is suggested that the thresholds for such effects, which include decreased number of spawnings and decreased number of eggs produced, are in the range from 5 to 240 μg/L Zn. These laboratory derived concentrations are consistent with zinc concentrations in contaminated lakes where recruitment failure has occurred (Van Loon and Beamish, 1977). Rachlin and Perlmutter (1968) suggested that low levels of zinc may act directly upon the nuclei of dividing cells, and this in turn may be related to the central role of zinc proteins in replication and transcription of DNA. Zinc is therefore likely to have important toxic actions on gametogenesis (Spear, 1981).

Sublethal Effects of Zinc on Disease Resistance

Cestode infection of sockeye salmon decreases tolerance to zinc. Similarly, zinc exposure increases the susceptibility to infection (Boyce and Yamada, 1977).

Sublethal Effects of Zinc on Growth

In general reproduction and alevin survival are more sensitive indicators of zinc toxicity than growth. Given interactions between calcium and zinc uptake, it is not surprising that a wide range of threshold concentrations exist for both salmonids and for other fishes (Spear, 1981). The effective growth inhibiting concentration in rainbow trout in hard water (360 mg/L $CaCO_3$) was 1.12 mg/L Zn over 85 days (Watson and Mckeown, 1976). In contrast, inhibition of growth occurred at 450 μg/L Zn in soft water over 30 days (Hogstrand et al., 1995).

4.4 OVERVIEW OF HEAVY METALS TOXICITY TO OTHER MARINE ORGANISMS

4.4.1 Toxic Effects of Copper on Marine Organisms

Spear and Pierce (1979) completed a thorough review of toxic effects of copper on marine organisms. As indicated in Section 4.3, most of these studies were conducted at circumneutral pH. For marine invertebrates, concentrations as low as 6-10 $\mu g/L$ Cu may be lethal to the most sensitive life stage of a copepod species or to pelecypods (e.g., oysters and clams) chronically exposed. Modifying factors that affect the toxicity of copper include salinity, organic complexation, and adsorption to clays (Spear and Pierce, 1979). With increasing salinity, tolerance to copper is expected to increase because of inorganic complexation (Olson and Harrel, 1973). Organic complexation also affects copper tolerance in marine invertebrates. The lethal tolerance of two barnacles, *Balanus balanoides* and *Balanus eburneus*, varied with the form of cupric salt added. Copper added as citrate, tartrate, salicylate and para-aminoenzoate complexes was less potent than was copper carbonate. Organic extracts of sediments, possibly humic compounds, were found to increase the tolerance of copepod eggs and larvae to copper (Lewis et al., 1972,1973). Absorption to clays was found to increase the percent survival of copepod larvae (Lewis et al., 1972,1973).

4.4.1.1 Copper Toxicity to Algae

A variety of sublethal effects have been reported on the toxicity of copper to algae. These include:

Photosynthesis — A copper concentration of 100 $\mu g/L$ Cu caused a 50% decrease in photosynthesis in the giant kelp, *Macrocystis pyrefera* (EPA, 1985).

Growth Reduction — Growth reduction in the red algae *Champia parvula* occurred in both the tetrasporophyte and female plants exposed to copper concentrations of 4.6 and 4.7 $\mu g/L$ Cu (Steele and Thursby, 1983 from EPA, 1985). Microalgae were equally sensitive to copper, with growth rates reduced by 50% after exposure to 5.0 $\mu g/L$ Cu for up to 5 days (EPA, 1985). Growth reduction in the diatom *Thalassiosila pseudonana* occurs at 5 $\mu g/L$ to 50 $\mu g/L$ Cu. (Erickson, 1972).

4.4.1.2 Copper Toxicity to Invertebrates

Mollusca

In general, the pelecypods (e.g., clams and oysters) are relatively tolerant to copper during short term exposures (Spear and Pierce, 1979). Embryos of the blue mussel and Pacific oyster are the most sensitive saltwater species tested with acute values of 5.8 and 7.8 $\mu g/L$ Cu (EPA,1985). The acute lethal concentrations of copper to the larval and embryo stages of marine molluscs ranged from 5.8 ug/L (Martin et al., 1981) to 39 mg/L Cu (Eisler, 1977). Sublethal effects include:

Behaviour — The burrowing ability of the clam, *Venerupis decussata*, was reduced at 10 $\mu g/L$ Cu (Stephenson, Taylor, 1975).

Reproduction — Oysters did not spawn at concentrations of 22 and 42 μg/L Cu. Spawning was profuse at 4 μg/L Cu (Mandelli,1975).

Growth — The 42 d ECSO for growth was reported at 5.8 ug/L Cu (*Argopecten irradians*; Pesch et al., 1979).

Crustaceans

The larval forms of crustaceans were found to be less tolerant to copper during short term exposure than adults. (Spear and Pierce, 1979). Calanoid copepods were the most sensitive crustacean with LC50s in the range of 17-55 μg/L Cu. A concentration of 6 μg/L Cu is reported to be lethal to copepod larvae, *Euchaeta japonica* (Lewis et al.,1973). Lethal concentrations for adult copepods were at least 2 orders of magnitude greater (Corner and Sparrow, 1956; Barnes and Stanbury, 1948). LC50 values for the Dungeness crab larvae are 49 μg/L Cu (EPA, 1985). Sublethal effects include:

Attachment — The cyprids of the barnacle *B. balanoides*, exposed for 12 hours to 10 μg/L Cu, did not complete attachment to a substrate (Pyefinch and Mott, 1948).

Chemosensory Attraction — During a 48 hr exposure of the lobster *H. Americanus* to a standard chemosensory stimulus at 40, 80, and 100 μg/L Cu, attraction was significantly reduced. Normal attraction was observed in the subsequent 48 hour period in the absence of copper (McLeese, 1975)

Growth — Growth of the shrimp, *Pandalus danae*, was reduced at 41 ug/L copper (Gibson et al., 1976).

Echinoderms

Sublethal effects on Echinoderms include:

Development — A 16 hr exposure of the sea urchin *Arbacia punctulata* to cupric chloride concentrations ranging from 320 to 2500 μg/L Cu resulted in retarded or arrested development (Waterman, 1937). Gametes of the sea urchin *Psammechinus miliaris* underwent normal fertilization and cleavage when exposed to 64 μg/L Cu in natural seawater (Cleland, 1953).

Growth — Exoskeleton formation in larvae of the sea urchin *Echinometra mathaei* was significantly reduced at 20 and 50 μg/L Cu.

Annelids

The LC50 for saltwater polychaete worms ranges from 120 - 480 $\mu g/L$ Cu depending on the species (EPA, 1985).

4.4.1.3 Copper Toxicity to Vertebrates

Acute values for copper toxicity for saltwater fishes ranged from 13.93 to 411.7 $\mu g/L$. The lowest value was obtained in a test with embryos (EPA, 1985). The following sublethal effects have been documented:

Behaviour — The responsiveness of herring (*Clupea harengus*) to light stimulus was affected at 90 to 130 ug/L Cu (Blaxter, 1977)

Reproduction — Larval emergence was reduced by 50% for the mummichog, *Fundulus heteroclitus* at 250 ug/L Cu. In addition, hatching time of mummichog eggs was delayed at 500 ug/L Cu (Gardner and Laroche, 1973).

Morphology — The gill ultrastructure of the winter flounder was altered (*Pseudopleuronectes americanus*) at 180 ug/L Cu (Baker, 1969); lesions to the lateral lines and olfactory organs of the Atlantic silverside (*Menidia menidia*) were observed at 500 ug/L (Gardner and Laroche, 1973).

4.4.2 Toxic Effects of Zinc in Marine Organisms

In seawater, inorganic zinc is divided between free metal ion, chloro complexes and the carbonate form (Florence and Batley, 1980). Note that it is generally accepted that ionic forms of a metal are the most biologically available and thus the most toxic. (Sanders et al., 1983; Sunda et al., 1983; Sanders and Jenkins, 1984; Jenkins and Sanders, 1986). At a pH of 8, less than 0.1% of zinc is predicted to be adsorbed (Florence and Batley, 1980). Zinc complexes are soluble in neutral and acidic solution, and zinc can complex with organics as well as anionic, cationic, neutral and more complex ligands. High solubility makes zinc one of the most mobile of the heavy metals (EPA, 1987).

Concentrations of zinc in uncontaminated seawater range from 0.001 to 10 $\mu g/L$ Zn and tend to increase with depth (Florence and Batley, 1980). In seawater, zinc typically occurs in the following forms (in decreasing order of prominence): zinc hydroxyde, $Zn(OH)_n$; Zn^{+2}; zinc monochloryde, $ZnCl^+$; and zinc carbonate, $ZnCO_3$ (Florence and Batley, 1980).

Spear (1981) conducted a comprehensive review of the literature prior to 1981 on the effects of zinc on marine invertebrates. He notes that the survival of marine invertebrates is adversely affected by zinc concentrations of greater than 100 $\mu g/L$ Zn and zinc concentrations ranging from 100 to 350 $\mu g/L$ Zn caused significant mortality in the most sensitive annelid and arthropod species, in the larvae and eggs of pelecypods and in bryozoans. In contrast, adult gastropods and echinoderms are able to survive brief exposures to concentrations greater than 200 μg/L Zn (Spear, 1981). The effects of zinc are greatest at high temperatures (Eisler, 1977) and low salinities (Bryan and Hummerstone, 1973; Jones, 1975). In general, zinc is less toxic than copper.

4.4.2.1 Zinc Toxicity to Algae

Many studies have been conducted on the toxicity of zinc to saltwater algal species (EPA, 1987). Concentrations ranging from 15 to 30,000 μg/L Zn have been reported to elicit toxic effects in algae (Spear, 1981). Endpoints are usually sublethal, i.e., reduced growth and reduced chlorophyll a. The range of zinc toxicities to various classes of marine algae are:

- diatoms: 100 to > 33, 600 μg/L Zn
- green algae: 13,000 to > 33, 600 μg/L Zn
- brown macroalgae: 250 to 7,000 mg/L Zn

4.4.2.2 Zinc Toxicity to Invertebrates

Mollusca

The acute lethal concentrations of zinc to adult marine molluscs ranges from 166 to 750,000 μg/L Zn. The lowest reported effective concentration of zinc to the larval and embryo stages of marine molluscs was 175 μg/L Zn, which was shown to affect the normal development of blue mussel (*Mytilus edulis*) embryos (Mance, 1987); highest values were 3,800 μg/L Zn, also for the blue mussel (Ahsanullah, 1976).

Sublethal responses to zinc exposure include:

Resistance to Stress — At 800 - 1,000 μg/L Zn, *M. edulis* experienced reduced resistance to thermal shock (Cotter et al., 1982).

Growth — The 48d EC50 for shell growth was reported as 60 μg/L Zn (*M. edulis*; Stromgren, 1979); 80 - 95 μg/L Zn reduced the growth of 16d old *Crassostrea gigas* larvae (Watling, 1982). In addition, 1,800 μg/L Zn inhibited byssal thread production (*M. edulis*; Martin et al., 1975).

Biochemical — Calcium uptake by the clam, *Mulinia lateralis*, was reduced at 17.6 μg/L Zn (Ho and Zubkoff, 1982).

Reproduction — The fertilization success of *C. gigas* sperm cells was reduced by 50% at a zinc concentration of 443.5 μg/L Zn (Dinnel et al., 1983).

Crustaceans

Reported acute toxicity concentrations for zinc to adult marine crustaceans range from 294 to 16,000 μg/L Zn (Dear and Chapman, 1992). The reported LC50s for most crustaceans occurred at $\leq$ 1,600 μg/L Zn; all crustaceans (copepods, amphipods, mysids, crabs) were similar in sensitivity. Acute zinc toxicity data for the larvae of two species of marine crustacean, Dungeness crab (*C. magister*) larvae (Dinnel et al., 1983) and green crab (*Cancer maenas*) larvae (Connor, 1972) were within the range documented for adult crustaceans, i.e., 586 μg/L Zn and 1,000 μg/L Zn, respectively.

Data on the sublethal toxicity of zinc to marine crustaceans are limited:

Growth — Zinc at a concentration of > 1,000 μg/L Zn inhibited leg regeneration in the fiddler crab (*Uca pugilator*; Weis, 1980).

Osmoregulation — Osmoregulatory ability was reduced in the isopod, *Idotea baltica* at a zinc concentration of 20,000 μg/L Zn (Jones, 1975)

Echinoderms

Although limited data are available on the acute lethality of zinc to echinoderms, a study conducted by Dinnel et al. (1983) determined the acute toxicity of zinc to both sand dollars and sea urchins using two sublethal endpoints, fertilization success and embryo development. At a zinc concentration of 23.1 μg/L Zn, the development of purple sea urchin embryos to the pluteus stage was affected. Based on the data presented by Dinnel et al. (1983) it appears that echinoderm gametes are the most sensitive of all marine invertebrates to zinc toxicity. Data on sublethal effects of zinc on echinoderms are limited.

Development concentrations of zinc ranging from 65 to 33 μg/L Zn were found to affect developing sea urchin embryos (Waterman, 1937; Kobayashi, 1971). Effects reported included abnormal cleavage, inhibition of gastrulation, and the lack of formation of the fertilization membrane.

Annelids

Reported acute toxicity of zinc to various annelids (Dear and Chapman, 1992) provides LC50s ranging from 900 - 12,000 μg/L Zn. Both *Neanthes arenaceodentata* and *Capitella capitata* juvenile polychaete larvae were slightly more sensitive than adults to acute zinc toxicity (900 - 1,700 *versus* 1,800 - 3,000 μg/L Zn, respectively; Reish et al., 1976). Data on sublethal effects on annelids are limited:

Reproduction — Reish and Carr (1978) exposed two polychaetes, *Ophryotrocha diadema* and *Ctenodrilus serratus* to 6 metals, including zinc for 96h and 21d. The 96h LC50 (1,400 μg/L Zn) was slightly higher than the concentration which inhibited reproduction (500 μg/L Zn) in *O. diadema* (Reish and Carr, 1978). The 96h LC50 of zinc (12,700 μg/L Zn) was much higher than the concentration which inhibited reproduction (500 μg/L Zn) in *C. serratus* (Reish and Carr, 1978).

4.4.2.3 Zinc Toxicity to Vertebrates

The toxic effects of zinc in marine fish have not been extensively reported in the literature. For temperate fishes, most of the literature focuses on the freshwater species and/or life stages of salmonids. Documented lethal effects of zinc toxicity are reported in the range of 364 to 85,000 μg/L Zn (Mance, 1987; EPA, 1987). As for marine invertebrates, the early life stages of fish tend to be most sensitive.

4.5 SUMMARY OF ENVIRONMENTAL EFFECTS OF THE ANACONDA BRITANNIA MINE DISCHARGE

4.5.1 Physical Conditions and General Metals Exposure

4.5.1.1 Physical Oceanography

The Anaconda Britannia Mine is located on the east coast of the inner basin of Howe Sound. Howe Sound is a coastal fjord located along the B.C. mainland coast. It consists of a deep outer basin, with depths up to about 250 m, and an inner basin with similar depths. These basins are separated by a sill whose depth is approximately 70 m. One effect of the sill is to prevent the continuous renewal of deep water in the upper basin, resulting in occasional hypoxia in the bottom waters (Drysdale and Pederson, 1992). The deep water inside the sill is fairly homogeneous and usually has fairly low oxygen levels. Dissolved oxygen concentrations measured between the inner sill and the Squamish River are typically about 6 to 10 mg/L in the top 50 m, declining to less than 1 mg/L at depths greater than 100 m (Waldichuk, unpublished). Partial or total replacements occur from time to time raising oxygen levels and density values. Replacements to the bottom occur in late fall or early winter at intervals of one to four years. Replacements of intermediate depths occur more often and sometimes occur during freshet in late spring (or other times associated with short high rainfall periods) as well as in the late fall-early winter period (Pond, 1992).

The dynamics of the surface currents are driven by the Squamish River discharge, tides and wind and have been described by Buckley (1977). Surface currents flow mainly south, but form a counterclockwise gyre in the vicinity of Britannia Beach. Sediment transport patterns in Upper Howe Sound agree well with known surface currents as well as with currents in deep water (>150m). A gyre could not be detected in a trend analysis. This suggests that out-flowing bottom currents may be negating the effects of surface circulation near Britannia Beach (McLaren and Cretney, 1992). The net sediment transport regimes near the Britannia Beach area are driven by the Squamish River, and are indicative of total deposition. Sediments have a mean grain size approximately 40-80 μm (Jenkins and Sanders, 1986; Syvitski and McDonald, 1981) and once deposited, undergo no further transport (McLaren and Cretney, 1992) and are not subject to reworking by strong bottom currents (Syvitski and Mcdonald, 1981).

Howe Sound is an estuarine system with significant freshwater inputs, largely from the Squamish River. The waters of the upper basin are strongly influenced by salinity, temperature and current changes associated with freshwater input. A pronounced stratification occurs during freshet of the Squamish River (May to September) and extensive silt loading and turbidity results. Progressive density differences between water outside and inside the sill, which entrains lower-density freshwater from the Squamish River, cause periodic renewal of the bottom water in the inner basin (Harding, 1992).

Metal Loadings to the Marine Environment

Currently, the two major sources of metals loadings to Howe Sound as a result of the Anaconda Britannia Mine drainage are Britannia Creek and the 4100 Portal and treatment plant outfall. The outfall runs through Britannia Creek and discharges at 30 m depth in Howe Sound. SRK (1991) indicated that the major source of loading to Britannia Creek arises from drainage of the underground mine workings.

Moore (1985) noted that minimum metals loadings from Britannia Creek occur during late winter, with dissolved copper and zinc concentrations approximating 4.8 and 10.2 kg/day respectively (at 670,000 m^3/day, using a dissolved copper concentration of 0.8 mg/L Cu and a dissolved zinc concentration of 0.17 mg/L Zn). During late spring, creek discharges (from snowmelt and precipitation) can be estimated at 750,000 m^3/day, with the dissolved copper and zinc concentrations approximating 338 kg/day and 263 kg/day respectively (data from June 21, 1983). The concurrent discharge from the Britannia Creek outfall (4100 Portal and treatment plant discharge) on June 21, 1983 approximated 485 kg/day of dissolved copper.

Goyette and Ferguson (1985) found mean copper loadings to Howe Sound from 1980 to 1984 from the Britannia Creek outfall to be 230 kg/day of dissolved copper. These loading data do not include copper loading from the creek itself.

SRK (1991) calculated total copper and zinc loadings to Howe Sound from Britannia Creek and the Britannia Creek outfall for two sampling periods; September and December 1990. The total copper loading to Howe Sound from Britannia Creek was 0.9 kg/day in September and 128 kg/day in December. Copper loading from the Britannia Creek outfall was 51 kg/day in September and 289 kg/day in December. Zinc loadings in September from the creek and the outfall were 1.9 kg/day and 110 kg/day respectively. Zinc loadings in December from the creek and outfall were 84 and 541 kg/day. Measured flows at the mouth of Britannia Creek were 97 and 1770 L/s during September and December sampling, respectively. December metal loadings are confounded by a bypass of the treatment plant due to high flows.

From the available data, it is clear that the highest metals loadings occur during freshet or high rainfall events.

4.5.1.2 Water Quality in the Marine Environment near the Anaconda Britannia Mine Site

Due to the metals loading from Britannia Creek and the Britannia Creek outfall, it appears that copper and zinc concentrations are elevated relative to background values observed in Howe Sound (Dunn et al., 1992). Dunn et al. (1992) found that samples taken at the mouth of Britannia Creek in July (1991) ranged between 100 and >250 μg/L for copper and for zinc, and exceeded safe limits for freshwater aquatic life (Cu < 2 -4 ppb; Zn < 30 ppb; Canadian Water Quality Guidelines, 1987). Copper complexing work undertaken in 1990 near the Anaconda Britannia Mine site showed that copper concentrations radiating out from Britannia Beach, both in the surface water samples and those collected at depth were high - up to 888 μg/L Cu in the surface sample and 175 μg/L Cu at 15 m depth. In 1991, maximum copper concentrations in the water column near Britannia Beach were 175 μg/L Cu in a surface sample and 22 μg/L Cu in a sample collected at 15 m depth. The time of sampling was not indicated in the report (Moore and Swain, 1992). Earlier work in 1985 (Moore, 1985) characterized the extent of copper and zinc contamination emanating from the mine site. For example, mean dissolved copper levels in Howe Sound in September 1984 outside of the Britannia Beach area do not exceed 1.9 μg/L Cu. Surface levels of copper near Britannia Beach were at 4.7 μg/L Cu. Dissolved zinc concentrations in September 1984 ranged from 7-10 μg/L Zn. Thus, metals concentrations in the water column fluctuate, becoming elevated during flushing events. Sediments and suspended particulate matter may be binding copper, thereby acting as a sink and decreasing the toxicity of copper and zinc in the water column.

The BCMELP criterion (Nagpal et al., 1995) for the protection of aquatic life is 3 μg/L of total copper. The criteria for total zinc are: 86 μg/L Zn (4 day average); 95 μg/L Zn (1 hr average; and 19 μg/L Zn for some marine plants. Although none of the above studies indicated whether the copper and zinc concentrations were total or dissolved, the sampling concentrations reported for copper are substantially higher than these criteria and likely to cause adverse biological effects. Zinc appears to be problematic, in that concentrations in the water column are only sporadically high enough (>100 μg/L Zn) to cause adverse biological effects.

4.5.1.3 *Sediment Quality in the Marine Environment near the Anaconda Britannia Mine Site*

Dunn et al. (1992) sampled two nearshore bottom sediments in the Britannia Beach area, all of which had elevated copper and zinc concentrations (>500 ppm) compared to background values for sediments. Lead and antimony were also found to be enriched near Britannia Beach but other trace elements were found to be close to concentrations typical of background values for sediments (Dunn et al., 1992). In 1980, concentrations of copper and zinc in sediments offshore of Britannia Beach were 2700 and 829 ppm respectively (Ellis and Popham, 1983).

Drysdale and Pederson (1992) conducted an extensive two stage study of the Britannia tailings deposit to determine: the extent of the tailings and the degree to which they were being covered or diluted by natural sediments; the contribution of dissolved trace metals (specifically copper, zinc, manganese, iron, and lead) from the tailings deposit to the overlying waters. The areal distribution of copper and zinc indicated that the tailings have been considerably dispersed from their place of origin. However, Drysdale and Pederson (1992) found that concentrations of copper in surface sediment (0-2cm) were high (>400 ppm) only in close proximity to the mine outfall. Comparisons with Thompson and Paton (1976b) indicate that the tailings had been diluted by recent sedimentation. In a sediment core taken approximately 4 km downstream from the Anaconda Britannia Mine site, high copper and zinc concentrations were encountered at 14 cm below the sediment surface; this depth was interpreted to be the top of the tailings deposit (Drysdale and Pederson, 1992). With respect to concentrations of copper and zinc in the water column, Drysdale and Pederson (1992) concluded that the diagenetic environment of the tailings rich sediments indicates that there is little likelihood that the buried tailings deposit is contributing to the excess of copper and zinc in the water column. However, this may not be representative of shallower surface sediments.

The BCMELP copper criteria (Nagpal et al., 1995) for marine sediment quality range between 70 (low effects range) and 390 (median effects range). Sediment criteria for zinc range from 120 (low effects range) to 270 ppm (median effects range). Copper and zinc concentrations in surface sediments currently existing near Britannia Beach are elevated compared to BCMELP criteria.

4.5.2 Probable Effects on Juvenile Salmonids

4.5.2.1 Salmonids in Britannia Bay and Howe Sound

There is little specific information regarding salmonid usage of Britannia Bay and Britannia Creek. Downstream of the Dam near the Jane Creek confluence, Britannia Creek does not support any fish populations, due to physical as well as chemical unsuitability (Carlson, 1985). Upstream of the dam, a cutthroat trout *(Oncorhyncus clarkii clarkii)* population exists in the creek itself and in Park Lane Lake. Other nearby creeks such as McNab, Potlach, Foulger, Woodfibre, Mill Creek, Shannon, and Furry Creek support resident trout populations, i.e., cutthroat, steelhead (*Salmo gairdneri*) and rainbow (*Salmo gairdneri*). Some of these creeks also provide limited rearing and spawning habitat for coho (*Onchorhynchus kisutch*), chinook (*Onchorhynchus tshawytscha*), pink (*Onchorhynchus gorbuscha*), and chum salmon (*Onchorhynchus keta*) (Moore, 1985). In addition, prior to fisheries closures (in 1968), the Britannia Beach area was well known for its chinook fishery, as chinook salmon were known to collect in that area of the Sound before migrating to the Squamish River to spawn (Moore, 1985).

Considerable information exists regarding salmonid usage of the Howe Sound upper basin and associated tributaries. A comprehensive overview of the distribution of salmonids in Howe Sound was conducted by Levings and Riddell (1992) and Hatfield (1994). Before closure of commercial fishing in Howe Sound in 1968, Howe Sound was a major harvest area for salmon, particularly for chum, pink and chinook salmon (Anon, 1980 in Levings and Riddell, 1992). The Squamish River System in particular supports large numbers of salmon such as pink, chum, chinook and coho which are important to the local recreational, native and commercial fisheries. Sockeye salmon are not common but have been observed in the Squamish River System (Hatfield, 1994). In addition, Dolly Varden char (*Salvelinus malma*), anadromous and resident cutthroat trout, steelhead trout, and rainbow trout are common in the Squamish River System (Levings and Riddell, 1992) and in Howe Sound streams. The vast majority of escapement to Howe Sound is from the Squamish River.

Pink Salmon — Pink salmon return to the Squamish River in odd-numbered years. Adult pink are present in the Squamish River from August to early October. During even-numbered years, pink salmon smolt are likely present in the river between March and May, before entering Howe Sound (Heard, 1991). Pink salmon move to sea almost immediately after emergence and do not rear in estuaries (Levings and Riddell, 1992). Pink salmon have been reported from Rainy River and McNab Creek. The average spawning escapement to the Squamish River System (including tributaries) from 1981 to 1985 was 5,540. The average spawning escapement to Howe Sound streams from 1971-1980 was 25. (Farwell et al., 1987).

Chum Salmon — Chum return to the Squamish River in high numbers. They begin their spawning migration in October, with spawning occurring from November to January. Chum salmon have been reported spawning in almost all rivers and creeks draining into Howe Sound, except for Britannia Creek. Chum fry use the Squamish River estuary as a rearing area (Goodman and Vroom, 1992) and have been reported using foreshore habitat near the Rainy River (Birtwell and Harbo, 1980). Underyearling chum are present in the estuary for several weeks in the spring (Levings and Riddell, 1992). The average spawning escapement of chum to the Squamish River System (including tributaries) and Howe Sound Streams from 1981 to 1985 was 167,051 and 5,904 respectively (Farwell et al., 1987).

Chinook Salmon — Adult chinook return to the Squamish area in late June and spawn from July to early October (Hatfield, 1994). Juvenile chinook salmon have been observed in the Squamish River in autumn (Clark, 1987 in Levings and Riddell, 1992). These juveniles were probably about to begin winter rearing. Juvenile chinook have also been found in the Squamish Estuary during late spring and summer (Levy and Levings, 1978). The average spawning escapement to the Squamish River System (including tributaries) from 1981 to 1985 was 3,126. (Farwell et al., 1987).

Coho Salmon — Adult coho spawn in the Squamish River and its tributaries from September to January (Hatfield, 1994). Coho fry and smelt have been observed in the estuary, during spring and summer (Levy and Levings, 1978). Spawning coho have been reported from Howe Sound streams. Coho salmon fry rear in freshwater for at least one year and there is evidence that coho also use the Squamish estuary in the fry and smolt stage. The average spawning escapement to the Squamish River System (including tributaries) and Howe Sound streams from 1981 to 1985 was 12,781 and 101 respectively. (Farwell et al., 1987).

It is likely that some species of adult salmon use Britannia Bay before moving on to spawn in the Squamish River System. The timing of adult salmon migration (i.e., fall/winter) to spawning grounds coincides with lowered metal loadings to Britannia Bay. Although zinc and copper in Britannia Bay tend to be lower in the winter, adult salmonids may still be subject to a variety of sublethal effects on reproduction, growth, and swimming ability (see Sections 4.3.1.3 and 4.3.2.3). These sublethal effects can occur at concentrations as low as 5-20 ug/L Cu and 5-6 ug/L Zn, concentrations which have been known to occur near Britannia Bay.

Migrating juvenile salmon may also use Britannia Bay before moving out to sea (Moore, 1985). The extent to which juvenile salmon use Howe Sound is unclear. Juvenile salmon (i.e., coho) in Howe Sound spend up to a year in freshwater and are probably not exposed to the Anaconda Britannia Mine drainage until the yearling stage. In other salmon species, (i.e., pink), the young migrate almost immediately out to sea and may be exposed to the mine drainage during high metal loadings in the spring. Spring copper and zinc concentrations in

Britannia Bay may exceed 250 $\mu g/L$. Concentrations of this magnitude may cause lethality or a variety of sublethal effects (see Sections 4.3.1.3 and 4.3.2.3)

4.5.2.2 Salmonid Bioassays

Moore (1985) and Van Aggelen and Moore (1986) conducted some toxicity testing on salmonids. Moore (1985) attempted to determine toxicity at the acute and sublethal levels for chum fry using Anaconda Britannia Mine's final discharge water. Approximately 500-600 chum swim ups were obtained from the Inch Creek Hatchery and allowed to acclimate to a salinity of 19 ppm over a two and a half week period. The type of bioassay conducted was an LC50 replacement test with approximately 90% of the water exchanged and a ration of food every 96 hours for 30 days. The concentrations tested were 2860, 1660 and 1330 mg/L of mine water. There were two mortalities that occurred in 2860 mg/L concentration. These deaths occurred on the 24th and 26th days of testing. It is unclear whether the fish died as a result of effluent or other factors such as impairment in ability to acclimate to salt water.

A metallothionein bioassay was also conducted using two year old coho smelts (Van Aggelen and Moore, 1986). The fish were acclimated to a temperature of 8°C and were fed a daily ration for a two week period. A 21 d saltwater bioassay at a concentration of 2860 mg/L mine effluent (meant to simulate conditions that would occur 50 m from the outfall during winter) was conducted. No stressing or mortalities were observed. Test fish did not produce significantly more metallothionein than control fish.

In addition, bottom sediments taken from the mine site were tested for toxicity to chinook salmon after 30 days of exposure (Van Aggelen and Moore, 1986). The results did indicate some mortality (33%) with tailings taken south of Britannia Bay. These fish showed signs of stress, with tail and fin areas being severely eroded and a number of lesions around the mouth area.

Generally, these bioassays were limited in scope and inconclusive (Moore 1985). The testing was conducted in the laboratory and mine effluent was collected in the winter, a time of traditional lower metals loadings to Howe Sound. More detailed studies need to be conducted to provide a more complete overview of the toxicity of mine drainage to salmonids and to juveniles in particular.

4.5.3 Probable Effects on Marine Organisms

4.5.3.1 Benthic Invertebrates

There have been numerous studies and qualitative observations regarding the paucity of benthic invertebrates near the Anaconda Britannia Mine site. For example, Goyette and Ferguson (1985) noted that 1984 submersible observations off the Anaconda Britannia Mine site revealed an almost complete absence of benthic invertebrates and there were no signs of burrowing activity.

In a 1990 study (Ellis and Hoover, 1990), marine benthos colonizing the Anaconda Britannia Mine tailings discharge was compared to an adjacent area approximately 1 km north of Furry Creek. This study demonstrated that biological differences between tailings and non-tailing areas remained 12 years after the mine closure in 1974. While all stations had a similar number of organisms, tailings station fauna was less diverse than those at non-tailing stations. In summary, Ellis and Hoover (1990) observed increasing diversity from shallow to deep water and from the tailings site to the reference site. In addition, it was indicated that the shallow water reference station may have also suffered from copper contamination, due to extensive water column contamination downstream from the Anaconda Britannia Mine site. Unfortunately, no indication was made as to whether some of the differences in benthic communities could be attributed to changes in physical conditions in the sediments and tailings deposits, as opposed to metals concentrations.

Various studies have found elevated concentrations of copper and zinc in marine benthic tissues located downstream from the Anaconda Britannia Mine site. Copper concentrations in the mussel *M. edulis* were found to be elevated by a factor of 30 (358 to 431 $\mu g/g$ Cu) in Britannia Bay over Indian Arm reference populations (Moore, 1985). In results from June 1975, mussel (*M. edulis*) samples taken downstream from the Britannia Beach area showed copper and zinc to be 390 and 550 $\mu g/g$ respectively. Tissue concentrations in oysters *(C. gigas)* taken downstream of the mine ranged from 2100 to 2500 Cu (Goyette and Ferguson, 1985). Tissue Cu concentrations were highest at the mine site.

Levings and McDaniel (1976) noted that the industrial disruption of beaches at the Anaconda Britannia Mine site can be related to four sources: contamination from copper mining operations at the Anaconda Britannia Mine site; modification of sediments by disposal of mine tailings; discharge of sediments from the gravel concentrating facility; and shoreline disruption related to the construction of docks. Since the gravel concentration facility no longer exists and there is currently little construction in the area, it is likely that metals contamination from the mine in the water column and possibly contaminated surface sediments are contributing to the current lack of benthic diversity in the Britannia Beach area.

In summary, water column copper concentrations near the Britannia Beach area are periodically, and in some cases consistently, high enough to cause lethal effects and a variety of sublethal effects such as: reduced resistance to stress; reduced growth; biochemical changes; decline in reproductive success; and loss of osmoregulatory ability (see Section 4.4.1).

4.5.3.2 Macrophytic Algae

There is limited information regarding the effects on algae from the Anaconda Britannia Mine drainage. A study conducted by Dunn et al. (1992) showed that the shoreline 1.5 km both north and south of the mine site was devoid of seaweed. In addition, common rockweed (*Fucus gardneri*) found near the Anaconda Britannia Mine site (outside of the 1.5 km range) had copper concentrations exceeding 3,000 ppm and up to 1,540 ppm of Zn. Data from 1980 showed *Fucus sp.* near Britannia Beach contained 111 and 6 ppm copper and zinc respectively (Ellis and Popham, 1983).

As discussed in Section 4.4.1, water containing copper concentrations as low as 4.6 μg/L Cu can cause sublethal effects such as growth reduction and decrease in photosynthesis rates. Zinc concentrations ranging from 15 μg/L Zn have been reported to elicit sublethal effects such as reduced growth and chlorophyll (section 4.4.2). Since water concentrations in the Britannia Beach area have been shown to exceed these limits, it is likely that the Anaconda Britannia Mine discharge is at least partly responsible for the visible lack of marine algae near the site.

4.5.3.3 Plankton

With respect to phytoplankton, waters adjacent to the Anaconda Britannia Mine site were some of the least productive regions in Howe Sound. This observation may be partially due to severe light attenuation in the surface waters caused by the turbid, glacial Squamish River (Stockner, 1992). Plankton samples collected by tow in Howe Sound during May and June 1985 were analyzed for copper and zinc. Britannia Bay plankton averaged 1114 μg/g Cu and 464 μg/g Zn, while background concentrations averaged 443 μg/g Cu and 350 μg/g Zn (Moore, 1985).

Bioassay tests conducted by Moore (1985) on a marine bacterium (*Photobacterium phosphoreum*) and brine on shrimp (*Artemia salina*) showed that toxic effects can occur at dissolved copper concentrations (520 μg/L and 740 μg/L respectively) that have been measured in Britannia Bay (Moore, 1985). In addition, zinc concentrations in the water column greater than 100 μg/L, which has been measured in Britannia Bay, can cause sublethal effects to marine diatoms. Copper concentrations of 5.0 μg/L can reduce growth rates after exposure for 5 days (Section 4.4.2). As with algae, copper and zinc concentrations in the water column near Britannia Beach are periodically high enough to cause adverse lethal and sublethal effects.

4.5.3.4 Pelagic and Demersal Non Salmonid Fisheries

There is a general lack of information with regard to non-salmonid fisheries near the Anaconda Britannia Mine site. There have been qualitative observations regarding the absence of expected fish species (Goyette and Ferguson, 1985; Ellis and Popham, 1983). As acute values for copper toxicity for saltwater fishes can range from 13.93 to 411.7 $\mu g/L$ in the water column (Section 4.4.1), it is likely that non salmonid fish near the Anaconda Britannia Mine are affected by copper concentrations, either through lethal effects or through sublethal effects (e.g., avoidance, behaviour, growth, swimming performance). Juvenile fish are probably the most sensitive to high copper concentrations. The tolerance of fish for zinc appears to be higher than for copper. Documented lethal effects of zinc toxicity range from 364 to 850 $\mu g/L$ (Section 4.4.2). Zinc concentrations in the water column at Britannia Beach are probably well below 363 $\mu g/L$ for most of the year, except during high rain events or the spring freshet.

A suspension feeder (*Protothaca staminea*) caught near Furry Creek (6 km from Britannia Beach) had elevated copper and zinc concentrations in the viscera and gill compared to at reference site. Copper concentrations were 119 and 12 $\mu g/g$ in the viscera and gill. Zinc concentrations were 100 and 90 $\mu g/g$ in the viscera and gills (Ellis and Popham, 1983). A relationship between tissue bioaccumulation and potential for effects could not be determined.

4.6 OVERALL ASSESSMENT OF RISKS

There is a substantial body of literature indicating that copper and zinc concentrations in the water column can cause a variety of lethal and sublethal effects to salmonids and other marine organisms (see Sections 4.3 and 4.4). A potential for elevated risk to the Howe Sound marine environment exists due to high loadings of copper and zinc from Britannia Creek and the 4100 Portal and treatment plant outfall, and high concentrations of copper and zinc in sediments near the Anaconda Britannia Mine site (see Section 4.5). Overall, the published evidence suggests that elevated metal concentrations in Britannia Bay may have lethal and/or sublethal effects on salmonids and a significant effect on salmonid habitat and the surrounding marine environment.

Potential risk to salmonids is supported by:

- The presence of salmonids in numerous creeks in the Howe Sound drainage basin, with the exception of Britannia Creek. A cutthroat trout population does exist upstream of the dam in Britannia Creek where the mine has no influence. This suggests that Britannia Creek south of the dam may be unsuitable, either chemically and/or physically, to support salmonid populations.

- The possible usage of Britannia Bay as a stageing area for chinook before migrating to the Squamish River to spawn indicates that adult salmonids are potentially exposed to elevated metal loadings in the water column. Juvenile salmonids may also be exposed to high spring metal loadings from Britannia Creek due to spring migration to sea by some salmon species (e.g., pink salmon).

- Salmonid bioassays conducted by Moore (1985, 1986) were inconclusive but sediment toxicity tests did indicate tail and fin erosion, lesions around the mouth area, and some mortality (33%) with exposure to tailings taken south of Britannia Bay.

Potential risk to salmonid habitat and the Howe Sound marine environment is supported by:

- Studies indicating reduced benthic diversity near the Anaconda Britannia Mine site and elevated concentrations of copper and zinc in marine benthic organisms located downstream from the mine.

- The absence of macrophytic algae approximately 1.5 km both north and south of the mine. In addition, the rockweed (*Fucus gardneri*) found outside of this range contained high levels of copper and zinc.

- Bioassay tests conducted by Moore (1985) on plankton showed that toxic effects can occur at dissolved copper concentrations that have been measured in Britannia Bay.

- Qualitative observations regarding the lack of expected fish species near the mine site.

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5.0 CONCLUSIONS

The following conclusions and recommendations were derived based on the findings of Phases I, II, and III:

- Available data indicate high metals exposure, particularly copper and zinc, in water, sediments and biota in proximity to the Anaconda Britannia Mine site.

- These metals exposure levels have been associated with lethal and/or sublethal effects to juvenile salmonids and other marine organisms in other studies.

- There is a general lack of information and/or moderate to high levels of uncertainty associated with assessments of effects in the Howe Sound receiving environment.

The above conclusions further justify the need for a field assessment to quantify the spatial extent and magnitude of effects related to drainage from the Anaconda Britannia Mine. As detailed in Section 3, the use of an integrated assessment will provide a weight-of-evidence approach to formulate overall conclusions. The field assessment should emphasize the assessment of risks to juvenile salmonids and should address: metals exposure, metals bioavailability, direct toxicity (i.e., lethal and sublethal effects), behavioural responses (e.g., metals avoidance), and indirect effects on fish habitat. Metals bioavailability and effects to other resident marine organisms (e.g., blue mussels) should also be investigated to support the findings of the juvenile salmonid studies and to ensure that other components of the Howe Sound marine environment are adequately evaluated as part of the recommended integrated assessment.

APPENDIX A

List of Scientific Databases Searched Through DIALOG

List of Scientific Databases Searched Through DIALOG

NTIS
Oceanic Abstracts
Dissertation Abstracts Online
Pollution Abstracts
Aquatic Science Abstracts
GeoArchive
Life Sciences Collection
Geo-Ref
Pascal
Zoological Record Online
PAPERCHEM
GEOBASE
Scisearch
Current Contents Search
Ei Compendex
Energy SciTec
Water Resource Abstracts

APPENDIX B

List of Contacts

List of Contacts

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APPENDIX C

Minutes of the Workshop on Recommendations for Field Assessment

BRITANNIA WORKSHOP MINUTES

EVS CONSULTANTS, FEBRUARY 24, 1997

1. Introduction

A brief overview of the project was provided, following round-table introductions. The purpose of this project is to provide a first step towards assessing environmental risk to juvenile salmonids from the Britannia Mine drainage. The first phase of this project was a data compilation which was synthesized into a Data Matrix. This workshop was intended to invite comments on the Data Matrix, identify data gaps, and discuss alternative study designs and technical approaches.

2. Data Matrix Presentation and Discussion

A presentation and hand-out were provided by EVS relative to the workshop objectives.

The question was raised regarding what "not applicable" meant in the data matrix. The answer is that in these cases there were no data. It was agreed to replace "not applicable" with "not tested". It was clarified that if studies had been conducted but were inconclusive or full details were not available, then additional studies were felt to be necessary.

The point was noted that the "Recommendations" field in the Data Matrix reflected the types of studies that might be required to fill the data gaps, not necessarily those being recommended at this time.

The points were made that studies should be:

- Cyclical based on biological and hydrographic conditions
- Conducted over time given the fact that the Britannia mine drainage discharges are not predicable and are, in fact, highly variable (high frequency sampling is probably needed). The nature of this discharge makes the Britannia situation unique. This supports the idea of field measurements of metal uptake and toxicity (i.e., caged fish experiments). Laboratory uptake/toxicity tests will not provide the variability found in the field.
- Inclusive of "worst case" (greatest flushing of the mine occurs in the spring; which is timed with the Squamish freshette. The freshette dilutes metal concentrations but also results in water stratification); highest metal loads in receiving waters appear to occur from April to late June. It was also noted that "worst case" loadings could occur during summer/fall storm events.

- In terms of salmonids, sampling has to occur around April to be meaningful for juvenile downstream migration from the Squamish estuary.
- Design should be based on the science not the timing available for soliciting contractors.

There was discussion regarding sediments and the near-shore area which should be of primary utility to salmonids. There appears to be precipitation and flux of metals to the sediments which may be of more concern than the mine tailings (for which it is unclear whether release of metals might occur). Although offshore tailings do not appear to be contributing to water column metals levels, there is a possibility that metal flux is occurring in shallow littoral sediments. However, in general there appeared to be consensus that the primary concern is metals in the water column; sediments appear to be a secondary concern.

3. Study Designs and Technical Approaches

A presentation and handout were provided by EVS.

It was noted that turbidity currents occur. There was discussion regarding variability. The following points were noted:

- The sub-surface outfall is at a 30m depth and includes acid mine drainage from the 4100 portal mixed with sewage from Britannia Beach. There appear to be no other major discharge points other than Britannia Creek.
- Hepatic copper levels in caged fish could be used to determine exposure, compared with laboratory studies to assess uptake versus release. The unique water chemistry, flow variability at Britannia would be very difficult (and expensive) to duplicate in the lab toxicity test.
- Britannia Creek flow is now being measured by Water Survey and those data are available (flow, temperature, pH, salinity).
- There is no correlation between pH and metal concentrations (other than Fe) on a fine scale of analysis.
- A temperature and conductivity monitor (e.g., HOBO) should be attached to the fish cages to estimate the variability in hydrographic conditions and the presence/absence of the copper containing freshwater plume. Temperature and salinity should be measured on any caged-fish studies to assess the plume.
- Caged-fish studies should be matched with *in situ* studies of fish population.

There was discussion regarding the relationship between remediation and field studies, given that remediation of some sort may be occurring prior to any more scientific study. In answer it was noted that: (1) information is needed to evaluate current risks regardless of remediation being considered; (2) there is consideration of combining all contaminated mine water flows into the existing mid-water (30 m) discharge on the assumption that this will reduce or eliminate exposure to salmonids who will be found in the upper few meters of the water column.

There was extensive discussion regarding linking exposure with effects in light of varying exposures related not to the seepage but rather to water flow. It was agreed that the focus should be on the water not sediment.

There are several salmonid species which may use Howe Sound as a nursery area: chinook, coho, and steelhead. Pink and chum probably move out of the Howe Sound area. There may be a rearing population of chinook in the Sound; trout may previously have been found in Britannia Creek (but there are no data).

Lowest concentrations in the receiving environment occur in the fall, which may be the best period for *in situ* fish surveys. Previous caged-fish studies in Howe Sound have not required feeding for up to 4 weeks of exposure.

There was extensive discussion regarding the use of caged-fish. Given issues such as the variability of the plume, a suggestion was made that these studies be done in the laboratory. The pros and cons of the laboratory versus the field were discussed. It was recommended that studies involve laboratory and field experimental components together with field surveys of resident fish populations. It was pointed out that the laboratory work: can be done at any time of year; and provides the best chance to tease out relationships between exposure and effects. Al Lewis suggested that there are numerous "laboratory" toxicity values for selected salmonids and what should be done is to determine what is happening at Britannia. Laboratory uptake/toxicity studies will not be able to duplicate Britannia conditions.

Tony Farrell noted work in the U.K. has shown that in fresh and salt water, brown trout will survive at low pH and elevated copper levels but will not swim. Specifically, after 96-h exposure periods brown trout were allowed to swim in clean water (pH=5; soft water 50 umol Ca /L). Trout swimming performance was reduced by 50% at 0.08 umol Cu /L (5 deg C); reduced by 30% at 0.08umol Cu /L (15 deg C); and was incapacitated at 0.47 umol Cu /L (5 deg C). It was noted that in late August the 2200 portal which discharges into Britannia Creek dries up, so there would be no metals entering Howe Sound from this source, but only from 4100 at depth.

It was agreed that the best study would combine caged-fish (survival, growth, metals tissue concentrations, life-history characteristics, behaviour), caged-mussels (survival, growth, metals tissue concentrations), laboratory fish studies (toxicity and swimming performance) and field surveys of salmonids, specifically salmon presence/distribution within the vicinity of the mine discharge. If timing and funding does not allow for field studies in 1997, laboratory studies could be useful prior to conducting the full suite of studies in 1998.

Coincident water quality sampling (total and dissolved metals, salinity, temperature, DO, pH) would be conducted concurrently with the exposure of fish and mussels. It was noted that both dissolved and particulate metal concentrations should be measured and their relative contribution to toxicity in fish should be determined. Whether uptake occurs through the gills or food to salmonids should be determined. It was noted that plankton tows could and should be done in conjunction with any caged-fish and caged-mussel studies.

EVS recommended a gradient approach to field sampling. Since the Data Matrix indicates low uncertainty in water and sediment metals concentrations, collection of further data could be focussed on assessing gradient effects that might be found, specifically if a gradient approach is used in the study design. There was some discussion about the number of replicates needed per sampling station. EVS recommended that a large number of stations is more important in this case than the number of replicates. It was noted that stations could be grouped into sites during analysis of data (e.g., three sites with five replicates formed from 15 stations), and between versus inside variability assessed.

4. Attendees

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