COMPARISON OF POREWATER AND ELUTRIATE BIVALVE LARVAL DEVELOPMENT TOXICITY TESTING IN A SEDIMENT QUALITY TRIAD FRAMEWORK

by

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ABSTRACT

The magnitude and extent of contaminated marine sediment are frequently assessed using a Sediment Quality Triad framework involving multiple toxicity tests using a variety of species, toxicological endpoints and exposure systems. Data are integrated into an overall assessment of sediment quality using a weight of evidence approach. Guidance on the selection of appropriate toxicity tests recommends a wide range of organism type, life-cycle, exposure route, and feeding type be included—porewater bivalve larval development tests increases the number of exposure routes under consideration.

Interpretation of porewater toxicity data are subject to limitations, in part, related to the extensive chemical manipulations associated with the extraction of *in situ* anoxic porewater and the subsequent aeration required for the laboratory-based toxicity test. The ability of the porewater-only test to represent ecologically relevant adverse effects in the field appears minimal. Results from concurrent porewater and elutriate toxicity testing using newly fertilized larvae of the bivalve, *Mytilus galloprovincialis* are provided to illustrate these problems. Porewater samples were consistently more toxic than the corresponding elutriate sample, and ammonia, a common confounding factor in sediment toxicology, was identified as the most likely toxic agent for the majority of samples. Porewater toxicity testing using *M. galloprovincialis* is therefore not recommended as a routine element of the sediment quality triad unless additional chemistry (samples used for testing as well as *in situ*) are available to facilitate an appropriate interpretation of the resulting toxicity data.

DEDICATION

For all the support and understanding from my beloved wife, Elana.

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1.0 INTRODUCTION

Marine sediments collect a substantial fraction of atmospheric, aqueous and terrestrial contamination released to the environment as a result of development and industrialization. Contaminated sediments are a ubiquitous issue—for example, USEPA (2001) reported that 52% of all samples included in the US National Sediment Inventory program (10,124 out of 19,470 stations) demonstrated probable or possible adverse effects to aquatic life, based on sediment chemistry, toxicity and the potential for bioaccumulation. Summers (2001) concluded that nearly 30% of the total US estuarine area displayed poorer than expected biological condition, based on sediment toxicity, benthic and fish community composition and fish pathology, while Long et al. (1996) found that nearly 11% of the total area (277 km^2 of 2532 km^2) of 22 major US estuaries exhibited significant acute toxicity to amphipods. Local waterbodies demonstrate similar patterns—for example, PSAMP (2002) concluded that 63% of the total area of Puget Sound was degraded, based on sediment chemistry, toxicity and benthic community structure. Sediment quality degradation can lead to substantial economic costs (e.g., fisheries closures, reduction in recreational uses, increased costs to maintain marine transportation links), as well as threaten human health and aquatic life through contamination of the food chain.

A common approach to assessing the potential risks associated with contaminated sediments involves a weight-of-evidence assessment, such as the Sediment Quality Triad

(SQT; Long and Chapman, 1985; Chapman et al., 1987, Chapman, 1990; Chapman, 1996; Chapman et al., 1997). SQTs consist of three unique and complementary components: sediment chemistry, which measure contamination, sediment toxicity tests, and *in situ* parameters (e.g., benthic community structure), which measure alteration (Chapman, 1990). Data from the multiple lines of evidence are typically analyzed using both weight-of-evidence and multivariate statistical approaches to evaluate the overall magnitude and potential causes of any observed sediment quality impairment.

A guiding principle of the SQT is that the sediment toxicity component should "cover as wide a range as possible of organism type, life-cycle, exposure route, and feeding type" (Chapman, 1990). Chapman et al. (1997) suggested that toxicity test selection should account for "differences in routes of exposure and in organism physiology". Although specific guidance requiring porewater toxicity testing was not recommended, it is an obvious choice given the available guidance (Chapman, 1990; Chapman et al., 1997), and has been incorporated into many SQTs published in the peer-reviewed literature (e.g., Long et al. 1990; Carr et al., 1996a,b; Carr et al., 2000; Anderson et al., 2001a; Hunt et al., 2001). Porewater toxicity tests have been developed for various organisms and toxicological endpoints, including polychaete reproduction (Carr et al., 1989), sea urchin fertilization and development (Carr and Chapman, 1992), copepod survival (Carr et al., 1996a) and kelp spore germination (Hooten and Carr, 1998).

There are two schools of thought regarding the appropriate use of porewater toxicity testing in a SQT framework. Several authors have argued that porewater toxicity tests are

highly advantageous due to the tests' increased sensitivity to chemical contaminants, overall ecological relevance and their ability to avoid confounding factors (e.g., grain size) common to whole-sediment toxicity tests (e.g., Carr et al., 2001a). Other authors have cautioned that porewater toxicity testing has many inherent liabilities that may limit its utility for routine sediment quality investigations (e.g., Chapman et al., 2002). Conclusive evidence for or against the inclusion of porewater toxicity testing in a SQT framework has not yet been presented in the scientific literature—the question as to whether porewater toxicity testing is appropriate is clearly influenced by multiple factors, including the species being tested, the contaminants of potential concern, the unique biogeophysical characteristics of a given location, and the objectives of the investigation.

The specific objective of the study described herein was to determine whether or not the porewater BLD toxicity test should be included as a routine element of the SQT in order to maximize the number of exposure pathways under consideration (i.e., addition of a porewater exposure pathway to supplement whole-sediment exposure pathways typically considered in other toxicity tests). Data were collected from typical SQT projects conducted at four different urban harbour locations, ranging from shipyards and port facilities to shoreline redevelopment projects. Samples were similar for all projects: predominantly fine-grained samples collected from shallow near-shore environments, which were contaminated with metals and polycyclic aromatic hydrocarbons (PAHs). The overall objective was the same for all projects: evaluate the magnitude of sediment impairment using a SQT approach to determine whether or not active remediation (i.e., dredging and disposal) was required. Each project involved a total of two to five different

toxicity tests, which were evaluated using a weight-of-evidence approach in order to develop an overall estimate of sediment toxicity. Typical toxicity tests included in the SQT projects included a 10-d amphipod survival test and a 20-d polychaete survival and growth test, each using whole-sediment. Side-by-side elutriate and porewater versions of the 48-h *Mytilus galloprovincialis* bivalve larval development (BLD) toxicity test were also conducted.

The working hypothesis was that similar conclusions about the magnitude of sediment toxicity (and thus, the need for remediation) would result when either the porewater or the elutriate (i.e., a mixture of sediment and seawater) BLD toxicity tests were considered within a weight-of-evidence approach. If this working hypothesis proved false (i.e., the porewater BLD test demonstrated greater toxicity than the elutriate BLD test), then the relevance of the porewater BLD data in a SQT framework was evaluated. Relevance, in this context, focused on the likelihood of cause-effect relationships between contaminants of potential concerns and any observed toxicity, as well as factors that may influence the ecological realism of porewater toxicity testing. Recommendations regarding the inclusion of the porewater BLD toxicity test in routine SQT assessments based on the findings of the above-mentioned analyses are provided.

2.0 METHODS

2.1 Overview of testing program

Side-by-side porewater and elutriate BLD toxicity tests were conducted for a total of 25 samples collected for four different SQT investigations (Groups 1 – 4) between 2001 and 2003. Client confidentiality agreements limit the amount of information that can be provided about sample location and the specific nature of the upland activity at each site. However, all sediment samples were collected from industrial sites (e.g., shipyards and other port facilities) in otherwise urbanized harbors. Similar patterns of contamination (metals and PAHs) and substrate types (predominantly fine grained silts and clays) were noted for all samples. Group 1 included three samples (1A, 1B, and 1C) collected from uncontaminated reference sites in Puget Sound, while all other samples were from impacted urban harbour sites. Samples were divided into the following groups (one group per separate SQT investigation):

- Group 1 consisted of six samples with testing initiated November 21, 2001.
- Group 2 consisted of seven samples with testing initiated September 11, 2002.
- Group 3 consisted of eight samples with testing initiated September 17, 2002.
- Group 4 consisted of four samples with testing initiated August 12, 2003.

2.2 Sample collection

Sediment samples were collected using a van Veen grab sampler operated from a boat, with the exception of Group 4 samples, which were collected by divers. Each individual grab sample collected in Groups 1-3 was examined to verify that it met minimum acceptability criteria (i.e., grab sampler was properly closed; grab sampler penetrated at least 10 cm). Overlying water was removed from the sampler using a siphon, and surficial sediment (i.e., upper 10 cm) was transferred to a stainless-steel mixing bowl using a stainless steel spoon. Large stones and debris were removed. A minimum of three grab samples were collected from each sample location, and the contents of the mixing bowl were gently homogenized prior to transfer to sample containers (4-L high density polyethylene plastic pails or 1-L glass jars, depending on sample volumes). Sub-samples for chemical analyses were also collected, and placed in the appropriate sample containers (150- or 250-mL glass jars, depending on the analyses). All sample containers were kept at 4°C in the dark using coolers with ice packs or a constant environment room. Diver collection of samples in Group 4 involved completely filling a minimum of six 1-L glass jars with surface sediment and sealing the jars underwater. Jars were then transported to the surface and homogenized and sub-sampled in an identical fashion as the grab samples. Toxicity tests were conducted at EVS Environment Consultants (North Vancouver, BC) and chemical analyses were conducted by ALS Environmental (Vancouver, BC) or its subconsultants.

2.3 Bivalve larval development (BLD) toxicity testing

2.3.1 Overview of test method

The BLD toxicity test compares the number of normally-developed larvae after a 48-h exposure to contaminated sediment or porewater to the number of normally-developed larvae in a clean seawater laboratory control. The general test method has been validated by regulatory agencies for a number of West Coast species, including blue mussels (*Mytilus* sp) and oysters (*Crassostrea gigas*) (PSEP, 1995; USEPA, 1995), as well as sand dollars (*Dendraster excentricus*), red abalone (*Haliotis rufescens*), and purple sea urchins (*Strongylocentrotus purpuratus*) (USEPA, 1995). Researchers have also conducted this test with other sea urchin species native to different parts of the world (e.g., Carr and Chapman, 1992 using the sea urchin *Arbacia punctulata*).

2.3.2 Species selection and identification

All testing was conducted with larvae of the blue mussel, *Mytilus galloprovincialis*. Methods for identifying *Mytilus* species have changed substantially over the last ten years. Original nomenclature described the species as *Mytilus edulis* for all cold- and temperate-water locations on the western coast of North America; however, the following additional closely-related species of blue mussels have since been identified as a result of DNA analysis (McDonald and Koehn, 1988; McDonald et al., 1991):

- *M. trossulus* and *M. californianus*, both located from Siberia to central California, with overlapping distributions influenced by habitat factors such as wave action (USEPA, 1995).

- *M. galloprovincialis*, thought to have been transported to San Francisco from the Mediterranean and now common in central and southern California.

M. edulis is now generally used to describe the species along the eastern coast of North America and Europe (outside the Mediterranean), although *M. edulis* colonies have been transported to the west coast for aquaculture (Heath et al., 1995). Extensive hybridization occurs wherever two of species coexist (e.g., *M. edulis* and *M. galloprovincialis* along the coasts of England and France; *M. edulis* and *M. trossulus* in the Baltic Sea as well as Atlantic Canada (Sarver and Foltz, 1993; Innes and Bates, 1999; Rawson et al., 1999). The hybridization zone for *M. galloprovincialis* and *M. trossulus* covers nearly the entire coast of California (Rawson et al., 1999). The distribution of pure and hybridized *Mytilus* is primarily influenced by habitat conditions (Bierne et al., 2002), but even within a hybridization zone, populations of pure and hybrid mussels exist (Rawson et al., 1999). For example, McDonald et al. (1991) found pure populations of *M. trossulus* and *M. galloprovincialis* within a few meters of one another (one on an intertidal beach, and the other on a floating dock).

Identification of *Mytilus* species is not reliable by morphological characteristics (Sarver and Foltz, 1993). As a result, taxonomic identification of these "species" in the scientific literature is highly variable, depending on author and manner of species identification. Sampling location is often the primary determinant of species identification. Regulatory

guidance recognizes the difficulty in identifying blue mussels, and has typically opted to not distinguish between the different "species" (e.g., species reference in USEPA, 1995 is "*Mytilus* spp.").

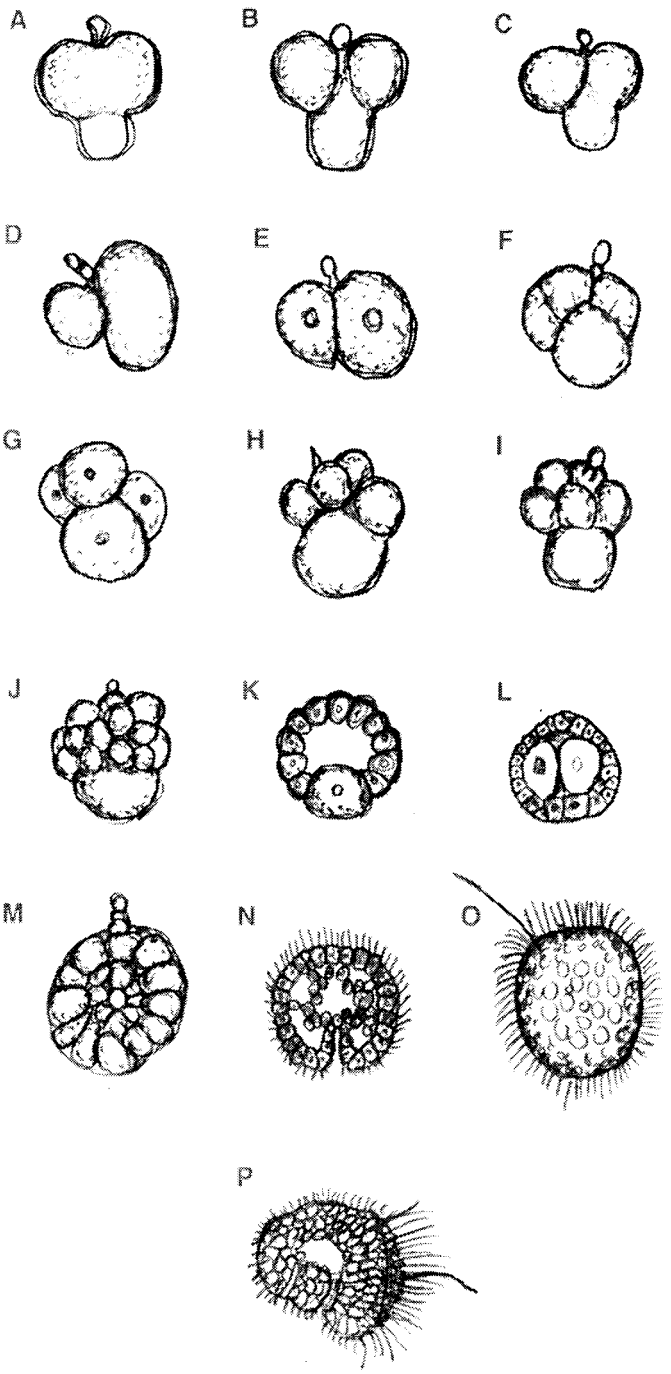
As a result of the difficulty in species identification, data from older literature reported for *M. edulis* (e.g., toxicity test data; biological characteristics) were also considered applicable for *M. galloprovincialis*. The mussel species used in this study was identified as *M. galloprovincialis* to conform to supplier location and historical laboratory practice.

2.3.3 Larval development in *Mytilus galloprovincialis*

In the absence of contaminants, *M. galloprovincialis* larvae rapidly develop from a single-cell, newly fertilized embryo to a free-swimming, fully shelled larvae (prodissoconch I stage) following a consistent pattern of cell cleavage and development (Figure 1). Cell division during cleavage is rapid, progressing from the first stage outlined in Figure 1A through to 1N by approximately 3 h post-fertilization (Field, 1923). A distinctive polar body forms approximately 20 min after fertilization and remains visible for approximately 1 h (see Figure 1A; modified from Field, 1923). Further cell division and differentiation leads to the formation of a differentiated cellular structure with an internal cavity by approximately 2-h post-fertilization (see Figure 1L). A free-swimming trochophore larva has formed by approximately 24 h after fertilization (see Figure 1O) which develops a characteristic D-shape by approximately 42 h (see Figure 1P – Field, 1923; Hayakaze and Tanabe, 1999). Overall dimensions of the D-shaped larvae are approximately 90 μm long by 70 μm at 48 h (Loosanoff et al., 1966; de

Schweinitz and Lutz, 1976) at this point—the D-shape is recognizable for approximately two or three weeks post-fertilization, depending on culture temperature (Saituito et al., 1994; Bayne, 1965). The fact that *M. galloprovincialis* larvae have a distinctive shape 48 h post-fertilization is critical to the test method. Examples of normal and abnormal bivalve larvae are provided in Figures 2 and 3.

Figure 1: Pattern of cleavage in bivalve larval development



Based on Field (1923). Magnification 375x

Figure 2: Normal *Mytilus galloprovincialis* larvae

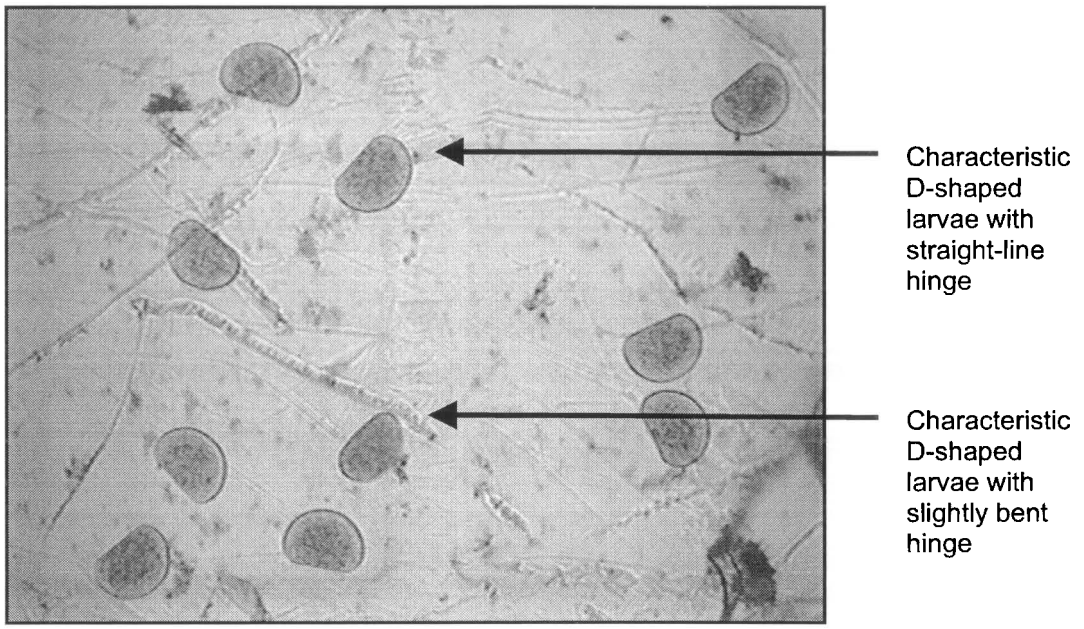
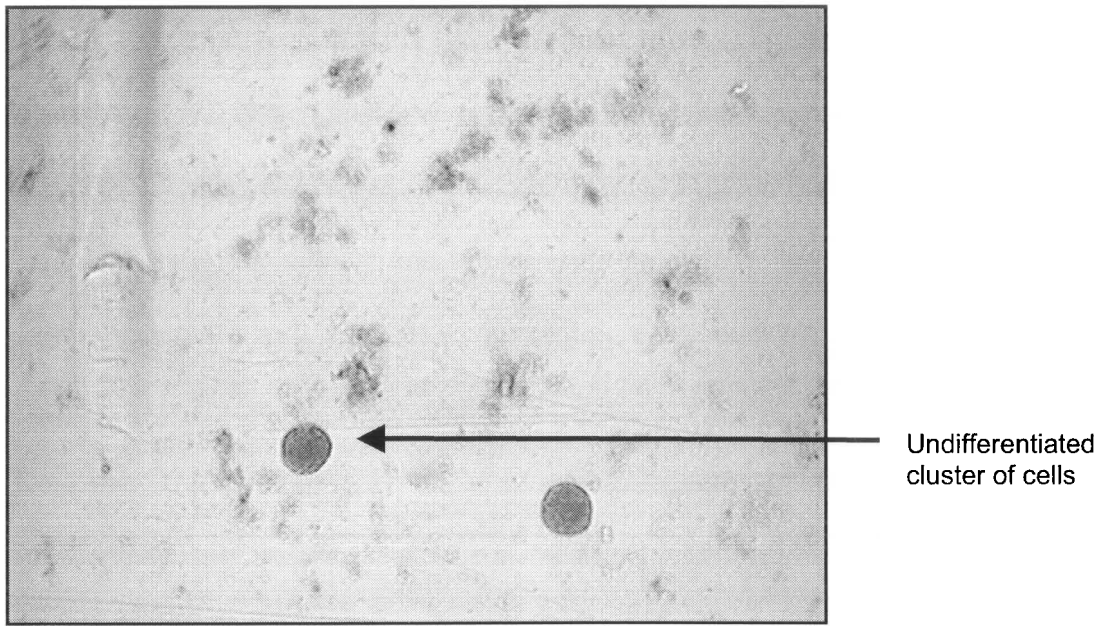


Figure 3: Abnormal *Mytilus galloprovincialis* larvae



2.4 Toxicity test methods

2.4.1 Seawater supply

Laboratory supplies of clean seawater were obtained from the Vancouver Aquarium, stored in a 4000-L high-density polyethylene (HDPE) tank, and replaced on a weekly basis. Seawater used for bivalve larval development toxicity testing was passed through a 0.45-μm inline filter and ultraviolet light sterilizer, and acclimated to test temperature under vigorous aeration for 24-h prior to use. Salinity was adjusted to 28 ± 1 ppt using hypersaline brine (prepared by repeated freezing of clean seawater) as necessary. Clean seawater was used for organism spawning, sample dilution, elutriate preparation, reference toxicant testing and negative controls..

2.4.2 Organism supply and gamete collection

Mature *M. galloprovincialis* were shipped overnight from Carlsbad Aquafarms (Carlsbad, CA, USA) in insulated containers. On arrival, approximately 50 bivalves were scrubbed to remove attached detritus, and placed in a shallow holding tank containing clean seawater. Spawning was stimulated by raising the water temperature from approximately 15 to 25°C, thereby simulating a seasonal shift from spring to summer. Organisms were sexed by the nature of gamete release. A thin milky white release identified a spawning male while a more globular, yellowish release identified a spawning female. Spawning organisms were removed from the holding tank, rinsed with clean seawater, and placed in individual 300-mL glass beakers containing clean seawater to continue spawning.

Gamete quality for each individual spawning bivalve was evaluated by visual examination under a compound microscope (Nikon Model SC, Tokyo, Japan). Male gamete quality was considered acceptable if sperm were motile, and female gamete quality was considered acceptable if eggs were round and opaque. Acceptable eggs from multiple spawning bivalves were screened through a 0.25-μm mesh to remove gonadal material, combined with approximately 2-L of clean seawater, and fertilized by adding sperm from acceptable multiple bivalves. After 60 min, the egg:sperm suspension was rinsed a second time to remove excess sperm, and the number of fertilized embryos counted in a 1:100 diluted subsample. The density of the egg suspension was adjusted to approximately 30,000 or 3,000 fertilized eggs/mL (for the elutriate and porewater tests, respectively) by decanting excess overlying water or adding clean seawater.

2.4.3 Sample preparation

For the elutriate toxicity test, five replicates were prepared for each sample by placing 18.0 ± 0.5 g of sediment in a 1-L glass jar using a digital top-loading balance (Sartorius BA 2100, Gottingen, Germany) and then adding 900-mL of clean seawater using a Class-A graduated cylinder. Samples were vigorously stirred with a glass rod for 10 sec. Five replicates for the negative control, and five replicates for the "time-zero" controls (described below), each consisting of 900-mL of clean seawater without sediment, were also prepared. Sediment samples were prepared one day in advance in order to allow a 24-h settling period (at test temperature) prior to inoculation. A 24-h settling period (instead of the 4-h settling period specified in PSEP, 1995) reduces the amount of larvae

lost to entrainment (i.e., larvae that are smothered by the settling sediment) and is now a routine modification to the test protocol.

For the porewater toxicity test, sufficient porewater was extracted by centrifuging bulk sediment for 30 min at 3000 rpm using a Model PR-6 centrifuge (International Equipment Company, Needham Heights, MA, USA). Centrifuge refrigeration was set to 15°C. The resulting supernatant was gently removed from the centrifuge containers using a glass pipette in order to minimize sediment resuspension, and transferred to a glass jar prior to distribution to the test containers. Four replicate treatments consisting of 10 mL of 100% porewater in a test tube were prepared for each sample. Five replicates for the negative control, and five replicates for the "time-zero" controls (described below), each consisting of 10-mL of clean seawater, were also prepared. Porewater was extracted on the same day as test initiation (i.e., a 24-h settling period was not necessary).

All test containers (1-L glass jars for elutriate or test tubes for porewater) were placed in a constant environment room with temperature set to 16 ± 1°C, and a photoperiod set to 14h:10h (light:darkness).

2.4.4 Test initiation and monitoring

Water quality parameters were measured for each sample using calibrated hand-held meters (Thermo-Orion, Beverly, MA, USA). Parameters included temperature, dissolved oxygen (Orion Model 835A), salinity (Orion Model 135A) and pH (Orion Model 266S). Measurements were made in a separate water quality replicate for the elutriate test, and in

a separate 30-mL beaker for the porewater test. All replicates (except the water quality replicate) were inoculated within 2 h after the egg suspension was first fertilized.

For the elutriate toxicity test, individual replicate containers (including negative controls and "time-zero" controls) were inoculated with 1-mL of the fertilized embryo suspension (see Section 2.4.2). Optimal density in the test container was approximately 33 gametes/mL (i.e., 1 mL of 30,000 gametes/mL suspension inoculated in a total volume of 901 mL). Time-zero controls were immediately preserved with formalin and used to determine the average number of gametes actually inoculated in each test replicate container.

For the porewater toxicity test, individual replicate containers (including negative controls and "time-zero" controls) were inoculated with 0.1-mL of the fertilized embryo suspension (see Section 2.4.2). Optimal density in the test container was approximately 30 gametes/mL (i.e., 0.1 mL of a 3,000 gamete/mL suspension inoculated in a total volume of 10.1 mL). Time-zero controls were immediately preserved with formalin and used to determine the average number of gametes actually inoculated in each test replicate container.

2.4.5 Reference toxicant test

A reference toxicant (positive control) test using copper was conducted with each bivalve shipment in order to verify the performance of the organisms (i.e., sensitivity to a known toxicant) relative to previous shipments. Reagent grade cupric chloride dihydride

(Anachemia Science, Lachine, Quebec) was dissolved in clean seawater to achieve a nominal dilution series of 2.5, 5, 10, 20 and 40 µg/L Cu, plus a negative control. Four replicates of 900-mL each were set up for each test concentration. Data from the reference toxicant test were used to determine an EC_{50} (with 95% confidence intervals) for bivalve normal survival using ToxCalc 5.0 (Tidepool Scientific Software, McKinleyville, CA, USA), which was compared to the mean EC_{50} ± 2 standard deviations (based on the most recent 20 previous reference toxicant tests).

2.4.6 Test termination

Water quality parameters were measured for each sample, and the test terminated 48-h after initial fertilization of the egg suspension. Elutriate toxicity tests were terminated by gently decanting the overlying water from individual replicates into clean 1-L jars, and stirring the overlying water with a glass rod. A 10-mL subsample of the overlying water was transferred to a test tube using an automatic pipettor, and preserved by adding 1-mL of 50% buffered formalin. The porewater toxicity tests were terminated by adding 1-mL of 50% buffered formalin directly to each test tube.

2.4.7 Larval counting and analyses

The number of normal and abnormal larvae in each replicate test container were counted using a 1-mL Sedgewick-Rafter counting chamber and a compound microscope (Nikon Model SC, Tokyo, Japan) with a total magnification of 40 x (10x ocular, 4x objective lens), This allowed for direct measurement of:

$$\text{Percent Survival} = \frac{\text{Total number of larvae at 48 - h (normal + abnormal)}}{\text{Total number of larvae at 0 - h}} \quad (1)$$

$$\text{Percent Normal} = \frac{\text{Total number of normal larvae at 48 - h}}{\text{Total number of normal larvae at 48 - h (normal + abnormal)}} \quad (2)$$

The total number of larvae at 0-h (i.e., test initiation) was the average number of larvae in the five "time-zero" controls. The toxicological endpoint under consideration was percent normal survival, obtained by multiplying the percent survival and percent normal measurements (i.e., equation 1 x equation 2). Percent normal survival measures the percentage of normally-developed larvae relative to the number of fertilized embryos introduced at test initiation—it is the preferred toxicological endpoint since from an ecology perspective, abnormally-developed larvae and dead larvae have an identical functional value (i.e., no value in terms in perpetuating the next generation).

2.4.8 General test acceptability criteria

Several factors are considered to determine if the results of a BLD toxicity test are considered acceptable, as follows:

- Percent normal survival in the negative control must be equal to or greater than 70%.

- Performance of the reference toxicant test (i.e., the calculated EC_{50}) must be within two standard deviations of the mean EC_{50} from previous reference toxicant tests.

- Water quality parameters (pH, dissolved oxygen, salinity and temperature) measured during the test must be within appropriate ranges (see Section 3.1.2.3)

2.5 Interpretation of toxicity data

Interpretation of BLD toxicity test data involves comparison of the number of normally developed larvae in the test containers to the number of normally developed larvae in the negative control. All data were normalized to negative control performance in order to remove the influence of multiple negative controls involved in testing different batches of samples, as follows:

$$\text{Control normalized \% normal survival} = \frac{\text{\% normal survival in test containers}}{\text{\% normal survival in negative control}}$$

The typical convention for interpreting the magnitude of the toxicological response in a sediment quality triad approach is as follows:

- Greater than a 20% reduction in the number of normally developed larvae (relative to the negative control) is considered indicative of a moderate biological effect (i.e., percent normal survival scores of less than 0.80 after normalization to negative control performance).

- Greater than a 50% reduction in the number of normally developed larvae (relative to the negative control) is considered indicative of a severe biological effect (i.e., percent normal survival scores of less than 0.50 after normalization to negative control performance).

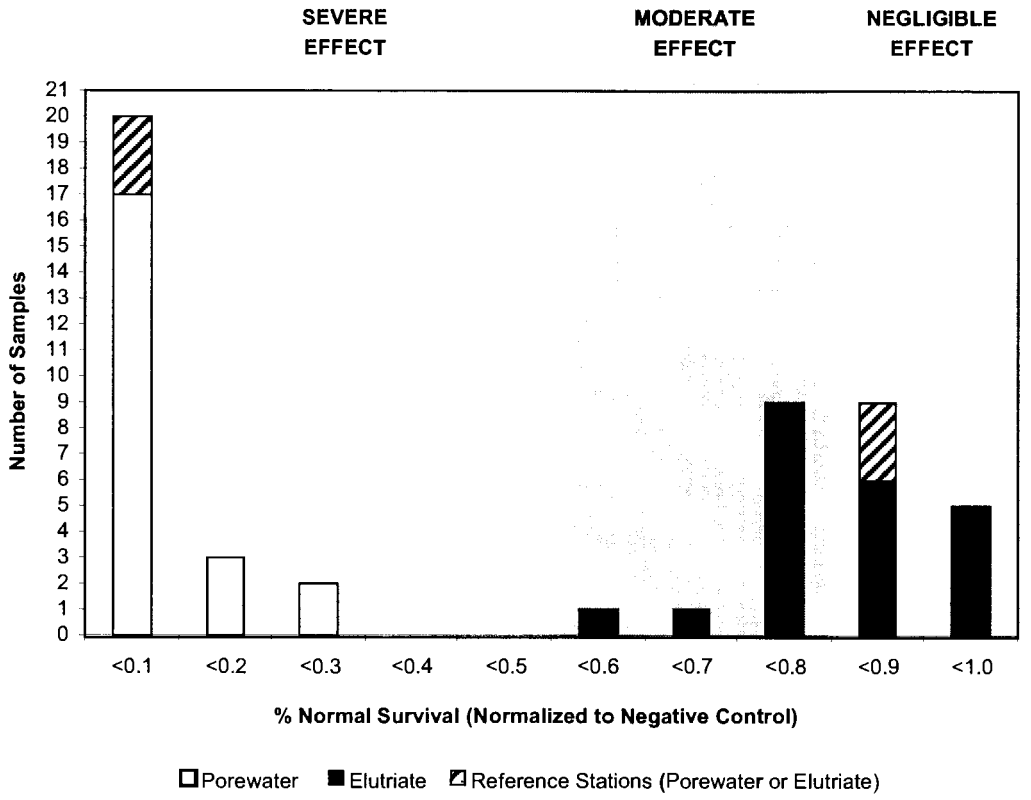
3.0 RESULTS

3.1 Toxicity test results

3.1.1 Comparison of elutriate and porewater BLD toxicity data

A comparison of the control normalized toxicity data obtained from the porewater and the elutriate tests is presented in Figure 4. A clear difference in test performance was observed. For the 22 samples collected from impacted urban harbour sites, the elutriate BLD toxicity test indicated negligible effect (i.e., control normalized results were greater than 0.80) in 11 samples. The remaining 11 samples indicated a moderate effect (i.e., control normalized results were greater than 0.50). Conversely, the porewater BLD toxicity test indicated that all 22 samples demonstrated a severe effect (i.e., control normalized results were less than 0.50)—in fact, the majority of the porewater samples (14 of 22 samples) had zero normally developed bivalve larvae at test termination. A similar pattern was observed in the three samples collected from unimpacted reference locations (indicated on Figure 4 with cross-hatching). All three samples were classified as demonstrating negligible effects in the elutriate BLD toxicity test, while demonstrating severe effects in the porewater BLD toxicity test.

Figure 4: Comparison of elutriate and porewater BLD toxicity data



Porewater toxicity test data indicated in white, and shows that all samples were classified as a severe effect. Elutriate toxicity test data indicated in black, and shows that all samples were classified as either moderate or negligible effects. Reference station toxicity data indicated with cross-hatching, and shows that the reference stations demonstrate the same pattern as the other porewater and elutriate stations collected from contaminated sites.

3.1.2 Acceptability of toxicity test data

3.1.2.1 Negative control performance

Percent normal survival was greater than the minimum test acceptability criterion of 70% for all negative controls. Percent normal survival values ranged from 85.8 to 96.3% in the four negative controls for the elutriate BLD toxicity tests, and ranged from 84.0 to 92.2% in the four negative controls for the porewater BLD toxicity tests.

3.1.2.2 Reference toxicant test performance

Reference toxicant test performance was acceptable for all four groups of samples, as follows:

- The reference toxicant test for the first group of samples had an EC_{50} of 11.8 (95% confidence interval: 10.8 – 12.8) µg/L Cu, relative to a mean ± 2SD of 11.3 ± 4.3 µg/L Cu.

- The reference toxicant test for the second group of samples had an EC_{50} of 14.5 (95% confidence interval: 13.5 – 15.5) µg/L Cu, relative to a mean ± 2SD of 11.6 ± 3.1 µg/L Cu.

- The reference toxicant test for the third group of samples had an EC_{50} of 13.5 (95% confidence interval: 13.0 – 14.0) µg/L Cu, relative to a mean ± 2SD of 10.0 ± 4.3 µg/L Cu.

- The reference toxicant test for the fourth group of samples had an EC_{50} of 12.1 (95% confidence interval: 11.5 – 12.7) µg/L Cu, relative to a mean ± 2SD of 10.8 ± 4.5 µg/L Cu.

3.1.2.3 Water quality parameters

Water quality parameters (temperature, pH, dissolved oxygen and salinity) for all samples were within acceptable ranges to support proper larval development. Optimal ranges (PSEP, 1995; USEPA, 1995) include:

- Acceptable salinity values range from 27 to 32 ppt (i.e., encompassing the range of 30 ± 2 specified by USEPA, 1995, and the range of 28 ± 1 specified in PSEP, 1995). Minor variations from this range are unlikely to substantially alter test results, given that His et al. (1989) found no effects on the normal development of *M. galloprovincialis* at salinities ranging from 20 and 35 ppt.

- Temperature: 15 – 17°C. Minor variations from this range are unlikely to substantially alter test results given that Bayne (1965) found that larval development in *M. edulis* occurred naturally within the range of 8-18°C. Temperature-related effects on larval development were not noted until temperatures were less than 5°C or above 20°C.

- PSEP (1995) and USEPA (1995) recommend a minimal dissolved oxygen concentration of 4.0 mg/L for all marine sediment toxicity testing. However, *Mytilus* larval development appears relatively insensitive to low dissolved oxygen concentrations. Wang and Widdows (1991) found no detectable effects on *M. edulis* larval development under low dissolved oxygen conditions, provided that the oxygen partial pressure remained higher than 3.16 kPa (i.e., approximately 1.5 mg/L at 15°C). Artificial oxygenation of larval development tests is not

recommended due to the adverse effects of the resulting turbulence on larval survival and growth (His et al., 1999).

- An optimal pH range for larval development was not established by USEPA (1995) and PSEP (1995), however measurements substantially outside the range of 7.5 – 8.5 may contribute to observed toxicity, and mask the effects of other contaminants (USEPA, 1995). Values within or near the range of 7.5 to 8.5 are considered acceptable for proper larval development.

Water quality measurements at test initiation and termination are summarized in Table 1 and Table 2, respectively, and were within acceptable ranges given the above considerations.

Table 1: Water quality parameters at test initiation

Sample	Porewater				Elutriate			
	T (°C)	pH	DO (mg/L)	Salinity (ppt)	T (°C)	pH	DO (mg/L)	Salinity (ppt)
1A	16.0	7.9	7.1	29	17.0	8.0	7.2	29
1B	16.0	7.8	7.0	29	17.0	8.0	6.9	29
1C	16.0	8.0	6.9	29	17.0	8.0	7.2	29
1D	16.0	7.7	7.6	26	17.0	8.0	5.6	29
1E	16.0	7.7	6.8	28	17.0	8.0	5.8	29
1F	16.0	7.6	7.2	27	17.0	7.9	6.0	29
2A	16.0	7.3	7.2	27	16.5	7.6	5.3	30
2B	16.0	7.8	7.3	27	16.5	7.6	5.9	30
2C	16.0	7.6	7.1	28	16.5	7.5	6.0	30
2D	16.0	7.6	6.9	28	16.5	7.6	5.8	30
2E	16.0	7.4	7.0	28	16.5	7.6	5.8	30
2F	16.0	7.9	6.9	27	17.0	7.7	5.8	30
2G	16.0	8.1	7.3	27	16.5	7.7	6.2	30
3A	16.0	8.0	6.7	27	16.5	7.7	4.3	28
3B	16.0	7.6	7.1	27	16.5	7.6	4.6	28
3C	16.0	7.5	6.8	27	16.5	7.6	4.8	28
3D	16.0	7.5	6.9	27	16.5	7.6	4.9	28
3E	16.0	7.7	7.0	27	16.5	7.6	5.1	28
3F	16.0	7.6	7.1	27	16.5	7.6	5.6	28
3G	16.0	7.6	7.1	28	16.5	7.6	5.8	28
3H	16.0	8.0	6.9	27	16.5	7.6	6.0	28
4A	16.0	7.7	7.7	31	15.0	7.7	6.9	29
4B	16.0	7.6	7.5	31	15.5	7.7	7.2	29
4C	16.0	7.5	5.6	30	15.5	7.7	7.0	29
4D	16.0	7.4	6.5	30	15.5	7.6	6.8	29

All water quality parameters are within physiological tolerance limits for *Mytilus galloprovincialis.* (T = temperature; DO = dissolved oxygen)

Table 2: Water quality parameters at test termination

Sample	Porewater				Elutriate			
	T (°C)	pH	DO (mg/L)	Salinity (ppt)	T (°C)	pH	DO (mg/L)	Salinity (ppt)
1A	16.0	7.9	6.5	29	16.0	8.1	8.0	29
1B	16.0	7.8	6.3	29	16.0	8.1	8.0	29
1C	16.0	8.0	6.7	29	16.0	8.1	8.0	29
1D	16.0	7.7	7.7	27	16.5	8.1	8.0	29
1E	16.0	7.7	6.4	28	16.0	8.1	8.0	29
1F	16.0	7.6	7.5	27	16.0	8.1	7.9	29
2A	16.0	7.3	7.2	26	16.5	7.6	5.2	30
2B	16.0	7.8	7.2	26	16.5	7.6	5.3	29
2C	16.0	7.6	7.2	28	17.0	7.7	5.1	30
2D	16.0	7.6	7.0	28	16.5	7.6	5.2	30
2E	16.0	7.4	7.1	28	16.5	7.6	5.2	30
2F	16.0	7.9	6.8	27	16.5	7.6	5.3	30
2G	16.0	8.1	7.4	27	16.5	7.6	5.3	29
3A	16.0	8.0	7.3	27	16.0	7.8	8.1	28
3B	16.0	7.6	7.2	27	16.0	7.6	8.1	28
3C	16.0	7.5	6.9	27	16.0	7.8	8.0	28
3D	16.0	7.5	6.9	27	16.0	7.9	8.1	28
3E	16.0	7.7	6.9	27	16.0	7.9	5.1	28
3F	16.0	7.6	7.4	27	16.0	7.9	5.6	28
3G	16.0	7.6	7.4	28	16.0	7.7	5.8	28
3H	16.0	8.0	6.3	27	16.0	7.9	6.0	28
4A	15.0	7.9	5.6	30	16.0	7.6	5.5	30
4B	15.0	7.8	7.4	30	16.0	7.6	6.4	30
4C	15.0	7.5	8.2	30	16.0	7.6	6.2	30
4D	15.0	7.8	8.1	30	16.0	7.5	6.4	30

All water quality parameters are within physiological tolerance limits for *Mytilus galloprovincialis*. (T = temperature; DO = dissolved oxygen)

3.2 Chemical concentrations in sediment and porewater

3.2.1 Chemical concentrations in bulk sediment

A table of values showing the results of the chemical analyses on the bulk sediment are presented in Appendix A. Multiple contaminants of potential concern (COPCs) exceeded provincial numerical sediment quality criteria (BCWLAP, 2003). Concentrations of multiple individual PAHs (e.g., acenaphthene, benz[a]anthracene, fluorene, phenanthrene, pyrene), as well as copper and zinc were greater than the provincial numerical sediment quality criteria in all 22 samples collected from impacted sites. Concentrations of arsenic (9 samples), cadmium (8 samples), lead (20 samples) and mercury (12 samples) were also higher than the provincial sediment criteria. Concentrations of metals and PAHs were less than analytical detection limits or substantially less than provincial sediment criteria in the three samples collected from unimpacted reference sites (i.e., 1A, 1B and 1C).

Statistical evaluation of the relationships between bulk sediment chemistry and the observed differences in toxicity was not feasible (i.e., correlations between toxicity and chemistry could not be established when nearly all porewater samples demonstrated 100% toxicity while nearly all elutriate samples demonstrated negligible toxicity). However, an examination of the available toxicity data indicated that porewater from the three reference samples had zero normally-developed bivalve larvae at test termination, despite bulk sediment contaminant concentrations that were substantially less than the BCWLAP (2003) numerical standards. In fact, PAH concentrations were less than

analytical detection limits in all three samples. This observation suggested that metals and PAHs were not the primary cause of porewater toxicity in those samples.

3.2.2 Chemical concentrations in porewater

Porewater from the fourth group of samples were analyzed for metals (4 samples) and PAHs (2 samples) in order to directly evaluate the potential contribution of those COPCs to the observed porewater toxicity. There was insufficient sample volume to conduct all PAH analyses on all four samples, and porewater chemistry data were not conducted for samples in Groups 1-3. Porewater chemistry data are presented in Table 3.

Table 3: Measured chemical concentrations in selected porewater samples

	4A	4B	4C	4D
Dissolved metals (mg/L)				
Aluminum	<0.5	<0.5	<0.5	<0.5
Antimony	<0.05	<0.05	0.1	<0.05
Arsenic	<0.1	<0.1	<0.1	<0.1
Barium	<0.2	<0.2	<0.1	<0.1
Beryllium	<0.1	<0.1	<0.1	<0.1
Boron	4	4	4	3
Cadmium	<0.00002	0.00007	0.00005	<0.00002
Chromium	<0.1	<0.1	<0.1	<0.1
Cobalt	0.00005	0.00008	<0.00005	0.00009
Copper	0.00033	0.00106	0.00162	0.00017
Iron	0.45	0.02	0.03	0.01
Lead	0.0002	0.00024	0.00022	0.00014
Manganese	0.029	0.0713	0.0735	0.00729
Mercury	<0.00005	<0.00005	<0.00005	0.00007
Molybdenum	<0.1	<0.1	<0.1	<0.1
Nickel	0.00167	0.00075	0.00086	0.00099
Selenium	<0.2	<0.2	<0.1	<0.1
Silver	<0.002	<0.002	<0.01	<0.01
Thallium	<0.02	<0.02	<0.01	<0.01
Tin	<0.05	<0.05	<0.01	<0.01
Titanium	<0.1	<0.1	<0.1	<0.1
Uranium	0.00919	0.00651	0.00417	0.00176
Vanadium	<0.3	<0.3	<0.3	<0.3
Zinc	0.0019	0.0032	0.0017	0.0005

	4A	4B	4C	4D
PAHs (mg/L)				
Acenaphthene	-	-	<0.0001	<0.0001
Acenaphthylene	-	-	<0.0001	<0.0001
Acridine	-	-	<0.0001	<0.0001
Anthracene	-	-	0.0001	0.0003
Benz(a)anthracene	-	-	0.0001	0.0002
Benzo(a)pyrene	-	-	0.00026	0.00023
Benzo(b)fluoranthene	-	-	0.0005	0.0004
Benzo(g,h,i)perylene	-	-	0.0002	0.0001
Benzo(k)fluoranthene	-	-	0.0002	0.0002
Chrysene	-	-	0.0001	0.0003
Dibenz(a,h)anthracene	-	-	<0.0001	<0.0001
Fluoranthene	-	-	0.0003	0.0011
Fluorene	-	-	<0.0001	<0.0001
Indeno(1,2,3-c,d)pyrene	-	-	0.0002	0.0001
Naphthalene	-	-	<0.0001	<0.0001
Phenanthrene	-	-	<0.0001	<0.0001
Pyrene	-	-	0.0006	0.001
Quinoline	-	-	<0.0001	<0.0001

Insufficient volume available for PAH analysis on samples 4A and 4B.

3.2.3 Ammonia concentrations in porewater

Ammonia concentrations in porewater samples used for BLD testing were not available for the first three groups of samples; instead, interstitial ammonia data from concurrent amphipod toxicity testing were used as a surrogate. Interstitial ammonia concentration data from amphipod testing are likely lower than ammonia concentrations in the original porewater samples, since sediment samples for amphipod toxicity testing were overlain with 900-mL of clean seawater for 24-h prior to ammonia sample collection. Repeated replacement of overlying seawater is widely used to reduce interstitial ammonia concentrations in whole-sediment toxicity tests (Ferretti et al., 2000). Ammonia measurements were conducted on 100% original porewater from the fourth group of samples. Unionized ammonia concentrations were calculated from the available total ammonia data using sample specific measurements of pH, temperature, and salinity and the mathematical model developed by Whitfield (1974) and experimentally validated by Khoo et al. (1977). Total and unionized ammonia data are summarized in Table 4.

Table 4: Summary of total and ionized ammonia concentrations and control normalized percent normal survival data

Sample	Porewater			Elutriate		
	Total Ammonia (mg/L N)	Unionized Ammonia (mg/L N)	Control Normalized % Normal Survival	Total Ammonia (mg/L N)	Unionized Ammonia (mg/L N)	Control Normalized % Normal Survival
1A	15.00	0.094	0	0.27	0.004	0.848
1B	18.00	0.175	0	0.26	0.004	0.841
1C	17.90	0.140	0	0.29	0.004	0.888
1D	10.40	0.259	0	0.09	0.001	0.814
1E	9.20	0.091	0	0.18	0.003	0.850
1F	7.70	0.122	0	0.21	0.003	0.892
2A	3.01	0.047	0	0.11	0.001	0.804
2B	3.53	0.022	0.015	0.12	0.001	0.800
2C	3.18	0.016	0.239	0.08	0.001	0.775
2D	19.0	0.095	0	0.50	0.003	0.762
2E	9.44	0.075	0	0.30	0.002	0.777
2F	5.25	0.033	0.109	0.11	0.001	0.932
2G	4.93	0.031	0.108	0.17	0.001	0.927
3A	5.51	0.086	0.030	0.10	0.001	0.920
3B	1.30	0.010	0.140	0.06	0.001	0.540
3C	8.98	0.142	0	0.50	0.002	0.780
3D	3.28	0.032	0	0.14	0.001	0.722
3E	5.40	0.043	0	0.18	0.001	0.750
3F	3.81	0.030	0	0.26	0.002	0.811
3G	7.61	0.122	0	0.39	0.003	0.718
3H	20.50	0.411	0	1.18	0.007	0.682
4A	17.50	0.134	0	0.23	0.002	0.788
4B	3.77	0.023	0.210	1.74	0.014	1.063
4C	5.15	0.025	0.018	0.26	0.002	0.953
4D	8.00	0.031	0	0.44	0.003	0.788

Notes: Porewater ammonia measurements for samples in groups 1 through 3 are from Day 0 interstitial water samples collected from concurrent amphipod testing. Percent normal survival data were normalized to control performance. Low percent normal values (i.e., less than 0.25) for correspond to higher unionized ammonia concentrations in the porewater toxicity test while higher percent normal survival values correspond to lower unionized ammonia concentrations in the elutriate toxicity test.

3.3 Ammonia reference toxicant test

An ammonia reference toxicant test was also conducted in order to investigate the actual response of *M. galloprovincialis* larvae to unionized ammonia. This reference toxicant test was conducted in a similar fashion to the copper reference toxicant tests used to evaluate the overall health of each batch of organisms (Section 2.4.5), and was conducted with the same batch of organisms used to evaluate the fourth group of samples.

Reagent grade ammonia chloride (Anachemia Science, Lachine, Quebec) was dissolved in clean seawater to achieve a dilution series of six test concentrations (i.e., 25 mg/L N followed by a serial dilution of five additional test concentrations) plus a negative control. Five replicates of 10-mL each were set up for each test concentration. Table 5 provides a summary of water quality parameters monitored during the test. All water quality parameters fell with acceptable ranges (Section 2.4.8)

Total ammonia concentration was measured for each test concentration at test initiation and termination. An EC_{20} (with 95% confidence intervals) for bivalve normal survival was calculated using a maximum-likelihood probit analysis (ToxCalc 5.0, Tidepool Scientific Software, McKinleyville, CA, USA). The average total ammonia concentrations at test initiation and termination were used as the basis for the dose-response relationship. The unionized ammonia fraction was estimated for the calculated EC_{20}, and its confidence interval using the approach described in Section 4.2.4.1. Total and unionized ammonia concentrations are summarized in Table 6. The EC_{20} was 2.82 (95% confidence interval: 2.71 – 2.93) mg/L total N, or 0.028 (95% confidence interval:

0.027 – 0.029) mg/L unionized N. The EC_{50} was 3.67 (95% confidence interval: 3.56 – 3.76) mg/L total N, or 0.036 (95% confidence interval: 0.035 – 0.037) mg/L unionized N.

Table 5: Water quality parameters in the ammonia reference toxicant test

Nominal Concentration (mg/L N)	Test Initiation				Test Termination			
	T (°C)	pH	DO (mg/L)	Salinity (ppt)	T (°C)	pH	DO (mg/L)	Salinity (ppt)
Control	16.0	7.8	7.8	28.4	15.0	7.8	8.3	28.5
1.25	16.0	7.8	7.7	28.8	15.0	7.9	8.3	28.4
2.5	16.0	7.8	7.7	28.6	15.0	7.9	8.3	28.4
5	16.0	7.8	7.7	28.4	15.0	7.9	8.3	28.4
10	16.0	7.8	7.7	28.2	15.0	7.9	8.3	28.3
20	16.0	7.8	7.7	28.5	15.0	7.9	8.3	28.3
40	16.0	7.8	7.7	28.4	15.0	7.9	8.3	28.3

Table 6: Ammonia concentrations in the ammonia reference toxicant test

Nominal Concentration (mg/L N)	Test Initiation (mg/L N)		Test Termination (mg/L N)		Average Measured Unionized Ammonia (mg/L N)
	Total Ammonia (Measured)	Unionized Ammonia	Total Ammonia (Measured)	Unionized Ammonia	
Control	0.03	0.000	0.12	0.001	0.001
0.78	1.16	0.012	1.05	0.013	0.013
1.56	2.18	0.023	1.86	0.023	0.023
3.12	3.98	0.042	3.08	0.038	0.040
6.25	7.68	0.082	5.79	0.072	0.077
12.5	12.90	0.137	13.90	0.172	0.154
25	24.40	0.259	26.40	0.326	0.292

4.0 DISCUSSION

4.1 Interpretation of toxicity data

As discussed in Section 3.1.1, in the elutriate toxicity test, twelve samples demonstrated negligible effects, while eleven samples demonstrated a moderate effect, and two samples demonstrated a severe effect. Conversely, all samples demonstrated a severe effect in the porewater toxicity test.

Differences in individual ratings for elutriate and porewater toxicity data resulted in changes to the overall estimates of sample toxicity when considered in a weight-of-evidence assessment. Overall estimates of sample toxicity were upgraded from negligible to moderate in 12 samples, and from moderate to severe in 2 samples when the porewater data were considered in lieu of the elutriate data. Less than half (11 of 25) samples had the same overall estimate of sample toxicity regardless of which bivalve larval development toxicity test was used. Data from the other concurrent toxicity tests conducted for the original SQT (i.e., 10-d amphipod survival and/or 20-d polychaete survival and growth toxicity tests) were used to provide a realistic framework in which to evaluate the potential effects of including either elutriate or porewater BLD toxicity tests in a weight-of-evidence assessment. The decision criteria used to evaluate each individual toxicity test and integrate the findings into an overall assessment of sediment toxicity are described in Table 7, and the toxicological data are summarized in Table 8.

Table 7: Decision criteria for weight-of-evidence toxicity assessment

Evaluating individual toxicological endpoints:

- ○ Less than 20% reduction in endpoint performance relative to negative control
- ⊙ Greater than 20% reduction in endpoint performance relative to negative control
- ● Greater than 50% reduction in endpoint performance relative to negative control

Integrating multiple toxicological endpoints

- ○ All individual endpoints are "○"
- ○ Only one sublethal endpoint (i.e., *Neanthes* growth; *Mytilus* normal survival) is "⊙"
- ⊙ Only one lethal endpoint (i.e., survival) is "⊙"
- ⊙ More than one sublethal or lethal endpoints are "⊙"
- ⊙ Only one sublethal endpoint is "●"
- ● Only one lethal endpoint is "●"
- ● More than one sublethal or lethal endpoints are "●"

Interpreting the integrated toxicity evaluation

- ○ Potential for adverse ecological effects is considered negligible
- ⊙ Potential for adverse ecological effects is considered moderate
- ● Potential for adverse ecological effects is considered severe

Table 8: Weight-of-evidence toxicity assessment using either porewater or elutriate bivalve larval development data

	Whole-Sediment		Elutriate	Porewater	Overall Toxicity (Porewater BLD Excluded)	Overall Toxicity (Elutriate BLD Excluded)
	Amphipod Survival	Polychaete Survival-Growth	BLD % Normal Survival	BLD % Normal Survival		
1A	○	○ - ◉	○	●	○	◉
1B	○	○ - ◉	◉	●	◉	◉
1C	○	○ - ◉	◉	●	◉	◉
1D	○	○ - ◉	○	●	○	◉
1E	○	○ - ○	○	●	○	◉
1F	○	○ - ○	○	●	○	◉
2A	○	○ - ○	◉	●	○	◉
2B	○	○ - ◉	◉	●	◉	◉
2C	○	○ - ◉	◉	●	◉	◉
2D	○	○ - ○	◉	●	○	◉
2E	○	○ - ○	○	●	○	◉
2F	◉	○ - ●	◉	●	◉	●
2G	◉	○ - ●	◉	●	◉	●
3A	○	NA	○	●	○	◉
3B	◉	NA	○	●	◉	◉
3C	○	NA	◉	●	◉	◉
3D	◉	NA	◉	●	◉	◉
3E	◉	NA	◉	●	◉	◉
3F	○	NA	○	●	○	◉
3G	◉	NA	○	●	◉	◉
3H	○	NA	○	●	○	◉
4A	○	NA	◉	●	◉	◉
4B	○	NA	○	●	○	◉
4C	○	NA	○	●	○	◉
4D	○	NA	◉	●	◉	◉

Notes: Amphipod survival is based on a 10-d *Eohaustorius estuarius* toxicity test. Polychaete survival and growth is based on a 20-d *Neanthes arenaceodentata* toxicity test. NA = test not available. Note that benthic community data (typical component of a SQT) were not available for any of the samples included in this project.

The findings presented above have substantial implications for the management of contaminated sediments if one assumes that dredging and disposal will be required for any samples that demonstrated either moderate or severe effects. Controlling for false positives (i.e., demonstrating toxicity in a laboratory toxicity test method when, in fact, toxicity is not present under field conditions) is likely necessary for porewater BLD toxicity testing, given that severe effects were observed in all porewater samples, even those from reference stations without elevated COPC concentrations. False positives result in substantial additional costs and lengthy project delays, since upgrading the overall effect rating for a single sample translates into additional financial costs that can reach tens of thousands of dollars, depending on the spatial scale of the investigation (i.e., what is the surface area represented by that single sample?) and/or the decision to proceed to dredging and disposal or to additional sediment quality investigations to refine the initial effect rating.

Conversely, false negatives (i.e., demonstrating no toxicity in a laboratory test method when, in fact, toxicity is present under field conditions) do not appear to be a concern based on the available porewater toxicity testing data (i.e., all samples demonstrated an effect). This is not to suggest that false negatives should not be controlled for. Clearly, failure to control for false negatives may result in contaminated sediments being left in place, when, in fact, they should be remediated in order to protect ecological resources. However, the data demonstrate that the porewater toxicity test is more likely to

demonstrate false positives than false negatives, and therefore, the relevance of any porewater toxicity data that demonstrates an effect should be critically evaluated. Relevance, in this context, involves the following considerations:

- Is there a demonstrable relationship between COPCs and any observed porewater toxicity?

- Is the observed porewater toxicity in the laboratory representative of adverse biological effects under *in situ* conditions?

4.2 Relationship between COPCs and porewater toxicity

The potential influence of different COPCs on porewater toxicity was examined by comparing measured porewater COPC concentrations (where available) to toxicity data on individual COPCs obtained from the literature. Although the limited availability of porewater COPC chemistry data prevents this comparison from providing definitive conclusions regarding each individual sample, the general relationship between bulk sediment and porewater COPC concentrations (and thus the magnitude of the potential contribution of COPCs to the observed porewater toxicity) can be inferred for other samples with similar geochemical characteristics. This inference is increasingly valid when the measured porewater COPC concentrations are orders of magnitude less than concentrations expected to cause adverse effects to bivalve larval development.

4.2.1 Compilation of toxicity reference values

Toxicity reference values for the normal development of marine bivalve larvae are summarized in Table 9. 48-h EC_{50} values for metals are based on the exposure of newly-fertilized *Mytilus* (i.e., *M. galloprovincialis* or *M. edulis*) larvae to clean seawater spiked with varying concentrations of metal salts (i.e., chlorides or sulphates). Toxicity data regarding *Mytilus* larval development when exposed to PAHs were not available, and, therefore, data on the effects of PAHs on the larval development of other related species were substituted from the following papers:

- Pelletier et al. (1997) exposed newly-fertilized embryos of the marine bivalve, *Mulina lateralis,* to varying concentrations of anthracene, fluoranthene and pyrene dissolved in seawater using acetone as a solvent. Test duration was 48-h, after which an EC_{50} for normal survival was calculated.

- Pillai et al. (2003) exposed newly-fertilized embryos of the sea urchin, *Lytechinus anemesis*, to varying concentrations of fluoranthene, fluorene, phenanthrene, pyrene and quinoline dissolved in seawater using dimethyl sulfoxide (DMSO) as a solvent. Test duration was until the control embryos reached the late gastrula stage (i.e., approximately 48-h). The toxicological endpoint measured was percent exogastrulation. Exogastrulation refers to the protusion of sea urchin proto-gut tissues outside the embryo's periphery, which results in the development of a gastrointenstinal tract outside the sea urchin's body. Exogastrulated embryos remain viable—as a result, measurement of percent exogastrulation provided a sensitive endpoint relative to percent normal survival.

Screening quotients (i.e., the environmental concentration divided by the EC_{50}) based on the maximum observed porewater COPC concentration are summarized in Table 9. Screening quotients greater than 1 indicate a COPC potentially causing porewater toxicity, while screening quotients less than 1 indicate a COPC that is unlikely to be causing porewater toxicity.

Table 9: Toxicity reference values for *Mytilus* larval development

Compound	Maximum Observed Porewater Concentration (µg/L)	EC_{50} (µg/L)	Screening Quotient	Source
Ag	< 10	14	7.1×10^{-1}	Martin et al. (1981)
*As	< 100	> 3000	3.3×10^{-2}	Martin et al. (1981)
*Cd	0.07	2300	3.0×10^{-5}	Williams and Hall (1999)
*Cu	1.62	11.8	1.4×10^{-1}	Lowest reference toxicant test result in this study
*Cr	< 100	4469	2.2×10^{-2}	Martin et al. (1981)
Fe	450	10,000	4.5×10^{-2}	Okubo and Okubo (1962; cited in His et al., 1999)
*Hg	0.07	5.8	1.2×10^{-2}	Martin et al. (1981)
Mn	73.5	30,000	2.5×10^{-3}	Morgan et al. (1986)
Mo	< 100	147,000	6.8×10^{-4}	Morgan et al. (1986)
Ni	1.67	891	1.9×10^{-3}	Martin et al. (1981)
*Pb	0.24	476	5.0×10^{-4}	Martin et al. (1981)
Se	< 200	> 10,000	2.0×10^{-2}	Martin et al. (1981)
*Zn	3.2	140	2.3×10^{-2}	Williams and Hall (1999)
*Anthracene	0.3	4,260	7.0×10^{-4}	Pelletier et al. (1997)
*Fluorene	< 0.1	1,260	7.9×10^{-4}	Pillai et al. (2003)
*Fluoranthene	1.1	58.8	1.9×10^{-1}	Pelletier et al. (1997)
*Phenanthrene	< 0.1	410	2.4×10^{-4}	Pillai et al. (2003)
*Pyrene	1.0	> 11,900	8.4×10^{-5}	Pelletier et al. (1997)
Quinoline	< 0.1	4,300	2.3×10^{-4}	Pillai et al. (2003)

Notes: Asterisks indicate a compound identified as a COPC based on comparison of bulk sediment concentration to numerical sediment quality criteria (BCWLAP, 2003).

Screening quotients (i.e., the ratio of the maximum observed porewater concentration to the lowest available EC_{50} value) for PAHs include a 10-fold uncertainty factor due to potential differences in sensitivity between *M. galloprovincialis* and the respective test species.

4.2.2 Influence of PAHs on porewater toxicity

Measured concentrations of PAHs in porewater samples were often several orders of magnitude less than concentrations predicted to cause adverse effects on bivalve or sea urchin larval development, even when a 10-fold uncertainty factor was incorporated to compensate for potential differences in sensitivity between *M. galloprovincialis* and the test species. (Note: the 10-fold uncertainty factor was included in the calculated screening quotients summarized in Table 9). The only exception was fluoranthene, which had a screening quotient of 1.9×10^{-1}, which was the result of a maximum porewater concentration of 1.1 µg/L, a toxicity reference value of 58.8 µg/L from Pelletier et al. (1997), and a 10-fold uncertainty factor. However, fluoranthrene is still unlikely to be causing the observed porewater toxicity, given that the screening quotient is still less than one.

4.2.2.1 PAH bioavailability and solubility

The methods used in the PAH water-only toxicity tests should be considered when evaluating the potential contribution of PAHs to the observed porewater toxicity. For example, the measured porewater PAH concentrations are the sum of the dissolved fraction as well as those PAHs sorbed to suspended particulate matter in the sample—and therefore, the available PAH concentration data likely overestimate the true measure of dissolved PAH in the porewater sample. Harkey et al. (1994) found that the bioavailability (i.e., the fraction of the compound present in the medium that is taken up by the organism) of benzo(a)pyrene was lower in porewater than in elutriates due to the

fact that porewater samples had an increased proportion of dissolved organic carbon (DOC). Increased DOC concentrations provide an increased surface area for binding (from colloids and microparticles), which sorb more PAHs than an equivalent concentration when expressed as total organic carbon (TOC). Landrum et al. (1987) demonstrated that DOC in porewater sorbed organic compounds (benzo(a)pyrene, phenanthrene and pyrene) and thus resulted in reduced bioavailability to a freshwater amphipod. The bioavailability of PAHs in marine porewater is therefore assumed to be lower than in a spiked-seawater toxicity test without DOC.

Pelletier et al. (1997) and Pillai et al. (2003) used clean seawater and organic solvents to enhance PAH solubility. Several of the resulting EC50s are greater than the predicted solubility of the PAH in saline water. For example, Swartz et al. (1995) cited a worst-case estimate of solubility of anthracene in marine porewater of 44.6 µg/L (i.e., the solubility in distilled water was used as a worst-case estimate although solubility in marine water (28 ppt) is approximately 43% less than in distilled water). The calculated EC_{50} of 4,260 µg/L for anthracene (Pelletier et al., 1997) is therefore unachievable under field conditions. Similar differences were noted for pyrene (132 µg/L solubility in distilled water versus an EC_{50} of >11,900 µg/L), although the solubilities in distilled water were greater than the EC_{50} for all other PAHs considered.

4.2.2.2 Predicted PAH porewater concentrations

PAH concentrations in the porewater samples were less than the available EC_{50} values, and therefore, were considered unlikely to be the primary cause of the observed toxicity

in porewater from samples 4C and 4D. PAHs were also unlikely to be the primary cause in other porewater samples with similar bulk sediment PAH concentrations. Bulk sediment total PAH concentrations in samples 4C and 4D ranged from 12.06 to 13.37 mg/kg—as shown in Appendix A, the majority of samples have similar total PAH concentrations. Potential exceptions include samples 1D (72.32 mg/kg), 1E (51.66 mg/kg) and 4A (42.34 mg/kg), which had bulk sediment total PAH concentrations substantially above the bulk sediment total PAH concentrations in samples 4C and 4D. To address the potential contribution of PAHs in those samples, equilibrium partitioning was used to predict porewater concentrations for the three samples with the highest total PAH concentrations in bulk sediment using the approach described by Swartz et al. (1995):

$$PAH_{Porewater} = \frac{PAH_{Sediment}}{K_{OC} \times f_{OC}}$$

Data needed for the equilibrium partitioning formula includes K_{oc}, which is the PAH-specific total organic carbon partitioning coefficient and f_{oc}, which is the sample-specific total organic carbon fraction. Values for log K_{oc} were available from Swartz et al. (1995) and are summarized in Table 10. $PAH_{Sediment}$ and f_{oc} values were available for the three samples from chemical analyses conducted as part of the SQT.

Table 10: Predicted PAH porewater concentrations in selected samples with high total PAH bulk sediment concentrations

PAH	EC_{50} (µg/L)	Predicted dissolved porewater concentration (µg/L)			Log K_{OC}
		1D	1E	4A	
Anthracene	4,260	0.03	0.02	0.03	4.37
Fluorene	1,260	0.04	0.03	0.02	4.11
Fluoranthene	58.8	0.06	0.04	0.04	5.00
Phenanthrene	410	0.11	0.11	0.12	4.29
Pyrene	> 11,900	0.03	0.02	0.02	5.23
	TOC fraction (f_{OC})	2.52	2.26	1.96	

4.2.2.3 Overall contribution to porewater toxicity

PAHs are unlikely to play a significant role in porewater toxicity in any of the samples for the following reasons:

- Measured porewater PAH concentrations from two samples with representative sediment PAH concentrations are less than the available toxicity reference values. For the majority of PAHs, this difference is several orders of magnitude.

- Predicted porewater PAH concentrations from three samples with worst-case PAH concentrations in sediment are also less than the available toxicity reference values. Differences are two or more orders of magnitude for all PAHs with available toxicity reference values.

- Several factors limit the effect of PAHs in natural porewater samples, including decreased solubility and bioavailability relative to the laboratory-based exposure systems used to derive the EC_{50} values.

4.2.3 Influence of metals on porewater toxicity

The results presented in Table 9 indicated that the measured concentrations of metals in porewater samples were consistently less than EC_{50} values obtained from the literature. The difference between the maximum measured porewater concentration and the EC50 value was greater than an order of magnitude, with the exception of copper, which had a screening quotient of 0.14. However, copper is still considered unlikely to be causing to the observed porewater toxicity, given that the screening quotient is still less than 1.

4.2.3.1 Metal bioavailability

Extrapolating an EC_{50} derived from dissolving metal salts in clean seawater as representative of the potential effects in porewater is highly conservative, given the differences in bioavailability between the seawater used for toxicity testing and the porewater sample. Understanding the relationship between metal ions and other metal species, as well as the ability of the metal to form complexes with various ligands (e.g., humic substances), is essential when comparing porewater chemistry to water-only toxicity data (Chapman et al., 1998).

One key difference is that porewater contains DOC, including colloids (i.e., particles ranging in size from 1 to 1,000 nm). Most colloids can pass through a 0.45-μm filter and, therefore, are included in samples analyzed for dissolved metals. However, colloids form complexes with dissolved metals, which may lead to reduced bioavailability relative to the freely-dissolved metal fraction (Green et al., 1993; Cantwell and Burgess, 2001;

Boucher and Watzin, 1999). As a result, comparing dissolved porewater concentrations to data obtained from water-only testing using metal salts (which are completely dissociated) will overestimate the potential toxicity of the porewater sample.

4.2.3.2 Overall contribution to porewater BLD toxicity

Metals are unlikely to play a significant role in porewater BLD toxicity in samples 4A, 4B, 4C and 4D, since the measured porewater metal concentrations were less than the available EC_{50}s. Samples with similar (i.e., less than twice that observed in samples 4A through 4D) bulk metal concentrations are also inferred to have porewater metal concentrations that are less than the available EC_{50} values. This inference is only possible due to the fact that EC_{50} values are highly conservative estimates of metal toxicity to *M. galloprovincialis* larval development since, unlike PAHs, prediction of porewater concentrations from bulk sediment concentrations using an equilibrium partitioning approach is not possible due to the numerous geochemical interactions involved. A key consideration is that the majority of available equilibrium partitioning data are based on the relationship between metals and sulphides under anoxic conditions, which is not applicable to the conditions present in the porewater samples used for toxicity testing.

Without the means to accurately predict metal porewater concentrations based on bulk sediment concentrations, it would be inappropriate to conclude that metals were not influencing BLD toxicity in sediment samples that had bulk metal concentrations that were substantially above those observed in samples 4A though 4D (such as 1F, 2A, 2B, 2C, 2F, 2G, 3A, 3B, and 3C – see Appendix A for bulk sediment concentrations).

4.2.4 Influence of ammonia on porewater toxicity

4.2.4.1 Toxic mode of action for ammonia

Toxicity of ammonia is largely attributed to the unionized ammonia fraction (USEPA, 1989; 1999). In solution, ammonia exists in an equilibrium between unionized ammonia (NH_3) and the ammonium ion (NH_4^+).

$$H_2O + NH_3 \leftrightarrow NH_4^+ + OH^-$$

Several factors influence the ammonia equilibrium, including pH and temperature (which correlate positively with the concentration of NH_3), and salinity (which correlates negatively with the concentration of NH_3) (USEPA, 1989).

Ammonia is a byproduct of metabolism—the presence of elevated unionized ammonia in the intercellular environment interferes with normal diffusion and, as a result, internal NH_3 concentrations rapidly build up to toxic levels (Armstrong et al., 1978). Diffusion is the dominant method for NH_3 excretion—it is a neutral molecule and able to pass through biological membranes more readily than the ionized form (USEPA, 1999)—overall, biological membranes are relatively impermeable to the diffusion of ionized ammonia (Randall and Tsui, 2002). Elevated ammonia concentrations in sediment are primarily a by-product of microbial metabolism.

The precise mode of toxic action of NH_3 on saltwater bivalve larvae has not been described, although Armstrong et al. (1978) concluded that the presence of unionized ammonia in the environment disrupted the normal diffusion of endogeneous ammonia in prawn larvae. Similar findings were reported for lobster larvae, although the potential interference of ionized ammonia on cellular sodium transport could not be ruled out (Young-Lai et al., 1991; Armstrong et al., 1978), especially if the ionized concentrations were exceptionally high.

4.2.4.2 Comparison to existing regulatory threshold concentrations

For *M. galloprovincialis* larval development toxicity testing, PSEP (1995) adopted an ammonia threshold of 0.13 mg/L NH_3, based on testing using larvae of the Pacific oyster (*Crassostrea gigas*) (USEPA, 1993). NH_3 concentrations above this threshold concentration are considered to represent a potential false positive, which requires additional investigation to separate the potential effects of the contaminants of interest from the effects of ammonia (PSEP, 1995).

A review of the data presented in Table 4 indicates that elevated NH_3 concentrations are likely contributing to the observed porewater toxicity. Two of the three reference samples had NH_3 concentrations above the PSEP (1995) threshold of 0.13 mg/L, and a corresponding percent normal survival of 0%. Several of the porewater samples collected from impacted urban areas that had percent normal survival measurements of 0% also had NH_3 concentrations above the PSEP (1995) threshold. However, percent normal survival measurements of 0% were also observed in many samples that had NH_3

concentrations that were less than the PSEP (1995) threshold value of 0.13 mg/L, including several samples that had NH_3 concentrations of approximately 0.03 mg/L.

4.2.4.3 Summary of ammonia toxicity in larval development tests

Application of an oyster-based ammonia threshold concentration to interpret the potential for false positives in *M. galloprovincialis* larval development testing may not be appropriate. Data on the effects of ammonia on the normal development of bivalves, sand dollars and sea urchin larvae were compiled to evaluate the inherent assumption in PSEP (1995) that ammonia toxicity is similar between different bivalve species. Seawater was assumed to have a salinity of 30 ppt and a pH of 7.5 if data were not available from the original literature (Sims and Moore, 1995).

- Kobayashi (1980) calculated NOECs for total ammonia for one sand dollar species (*Peronella japonica*) and two sea urchin species (*Hemicentrotus pulcherrimus* and *Anthocidaris crassispina*) from Japan and Australia. NH_3 concentrations were estimated using study-specific values for temperature, and assumed values for salinity and pH.

- Geffard et al. (2002) determined an EC_{20} of 2.8 mg/L for larval development of the oyster, *Crassostrea gigas*. NH_3 concentrations were estimated using a study-specific temperature and assumed values for salinity and pH.

- Greenstein et al. (1996) determined a NH_3 NOEC for larval development of the Pacific purple sea urchin, *Strongylocentrotus purpuratus*.

- Nipper et al (2002) developed an NH_3 NOEC for larval development of the sea urchin *Arbacia punctulata.*

- Stronkhorst et al. (2003) determined a NOEC of 6 mg/L total ammonia for larval development of the sea urchin, *Psammechinus miliaris*, which was converted to an NH_3 NOEC using study-specific values for temperature, pH and salinity.

- Cesar et al. (2002) determined an EC_{25} for larval development in three different Mediterranean sea urchin species, which was converted to a NH_3 EC_{25} using study specific measurements of salinity and temperature, and an assumed value for seawater pH.

Table 11: Summary of ammonia toxicity data for species typically used in larval development tests

Source	Common Name	Species	Endpoint	Unionized Ammonia (mg/L N)
*USEPA (1993)	Oyster	*Crassostrea gigas*	EC50	0.130
USEPA (1993)	Oyster	*Crassostrea gigas*	NOEC	0.080
Carr and Chapman (1992)	Sea urchin	*Arbacia punctulata*	NOEC	0.090
Greenstein et al. (1996)	Sea urchin	*Strongylocentrotus purpuratus*	NOEC	0.060
Stronkhorst et al. (2003)	Sea urchin	*Psammechinus miliaris*	NOEC	0.042
USEPA (1993)	Sand dollar	*Dendraster excentricus*	NOEC	0.040
Kobayshi (1980)	Sand dollar	*Peronella japonica*	NOEC	0.022
Kobayshi (1980)	Sea urchin	*Anthocidarius crassispina*	NOEC	0.022
Geffard et al. (2002)	Oyster	*Crassostrea gigas*	EC20	0.019
Cesar et al. (2002)	Sea urchin	*Parocentrotus lividus*	EC25	0.013
Cesar et al. (2002)	Sea urchin	*Sphaerechinus granularis*	EC25	0.012
Cesar et al. (2002)	Sea urchin	*Arbacia lixula*	EC25	0.011
Kobayshi (1980)	Sea urchin	*Hemicentrotus pulcherrimus*	NOEC	0.003

Note: Test durations for Kobayshi (1980) was 12-h for *A. crassispina* and 24-h for *P. japonica* and *H. pulcherrimus*.

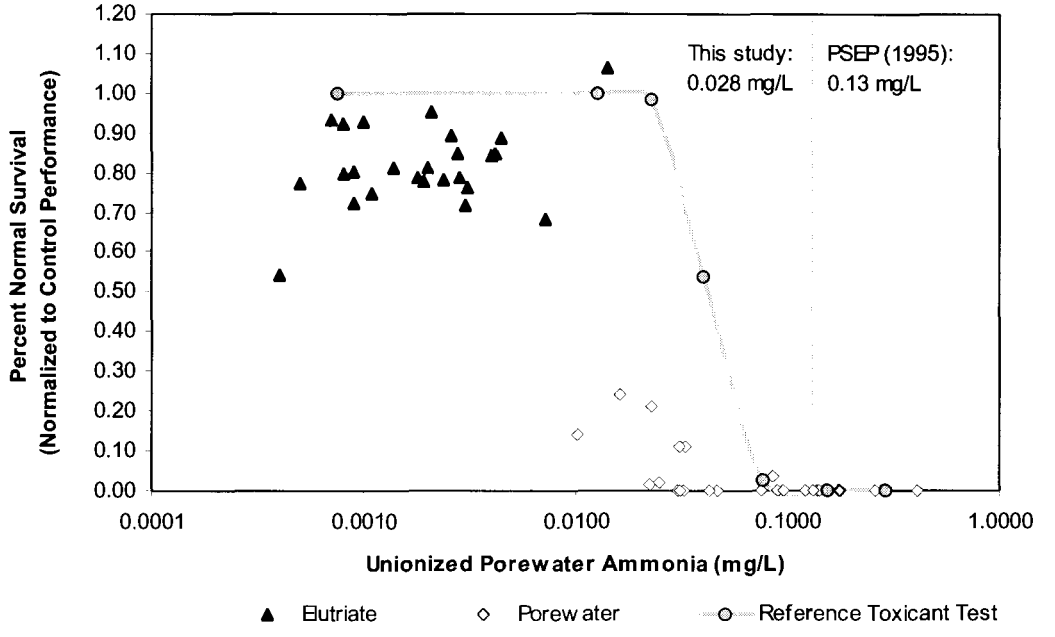
Asterisk indicates the citation used for the *M. galloprovincialis* ammonia threshold concentration

A review of the available data on the toxicity of unionized ammonia to species used in larval development toxicity tests (Table 11) suggests that the 0.13 mg/L toxicity reference value used by PSEP (1995) is unlikely to be protective of *M. galloprovincialis.* All other species used in larval development toxicity testing have demonstrated adverse effects sensitivity at concentrations substantially less than 0.13 mg/L NH_3.

Additionally, results from the ammonia reference toxicant test in the present study (Section 3.3) found that percent normal survival of *M. galloprovincialis* was reduced by

20% when exposed to concentrations as low as 0.028 mg/L NH_3. A 50% reduction in percent normal survival was observed at NH_3 concentrations of 0.036 mg/L N. As a result, the EC_{20} benchmark of 0.028 mg/L NH_3 obtained from the reference toxicant test was used as the basis for evaluating the potential contribution of ammonia to the observed toxicity in the porewater tests rather than the toxicity reference value from PSEP (1995).

Figure 5: Comparison of control-normalized percent normal survival and unionized ammonia concentrations in the porewater and elutriate toxicity tests and ammonia reference toxicant test



Threshold concentration of 0.028 mg/L NH_3 and the response of *M. galloprovincialis* larvae in the ammonia reference toxicant test provide a better fit to the available toxicity data than the PSEP (1995) threshold concentration of 0.13 mg/L.

For example, normal survival begins to increase to the left of the ammonia reference toxicant test threshold concentration, suggesting that *M. galloprovincialis* larval development may have been impaired in the toxicity tests as a result of the measured ammonia concentrations. Additionally, the relatively high percent normal development in the elutriate toxicity tests corresponds to unionized ammonia concentrations that are consistent with minimal effects on BLD observed in the reference toxicant tests.

There are several areas of uncertainty regarding the true effect of NH_3 on BLD toxicity that should be noted. The primary source of uncertainty is that the NH_3 concentrations for the majority of samples are based on measurements collected from Day 0 interstitial samples from concurrent amphipod toxicity testing—ammonia concentrations in the actual porewater samples are likely higher (see Section 3.2.3). Total ammonia concentrations were converted to the unionized fraction using point estimates of temperature, pH and salinity that may not account for minor variations during the test exposure. Finally, NH_3 toxicity to *M. galloprovincialis* larvae may vary by organism batch (due to factors such as seasonal effects and different organism stress levels).

Figure 5 also suggests that COPCs other than ammonia may be contributing to the observed toxicity in some samples. For example, sample 3C had one of the highest percent normal scores of all the porewater samples (0.239), and the unionized ammonia concentrations were lower (0.016 mg/L NH_3) than the ammonia reference toxicant test EC50 of 0.028 mg/L NH_3, suggesting that ammonia was not the primary cause to the observed BLD toxicity. However, sample 3C had relatively high bulk sediment

concentrations of copper, mercury and zinc (see Section 4.2.3.2) and as a result, metal concentrations in porewater cannot be excluded as potential contributors to observed porewater BLD toxicity in sample 3C.

4.2.4.4 Influence of ammonia toxicity in other porewater testing

Ho et al. (2002) reviewed the available published literature for porewater and whole-sediment toxicity identification evaluation (TIE) testing to determine if one or more groups of contaminants (e.g., metals versus PAHs) were responsible for observed toxicity. Ammonia was identified as a contributory toxic agent in 69% of the porewater TIE studies (and was the sole toxic agent in 23% of the porewater TIE studies). Conversely, ammonia was never a toxic agent in the whole-sediment TIE studies included in the review. Although the number of studies included in the review was relatively small (i.e., n = 13 for porewater; n = 5 for whole-sediment), it does indicate the potential magnitude of the influence of ammonia in porewater testing. Ho et al. (2002) suggested that the influence of ammonia may be an artifact of the test system (i.e., ammonia is water soluble, and therefore more likely to result in over-exposure in a porewater sample).

Several additional studies not included in the review conducted by Ho et al. (2002) also indicate the potential influence of ammonia in porewater toxicity testing. Stronkhorst et al. (2003) identified ammonia as the primary cause of toxicity to a sea urchin (*Psammechinus milaris*) in marine porewater TIE testing on samples collected from an urbanized harbour. Van Sprang and Janssen (1997) identified ammonia as the major toxic

agent in porewater TIE testing on samples collected from an industrialized river. O'Day et al. (2000) found that unionized ammonia was responsible for observed toxicity (i.e., 0% normal survival) in several subsurface sediment samples tested using embryos of the sand dollar, *Dendraster excentricus*. O'Day et al. (2000) used an unionized ammonia EC_{50} of 0.2 mg/L N (from unpublished data) as the threshold value for evaluating the contribution of ammonia; however, USEPA (1993) determined an EC_{50} of 0.03 mg/L for *D. excentricus* normality. As a result, the contribution of ammonia to the observed toxicity of subsurface samples may be greater than that assumed by O'Day et al. (2000).

4.2.5 Sensitivity of porewater toxicity testing

Porewater toxicity testing with sea urchin embryos (and by extension, bivalve embryos) has been described as significantly more sensitive than common whole-sediment tests (Nipper and Carr, 2001; Carr et al., 1996a), and provides "an indication of potential sublethal effects which could otherwise not be analyzed" (Nipper et al., 2002). Carr et al. (2001a) comment that porewater toxicity testing should be included in a SQT approach, in part, because "porewater toxicity testing may be an order of magnitude more sensitive than whole-sediment toxicity testing, which allows for further investigation for those sediments that may be causing more complex changes to the benthic community." However, this assumed sensitivity is meaningless unless a cause and effect relationship exists between the presence of COPCs and the measured adverse biological effects. Although it is true that concurrent porewater samples tend to be toxic for those samples that also demonstrate toxicity in a bedded sediment exposure system, it is not necessarily true that porewater testing is inherently more accurate than other exposure systems.

One of the first papers advocating the use of porewater toxicity testing was Ankley et al. (1991) who conducted side-by-side trials of the toxicity of freshwater porewater, sediment elutriate and bedded-sediment to fathead minnows (*Pimephales promelas*), amphipods (*Hyalella azteca*) and oligochaetes (*Lumbriculus variegatus*). Ankley et al. (1991) found that a larger percentage of samples that were categorized as toxic (i.e., greater than 50% mortality) in bedded sediment were also correctly categorized as toxic in porewater (86%) versus elutriate (45%). The authors concluded that the "data set clearly demonstrates that porewater is a reasonable test fraction for predicting the presence of toxicity in bulk sediments, whereas elutriate is a poor predictor of bulk sediment toxicity." Implications of this finding to a SQT approach are clear: one can presumably substitute acute porewater toxicity testing for whole-sediment exposure toxicity testing. Porewater testing is quicker, cheaper and easier to conduct than equivalent acute toxicity testing using whole sediment (Carr et al., 2001a).

In the same experiment described above, Ankley et al. (1991) found that non-toxic bedded sediment samples had toxic porewater in 26 of 75 comparisons (27%). This "relatively frequent observation of porewater toxicity in the absence of bulk sediment toxicity" was dismissed with the observation that "it is far preferable to have even a moderate percentage of false positives than a small percentage of false negatives." However, from a management perspective, using a test method that may have an inherent false positive rate greater than 1 in 4 is clearly unacceptable.

Two additional examples where marine porewater toxicity testing are described as more sensitive than whole-sediment toxicity testing are provided. Carr et al. (1996a) stated that porewater tests using sea urchins were considerably more sensitive than sediment amphipod testing, based on an evaluation of sediment toxicity in the vicinity of offshore oil & gas platforms. Long et al. (1996) found that tests involving the effects of 100% porewater on sea urchin fertilization (*Arbacia punctulata*), or sea urchin/abalone larval development (*Strongylocentrotus purpuratus, Haliotis rufescens*) led to the determination that 43% of the total area in major US estuaries was toxic, while tests involving the effects of whole sediment amphipod survival led to the determination that only 11% of the total area was toxic. Porewater toxicity testing, therefore, was considered more sensitive than amphipod toxicity testing on whole sediment.

As part of Carr et al. (1996a)'s argument that porewater toxicity testing was more sensitive than other toxicity test methods, statistically significant ($p < 0.005$) correlations were identified between sediment concentrations and porewater toxicity for multiple metals (e.g., barium, copper, lead and zinc) as well as total PAHs and naphthalene. However, a correlation between bulk sediment concentrations and porewater toxicity provides minimal information regarding the identity of toxic agents in the porewater exposure. Carr et al. (1996a) also measured unionized ammonia concentrations in porewater samples and demonstrated that ammonia concentrations were less than published values for adverse effects on sea urchin larval development. Measured copper and zinc porewater concentrations were greater than concentrations above which adverse effects on sea urchin larval development was predicted (i.e., no-observed effect

concentrations; NOECs). Although a true cause-effect relationship between the toxic agent and the observed effects on sea urchin development was not established, the fact that ammonia was excluded as a toxic agent while copper and zinc were identified as potential toxic agents supports the argument that the porewater sea urchin larval testing on those particular samples was more sensitive than bedded-sediment amphipod toxicity testing. This argument would have been strengthened if data on porewater concentrations of PAHs were available in addition to the previously-identified correlation between bulk sediment PAH concentrations and porewater toxicity.

Conversely, little evidence was provided by Long et al. (1996) to demonstrate increased sensitivity of porewater concentrations. Ammonia was not evaluated as a potential cause for the observed porewater toxicity in Long et al. (1996), making it likely that many of the samples categorized as "toxic" were in fact influenced by ammonia toxicity. Porewater concentrations of relevant COPCs (e.g., metals and PAHs) were not measured. As a result, porewater toxicity testing should not be considered more sensitive than other tests—it is simply demonstrating a greater degree of toxicity. Sensitivity implies that a cause-effect relationship exists.

Multiple authors have noted similar problems with the "sensitivity" of porewater larval development toxicity tests. For example, Burgess et al. (1993) conducted sea urchin (*Arbacia punctulata*) fertilization toxicity tests using whole-sediment, elutriate, and porewater from contaminated marine sediments, and found the porewater demonstrated greater toxicity than the whole-sediment or elutriate. However, toxicity was not

attributable to COPCs, and instead, was determined to be the result of elevated concentrations of ammonia and sulphides. Additional examples of the disproportionate role of ammonia in porewater toxicity testing are summarized in Section 4.2.4.4.

Anderson et al. (2001b) conducted sea urchin (*Strongylocentrotus purpatus*) larval development toxicity testing using marine sediments with varying degrees of metal contamination. Toxicity was consistently higher in porewater than in a corresponding homogenized whole-sediment exposure, which was linked to increased ammonia concentration in the porewater samples, rather than metals (which were the COPCs under investigation). This pattern of toxicity extended to a clean reference site, which had percent normal development of approximately 45% (toxic) and 90% (non-toxic) for the porewater and homogenized whole-sediment exposures, respectively. Similar findings of highly toxic porewater samples from uncontaminated reference samples were also identified in this study (i.e., the results from samples 1A, 1B and 1C discussed in Section 3.1.1).

Any argument for the increased sensitivity of the porewater BLD toxicity test must be able to demonstrate a cause-effect relationship—which will likely require data to demonstrate that confounding factors such as ammonia are not contributing to the observed toxicity, as well as data indicating how the actual COPCs are partitioning in sediment-porewater systems. Additional lines of evidence, such as critical body residue data (i.e., demonstrating that elevated COPC concentrations are accumulated by the test organisms), or TIE (i.e., identifying the toxic agent through iterative toxicity testing of

samples with varying chemical manipulations) should also be considered. Concluding that porewater testing in general is more sensitive than other test methods (e.g., Carr et al., 2001a) is inappropriate, based on the available data—for example, Nipper et al. (2002) found that the sea urchin larval development test using porewater was the least sensitive of four test species (one bedded-sediment and three porewater) used to evaluate the toxicity of ordnance compounds in marine sediment. The sensitivity of porewater toxicity testing is likely a sample- and COPC-specific property, and should be evaluated on a case-by-case basis.

4.2.6 Conclusions

Unionized ammonia is a significant factor in the observed differences in toxicity between the porewater and elutriate BLD tests. Data collected as part of this research project indicate that NH_3 concentrations in the elutriate samples were relatively non-toxic, while NH_3 concentrations in the majority of porewater samples were above the concentration that caused substantial reductions in normal survival of *M. galloprovincialis* larvae in an ammonia reference toxicant test.

PAH concentrations in porewater samples (both measured and predicted using equilbrium partitioning – see Section 4.3.2) are substantially less than PAH concentrations that cause adverse effects in other larval development tests, although data specific to *M. galloprovincialis* were not available. However, the magnitude of the difference, along with considerations regarding the solubility and bioavailability of PAHs in marine

porewater, strongly suggest that PAHs are unlikely to be contributing to the observed porewater BLD toxicity.

Concentrations of metals in four porewater samples analyzed as part of this research project were also less than water-only toxicity data for the effects of metals on *M. galloprovincialis* larvae. Several factors were identified (e.g., differential bioavailability as the result of DOC) that suggest that water-only toxicity data derived from testing metal salts in clean seawater will overestimate the actual toxicity data in porewater samples. As a result, porewater toxicity in the majority of samples tested in this project was considered unlikely to be the result of metals. However, bulk sediment metal concentrations in 9 of the 25 samples (1F, 2A, 2B, 2C, 2F, 2G, 3A, 3B, and 3C) were substantially higher than the four samples for which porewater chemistry data were available. Reliable models to predict porewater metal concentrations in toxicity test samples (which are well-aerated) were not available. As a result, metals cannot be excluded from consideration as a potential contributor to the observed toxicity in some of the samples included in this research project.

4.3 Ecological relevance of porewater BLD toxicity testing

Although the influence of COPCs may differ in elutriate and porewater toxicity tests, the underlying purpose of any laboratory-based toxicity test is to predict the potential for adverse effects under field conditions. Carr et al. (2001a) suggested that porewater toxicity testing was ecologically relevant and, therefore, appropriate for inclusion in a

SQT. However, the ecological relevance of conducting toxicity testing on porewater samples is questionable, due to numerous chemical and biological factors, as follows:

4.3.1 Comparison of porewater chemistry *in situ* and in laboratory testing

Carr et al. (2001a) concluded "it is nearly impossible to remove a porewater sample from sediment and expose organisms to it while preventing changes in the chemistry of its... constituents." These alterations are unavoidable, given that porewater is typically anoxic, yet toxicity testing requires an oxic environment for proper biological function. Demonstrated alteration in porewater chemistry as a result of aeration are described below.

4.3.1.1 Changes in organic compound geochemistry

Hunchak-Kariouk et al. (1997) found substantial differences in the equilbrium partitioning of 2,2',4,4'-tetrachlorobiphenyl (TeCB) (and by inference, other hydrophobic organic compounds) in freshwater porewater, depending on whether the porewater was oxic or anoxic. Under aerobic conditions, DOC coagulated with iron precipitates, reducing the amount of DOC available for binding TeCB. As a result, the amount of dissolved TeCB was higher (and thus, bioavailability assumed to be greater) in oxic porewater samples than in anoxic porewater samples.

4.3.1.2 Changes in metal geochemistry

A discussion of the current understanding of important aspects of metal geochemistry in sediment is provided by Chapman et al. (1998) and can be summarized as follows:

- Metals form insoluble metal sulphide precipitates under anerobic conditions and are remobilized as the sulphide precipitates are oxidized.

- Metals bind to particulate and dissolved organic carbon (i.e., humic acids), and are remobilized as the organic carbon is oxidized.

- Metals bind to iron and manganese oxyhydroxides (FeOOH and MnOOH).

These sediment-binding phases do not operate in isolation. Rather, a complex and interlocking series of chemical reactions occur as anoxic sediment porewater is aerated. To illustrate these connections, Simpson et al. (2002) placed thin segments of an intact, anoxic sediment core in anoxic seawater, and measured changes in porewater metal concentration as the system was gradually aerated. Dissolved iron concentrations rapidly increased, as a result of the dissociation of iron sulphides ($FeS \rightarrow Fe^{2+} + S^{2-}$) followed by a rapid decrease as the Fe^{3+} formed Fe^{2+} precipitates, which were then hydrolyzed ($Fe^{3+} + 2H_2O \rightarrow Fe(OH)_3 + 3H^+$). Manganese (i.e., MnS) demonstrated similar patterns, albeit at a slower rate than iron. Substantial decreases in porewater pH were noted as a result of the release of H^+ into the system, with porewater pH decreasing from approximately 8.0 to 6.5. Acidification alters the equilibrium of the iron and manganese oxyhydroxides ($FeOOH + Me^{2+} \leftrightarrow FeOOMe^+ + H^+$), favouring the production of metal ions.

Oxidation of dissolved organic carbon also increases the dissolved metal concentration (Cantwell et al., 2002).

Although the precise difference between metal concentrations in anoxic and oxic porewater is sample-specific, the results from Simpson et al. (2002) demonstrate the general magnitude of the issue. Changes in porewater pH from 8.5 to 7.5 resulted in an increase in zinc concentration from <3 μg/L to approximately 75 μg/L, with a further increase to approximately 400 μg/L as the pH dropped to 7.0. Other metals were also mobilized with pH decreases, with concentrations increasing from <3 μg/L to 3-5 μg/L (Cu), 3-8 μg/L (Cd), and 5-15 μg/L (Pb) as the pH dropped from 7.5 to 7.0. Teasdale et al. (2003) linked elevated porewater concentrations of copper during oxidation to an identical series of geochemical reactions (i.e., sulphide dissociation, iron precipitation/hydrolysis, and acidification).

4.3.1.3 Ecological relevance

Although intentional aeration of the sample itself was not necessary in the testing described in this study, the manipulations needed to prepare the sample for testing would have provided ample opportunity for oxidation. Examples of these necessary manipulations include homogenization of the bulk sample prior to centrifugation and the transfer of porewater from the centrifugation tubes to test tubes using a pipette. Samples were also exposed to air throughout testing. As a result, COPC concentrations in porewater samples used for toxicity testing bear little similarity to *in situ* concentrations—dissolved concentrations of metals are potentially much higher in

toxicity samples. Ho et al. (2002) identified several additional potential chemical alterations inherent in porewater toxicity testing, including additional pH variation due to CO_2 volatilization, and potential underexposure to high K_{OW} compounds that sorb to test containers.

Substantial alterations in porewater chemistry are an inevitable result of the sample aeration needed to conduct the 48-h *M. galloprovincialis* larval development toxicity test—porewater chemistry in the test bears little resemblance to porewater chemistry *in situ*. Extrapolation of porewater BLD toxicity data to represent potential ecological effects in the field is therefore questionable. Conversely, sample oxidation would be anticipated for an elutriate-like *in situ* exposure (i.e., resuspension of sediment particles as result of dredging, wave action or propeller wash)—the sample chemistry in the elutriate BLD test would be similar to that encountered in the field. Extrapolation of elutriate BLD toxicity data to represent potential ecological effects in the field appears more reasonable than extrapolation of porewater BLD toxicity data.

4.3.2 Consideration of multiple COPC exposure pathways

Early work suggested that porewater exposure was an important route for benthic organisms, since the desorption of sediment-sorbed chemicals is mediated by interstitial water (Knezovich et al., 1987) although, at the time, the relative contribution of porewater exposure and the mechanisms underlying contaminant uptake in aquatic food webs were not well understood. The assumption that porewater is the only significant route of exposure can also be linked to the development of sediment quality guidelines

(SQG) using equilibrium partitioning (Di Toro et al., 1991; Ankley et al., 1996; Di Toro and McGrath, 2001).

Equilibrium partitioning was proposed in response to the fact that interpretation of whole-sediment toxicity tests is often confounded by the effects of physical sediment characteristics (e.g., organic carbon content, grain size distribution), since many test organisms exhibit reduced biological performance if physical sediment characteristics are substantially different than their native environment. These physical sediment characteristics also influence the bioavailability of COPCs, and make predictions of adverse biological effects based on bulk sediment concentrations unreliable. As a result, equilibrium partitioning models and toxicity testing based on porewater exposures have been used for derive sediment quality criteria for metals (Ankley et al., 1996), nonionic organic compounds (Di Toro et al., 1991), PAHs (Di Toro and McGrath, 2000) and PAH mixtures (Swartz et al., 1995) based on observed correlations between porewater concentrations and adverse biological effects in different benthic organisms. The SQGs are estimated from an acceptable porewater concentration and mathematical formula intended to represent the relationship between porewater and bulk sediment COPC concentrations.

4.3.2.1 Routes of exposure in a bivalve larval development test

At a fundamental level, the ecological relevance of porewater testing should be considered in the context of the fact that bivalve larvae are never exposed to 100% porewater in their natural environment. However, even if this limitation is ignored, the

exposure pathways available in a porewater exposure system are unlikely to reflect natural conditions. Aquatic organisms are exposed to COPCs through multiple pathways, including:

- Direct contact with dissolved COPCs in the water column (i.e., diffusion of COPCs across biological membranes).

- Direct contact with COPCs associated with particulate matter (suspended or otherwise), colloids or complexed with other compounds.

- Ingestion of COPCs associated with particulate matter and/or food.

Of the three pathways, the first two are dominant in the 48-h bivalve larval development test, since D-shaped veliger larvae less than 48-h old have not yet developed the organs necessary for feeding (Bayne, 1965). The relative contributions of exposure to dissolved COPCs versus sorbed COPCs to bivalve larval toxicity are not well understood, although substantial evidence exists to suggest that entrainment of larvae in the elutriate version of the test as particles settle out is a significant confounding factor. A 24-h settling period is routinely applied to all elutriate bivalve larval development tests (instead of the 4-h period specified by PSEP, 1995) to compensate for this problem.

The importance of porewater as the basis for setting SQGs, and its advantage in avoiding the confounding factors associated with the bulk sediment, appears to have resulted in the assumption that porewater-only exposures are inherently superior to sediment exposure systems. However, porewater exposure systems are subject to extensive sample

manipulation that is potentially a greater confounding factor than variations in grain size and organic carbon content in whole-sediment.

As described in Section 4.3.1, the process of extracting porewater results in substantial alteration in contaminant geochemistry. Changes to the oxidative state of the colloidal carbon, and reduction in the fraction of coarse particulate matter are also expected. Mahony et al. (1996) found that the sediment organic carbon provided substantial binding capacity for metals (i.e., over and above the capacity of sulphide complexation). Removal of this coarse particulate fraction alters the available exposure pathways in the porewater toxicity test, effectively maximizing direct contact with dissolved COPCs while minimizing direct contact with COPCs bound to organic carbon.

Alterations in exposure pathways are even more pronounced if porewater samples are filtered, as was done by Carr et al. (1996a) and Long et al. (1990). Carr and Chapman (1995) found that unfiltered porewater samples were more toxic to sea urchin (*Arbacia punctulata*) fertilization than filtered samples. Percent fertilization ranged from 38% in the non-filtered sample to 67-71% in the filtered samples. Ankley and Schubauer-Berigan (1994) also noted that various methods of filtration reduced the concentrations of various organic compounds, including pesticides and PAHs via adsorption. Reduced toxicity in the filtered samples may also be due to the removal of a significant exposure route (i.e., direct contact with particle-bound COPCs).

Data from freshwater testing illustrate the effect of suspended particulate material on porewater toxicity. Ankley and Schubauer-Berigan (1994) conducted 48-h *Daphnia magna* survival tests on freshwater porewater extracted using a variety of methods, including low-speed (2,500 g), and high-speed (10,000 g) centrifugation, compression and dialysis. Filtered (1 μm) samples had lower concentrations of metals and substantially lower toxicity than unfiltered samples, regardless of the method of extraction. Toxicity also varied by the method of extraction, with the greatest toxicity observed in samples extracted with the method that left the largest amount of particulate matter (i.e., low-speed centrifugation). Similar findings were observed by Carr and Chapman (1995) who extracted porewater samples using different methods (centrifugation, squeezing) and filter types (none, fluorocarbon, glass, nylon) with varying porosity. Percent fertilization in a sea urchin (*Arbacia punctulata*) fertilization toxicity test ranged from 25.2 to 93.2%. Toxicity was highest when an 8-μm Nylon filter was employed, which was later attributed to the presence of leachable toxicants from the nylon itself. Of the remaining methods, percent fertilization was substantially increased in filtered samples relative to samples that were centrifuged without filtration. Carr and Chapman (1995) concluded that toxicity was correlated to the presence of particulate material in the sample.

4.3.2.2 Ecological relevance

Porewater samples for this investigation were extracted using low-speed centrifugation without filtration, and were tested immediately per recent guidance (Carr et al., 2001a). Data from Carr and Chapman (1995) suggest that this approach likely maximized

porewater toxicity in bivalve larval development relative to other methods of porewater extraction and manipulation described in the literature.

The ecological relevance of porewater extraction methods are unclear—Ankley and Schubauer-Berigan (1994) commented that the "true" composition of porewater will always remain unknown, including information regarding the amount of suspended particulate material available *in situ*. Presumably, *in situ* porewater exists within a stable sediment matrix with a lower amount of particulate material than would be present in a porewater sample extracted via any of the customary methods. As a result, the relative influence of direct contact with dissolved COPCs versus particle-sorbed COPCs, while not fully understood in the context of bivalve larval development, is clearly different in extracted porewater than would be expected for *in situ* exposures. Ho et al. (2002) noted that overexposure of organisms not normally exposed to 100% porewater and elimination of other, relevant routes of exposure were significant limitations in porewater toxicity testing.

5.0 CONCLUSIONS AND RECOMMENDATIONS

M. galloprovincialis larval development was consistently poorer in porewater-only exposures than in concurrent sediment elutriate exposures. Interpretation of the available BLD toxicity data led to a conclusion of severe effects for all samples in the porewater-only tests, but negligible or moderate effects for all samples in the elutriate tests. The differences in the magnitude of effects between the two exposure systems resulted in substantially different conclusions regarding overall sediment toxicity when integrated with data from other sediment toxicity tests using a weight-of-evidence framework, which could lead to unnecessary remediation or additional investigative costs. The increased toxicity evident in the porewater BLD test cannot be interpreted as evidence of increased sensitivity for the majority of samples, despite statements in the literature that porewater toxicity testing is inherently superior to other exposure systems.

Porewater samples collected from uncontaminated reference locations demonstrated severe reductions in bivalve larval development, despite an absence of COPCs. Measured and predicted porewater concentrations of COPCs (where available) were less than toxicity reference values derived from the literature. Ammonia was identified as a probable source of the false positive porewater results—ammonia concentrations in porewater samples were generally greater than the threshold concentration derived from a water-only reference toxicant test.

The overall ecological relevance of porewater BLD toxicity testing appears limited. Bivalve larvae are pelagic organisms and, by definition, will not be exposed to 100% porewater. The process of extracting anoxic porewater samples and aerating them for toxicity testing results in numerous geochemical alterations that lead to unrealistic concentrations of many COPCs. However, recent developments in larval development toxicity testing may provide an acceptable substitute that provides many of the advantages of porewater toxicity testing without its limitations.

Anderson et al. (2001b) proposed a method for conducting embryo larval toxicity testing which involved suspension of newly-fertilized sea urchin larvae (*Strongylocentrotus purpuratus*) above the sediment-water interface of an non-homogenized sediment core. Sediment cores were kept in their original polycarbonate core sampler sleeves and were maintained at 4°C in the dark in an oxygen-free atmosphere prior to testing. Overlying seawater was added to the core sleeves at test initation, and fertilized sea urchin suspended above the sediment-water interface in a tube screened with 25-µm mesh. This exposure system places the embryos in close proximity to the flux of metals and ammonia leaving the sediment core, without the extensive manipulation inherent in collection, extraction and aeration of porewater samples. This exposure system also provides advantages in that ecological realism is substantially improved over a traditional porewater toxicity test method, since bivalve larvae can reasonably be assumed to be present near the sediment-water interface, especially during settling and attachment.

If porewater toxicity testing is included in a SQT framework, sufficient data must be collected to properly assess its ecological relevance. Recommendations for additional data needed to evaluate porewater toxicity data in a SQT framework include:

- Sample-specific measurement of COPC concentrations. Data regarding *in situ* concentrations (e.g., obtained using dialysis membrane "peeper" devices – Azcue et al., 1996) as well as in the toxicity test samples would allow better understanding of the appropriate extrapolation of observed toxicity from the laboratory to the field. Measurement of COPC porewater concentrations in all samples included in this research project would have provided essential data—although metals could not be ruled out as the cause of porewater toxicity for several samples, nor could they be directly evaluated due to a lack of porewater chemistry for those samples.

- Sample-specific measurement of confounding factors such as unionized ammonia is required to exclude obvious false positive results. Ammonia reference toxicant testing on each batch of organisms used in porewater testing is recommended until species-specific data on the natural variability in ammonia sensitivity is available.

- Sufficient lines of evidence should be included in the SQT so that the potential influence of false positive results from a porewater toxicity test on the overall conclusions are mitigated. No guidance on what constitutes an acceptable number of lines of evidence is available in the literature at this point—the tendency for SQTs to utilize 2-4 toxicity tests is more a function of historical practice than the result of careful consideration.

Realistically, the additional data required for interpreting the results of porewater toxicity testing means that porewater toxicity testing is less desirable than other, readily available whole-sediment toxicity tests. For example, toxicity tests with longer test durations and multiple toxicological endpoints are now available (e.g., 28-d *Leptocheirus plumulosus* survival, growth and reproduction amphipod toxicity test; USEPA/USACE, 2001). Routine inclusion of porewater toxicity testing using larvae of the bivalve, *M. galloprovincialis* in a SQT cannot be recommended in light of the limitations of porewater toxicity testing described in this paper, and the availability of numerous other test species and exposure systems that have greater ecological relevance.

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APPENDIX A	SED QC_{SCS}	1A	1B	1C	1D	1E	1F	2A	2B
Metals (mg/kg)									
Aluminum	NC	6980	4760	3750	7400	6180	6970	19000	16800
Antimony	NC	<10	<10	<10	<10	<10	<10	79	36
Arsenic	25	2.2	3.2	2.7	20.9	24.6	**54.3**	**153**	**36**
Barium	NC	19	8	6	39	31	86	371	197
Cadmium	2.4	0.2	0.3	0.2	**3.7**	**4.3**	**3.1**	**4.1**	1.5
Chromium	110	12	18	14	26	27	26	51	60
Cobalt	NC	<1	<1	<1	<1	<1	<1	19	12
Copper	65	9	7	5	**216**	**167**	**567**	**834**	**574**
Iron	NC	10700	8690	6160	17800	14700	38000	64400	45900
Lead	70	<1	<30	<30	**89**	60	**222**	**480**	**143**
Manganese	NC	113	90	75	147	122	145	381	367
Mercury	0.42	0.04	0.05	0.05	0.17	0.14	0.12	**3.70**	**0.42**
Molybdenum	NC	<4	<4	<4	<4	<4	<4	23	4
Nickel	NC	6	16	13	15	14	13	40	30
Selenium	NC	0.3	0.3	0.2	0.7	0.6	0.5	<2	<2
Silver	NC	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	5	<2
Strontium	NC	24	15	12	53	64	99	246	148
Thallium	NC	0.1	0.2	0.2	0.3	0.2	0.3	<50	<50
Tin	NC	<5	<5	<5	<5	<5	6	15	24
Titanium	NC	366	377	281	320	244	241	691	597
Vanadium	NC	22	17	13	22	18	17	62	63
Zinc	200	22	19	15	**566**	**617**	**1670**	**1930**	**1180**
PAH (mg/kg)									
Acenaphthene	0.048	<0.005	<0.005	<0.005	**1.9**	**1**	**0.37**	**0.09**	**0.19**
Acenaphthylene	0.067	<0.005	<0.005	<0.005	**0.25**	**0.13**	0.065	**0.09**	<0.05
Anthracene	0.15	<0.01	<0.01	<0.01	**1.8**	**1.2**	**0.45**	**0.26**	**0.32**
Benz(a)anthracene	0.38	<0.01	<0.01	<0.01	**4.4**	**3.5**	**1.9**	**0.76**	**1.1**
Benzo(a)pyrene	0.43	<0.01	<0.01	<0.01	**3.3**	**3.4**	**1.2**	**2.01**	**1.2**
Benzo(b)fluoranthene	NC	<0.01	<0.01	<0.01	8.3	7.7	3.1	3.28	1.86
Benzo(g,h,i)perylene	NC	<0.01	<0.01	<0.01	1.4	1.4	0.64	1.32	0.7
Chrysene	0.48	<0.02	<0.01	<0.01	**5.3**	**5**	**2**	**1.21**	**1.32**
Dibenz(a,h)anthracene	0.071	<0.005	<0.005	<0.005	**0.37**	**0.4**	**0.13**	**0.39**	**0.19**
Fluoranthene	0.8	<0.01	<0.01	<0.01	**14**	**10**	**4.4**	**1.18**	**2.83**
Fluorene	0.083	<0.01	<0.01	<0.01	**1.4**	**0.85**	**0.28**	**0.11**	**0.19**
Indeno(1,2,3-c,d)pyrene	NC	<0.01	<0.01	<0.01	1.6	1.8	0.74	1.41	0.77
Naphthalene	0.21	<0.01	<0.01	<0.01	**11**	**0.88**	**0.23**	**0.3**	0.18
Phenanthrene	0.32	<0.01	<0.01	<0.01	**5.3**	**5**	**2**	**0.66**	**1.58**
Pyrene	0.78	<0.01	<0.01	<0.01	**12**	**9.4**	**3.5**	**3.59**	**2.75**
Conventional Parameters									
Moisture (%)		26.3	35	35.6	51.2	44.7	33.6	52.6	57.2
TOC (%)		NM	NM	NM	2.52	2.26	2	3.3	3.7
pH		8.3	8.2	8.5	8.2	8.2	8.4	8.01	8.18
AVS		NM	NM	NM	105	60.8	35.5	138	58.9
SEM		NM	NM	NM	2.3	1.1	1.8	10.4	19.6
AVS-SEM		NM	NM	NM	102.7	59.7	33.7	127.6	39.3
Percent Gravel		0	0	0	5.1	20.4	13.9	4.9	1
Percent Sand		70.1	13.6	45.3	40.6	56.3	66.3	30.5	27.6
Percent Silt		24.9	77.5	47.7	30.8	12.3	9.1	32.6	37.8
Percent Clay		5	8.9	7	23.5	11	10.7	32	33.6

APPENDIX A	SED QC_{SCS}	2C	2D	2E	2F	2G	3A	3B	3C
Metals (mg/kg)									
Aluminum	NC	15200	12200	13400	16800	17500	28200	26400	26000
Antimony	NC	14	14	11	29	71	134	25	14
Arsenic	25	**27**	**28**	14	**30**	**30**	**1210**	**105**	**60**
Barium	NC	102	105	66	194	326	163	185	165
Cadmium	2.4	1.8	1	1.5	1.3	1.8	**10**	**3.1**	**2.5**
Chromium	110	37	28	28	53	81	**137**	92	95
Cobalt	NC	9	8	8	12	17	21	9	9
Copper	65	**327**	**246**	**184**	**904**	**907**	**2480**	**341**	**688**
Iron	NC	36700	26800	28500	56300	68000	185000	63800	63000
Lead	70	**101**	**78**	69	**121**	**130**	**1460**	**398**	**342**
Manganese	NC	318	243	267	426	716	1310	419	400
Mercury	0.42	**0.42**	0.23	0.22	0.33	0.26	**0.48**	**0.71**	**6.35**
Molybdenum	NC	4	<4	<4	5	11	47	8	7
Nickel	NC	25	17	18	27	34	40	34	35
Selenium	NC	<2	<2	<2	<2	<2	<4	<2	<2
Silver	NC	<2	<2	2	<2	<2	3.2	1.9	1.6
Strontium	NC	77.2	98.9	73.1	138	292	586	331	445
Thallium	NC	<50	<50	<50	<50	<50	<1	<1	<1
Tin	NC	8	7	6	40	78	90	17	14
Titanium	NC	714	554	671	653	688	1030	1210	1180
Vanadium	NC	64	52	57	61	67	139	86	83
Zinc	200	**460**	**466**	**325**	**1440**	**2460**	**12200**	**2310**	**1590**
PAH (mg/kg)									
Acenaphthene	0.048	**0.16**	**0.12**	**0.08**	**0.48**	**0.3**	**0.05**	**0.11**	**0.18**
Acenaphthylene	0.067	<0.05	<0.05	<0.05	<0.05	<0.05	**0.10**	**0.21**	**0.19**
Anthracene	0.15	**0.27**	**0.24**	0.13	**0.54**	**0.35**	**0.26**	**0.45**	**0.56**
Benz(a)anthracene	0.38	**0.59**	**0.4**	**0.4**	**1.64**	**1.03**	**0.68**	**1.18**	**1.23**
Benzo(a)pyrene	0.43	**0.7**	0.36	**0.43**	**1.69**	**1.01**	**0.82**	**1.53**	**1.59**
Benzo(b)fluoranthene	NC	1.03	0.55	0.7	2.58	1.57	1.32	2.77	3.01
Benzo(g,h,i)perylene	NC	0.42	0.21	0.27	1.01	0.57	0.53	0.94	1.00
Chrysene	0.48	**0.71**	0.47	**0.52**	**2.06**	**1.36**	**0.95**	**2.02**	**2.76**
Dibenz(a,h)anthracene	0.071	**0.11**	0.07	0.07	**0.27**	**0.15**	**0.14**	**0.23**	**0.22**
Fluoranthene	0.8	**1.28**	**1.02**	**0.91**	**4.22**	**3.13**	**1.08**	**3.04**	**4.55**
Fluorene	0.083	**0.15**	**0.15**	**0.09**	**0.47**	**0.28**	**0.07**	**0.14**	**0.2**
Indeno(1,2,3-c,d)pyrene	NC	0.45	0.24	0.29	1.11	0.64	0.61	1.01	1.14
Naphthalene	0.21	**0.31**	0.14	0.17	**0.36**	**0.28**	0.17	**0.34**	**0.62**
Phenanthrene	0.32	**1**	**1**	**0.61**	**3.7**	**2.13**	**0.44**	**1.12**	**2.19**
Pyrene	0.78	**1.65**	**1**	**1.11**	**3.97**	**2.51**	**1.53**	**3.56**	**4.70**
Conventional Parameters									
Moisture (%)		47	39	47	52.1	58.2	56.4	59.6	60.5
TOC (%)		2.55	2.5	3.27	2.5	3.4	4.61	5.5	6.7
pH		7.94	8.09	7.84	8.16	8.28	7.69	7.66	7.71
AVS		31	33.2	34.7	111	142	178	116	102
SEM		3.4	4.4	5.1	14.9	49.9	97.1	31.4	19.3
AVS-SEM		27.6	28.8	29.6	96.1	92.1	81.0	84.6	82.7
Percent Gravel		0.9	5.4	0.8	1	0.1	4.7	9.1	32.5
Percent Sand		44.8	68.3	61.4	44.8	31.6	55.6	25.7	23.2
Percent Silt		31.4	13.9	21.4	28.5	36.9	16.9	29.8	18.7
Percent Clay		22.9	12.4	16.4	25.7	31.4	22.8	35.4	25.6

APPENDIX A	SED QC_{SC}	3D	3E	3F	3G	3H	4A	4B	4C	4D
Metals (mg/kg)										
Aluminum	NC	28000	29700	35900	32900	27600	NM	NM	NM	NM
Antimony	NC	<10	<10	<10	<10	<10	16	11	<10	<10
Arsenic	25	20	15	18	16	12	22	19	19	17
Barium	NC	124	129	156	143	119	82	87	173	162
Cadmium	2.4	1.9	1.5	1.8	1.7	1.4	1.0	0.8	1.6	**2.6**
Chromium	110	52	50	59	55	44	36	38	56	48
Cobalt	NC	9	9	11	10	9	7	7	10	8
Copper	65	**205**	**127**	**142**	**132**	**97**	**78**	**69**	**130**	**84**
Iron	NC	36900	43300	46600	44800	37400	NM	NM	NM	NM
Lead	70	**121**	**102**	**125**	**122**	**96**	**130**	**216**	**232**	**142**
Manganese	NC	350	360	420	403	343	NM	NM	NM	NM
Mercury	0.42	0.39	0.38	**0.50**	**0.48**	**0.46**	**0.46**	**0.45**	**0.47**	0.36
Molybdenum	NC	<4	<4	<4	<4	<4	<4	<4	<4	13
Nickel	NC	31	31	35	33	27	23	20	30	27
Selenium	NC	<2	<2	<2	<2	<2	<2	<2	<3	<2
Silver	NC	1.5	1.5	1.7	1.6	1.1	<2	<2	<2	<2
Strontium	NC	550	341	195	202	151	NM	NM	NM	NM
Thallium	NC	<1	<1	<1	<1	<1	<1	<1	<1	<1
Tin	NC	5	6	7	6	6	6	<5	9	13
Titanium	NC	1240	1300	1570	1490	1300	NM	NM	NM	NM
Vanadium	NC	78	77	91	85	72	54	51	88	97
Zinc	200	**546**	**329**	**403**	**440**	**289**	**220**	**255**	**270**	**218**
PAH (mg/kg)										
Acenaphthene	0.048	**0.11**	**0.15**	**0.13**	**0.12**	**0.11**	**0.38**	**0.07**	**0.09**	**0.13**
Acenaphthylene	0.067	**0.15**	**0.14**	**0.18**	**0.18**	**0.19**	**0.3**	**0.08**	**0.14**	**0.11**
Anthracene	0.15	**0.35**	**0.38**	**0.39**	**0.39**	**0.32**	**1.2**	**0.21**	**0.46**	**0.74**
Benz(a)anthracene	0.38	**1.08**	**0.80**	**0.76**	**0.93**	**0.77**	**3.11**	**0.4**	**1.03**	**0.86**
Benzo(a)pyrene	0.43	**1.07**	**1.02**	**1.05**	**1.28**	**1.00**	**3.4**	0.42	**0.92**	**0.77**
Benzo(b)fluoranthene	NC	1.59	1.31	1.37	1.66	1.17	4.76	0.64	1.38	1.34
Benzo(g,h,i)perylene	NC	0.58	0.69	0.70	0.80	0.59	1.78	0.21	0.41	0.39
Chrysene	0.48	**1.41**	<0.9	<0.8	<1	<0.8	**3.99**	**0.48**	**1.15**	**1.27**
Dibenz(a,h)anthracene	0.071	**0.14**	**0.16**	**0.16**	**0.19**	**0.15**	**0.52**	**0.08**	**0.15**	**0.2**
Fluoranthene	0.8	**1.73**	**1.47**	**1.41**	**1.68**	**1.25**	**7.06**	**0.8**	**2.12**	**2.99**
Fluorene	0.083	**0.14**	**0.19**	**0.21**	**0.2**	**0.16**	**0.45**	**0.09**	**0.13**	**0.21**
Indeno(1,2,3-c,d)pyrene	NC	0.63	0.69	0.69	0.78	0.6	2.09	0.23	0.47	0.44
Naphthalene	0.21	**0.32**	**0.39**	**0.57**	**0.48**	**0.43**	**0.64**	0.16	**0.24**	**0.46**
Phenanthrene	0.32	**0.80**	**1.11**	**0.92**	**1.16**	**0.69**	**4.73**	**0.48**	**0.84**	**0.67**
Pyrene	0.78	**2.60**	**1.98**	**2.14**	**2.41**	**2.06**	**7.93**	**1.15**	**2.53**	**2.79**
Conventional Parameters										
Moisture (%)		62	55.8	54.8	51.9	46.7	37.1	38	48.2	72.1
TOC (%)		4.7	3.5	2.9	2.8	2.2	1.96	1.08	1.8	10.6
pH		7.94	8.02	7.86	7.91	7.87	8.16	8.04	7.9	7.39
AVS		78.8	45.4	48.7	64	30.2	28.2	25.9	21.6	114
SEM		8.4	4.8	5.2	5.1	4.1	2.9	3.4	3.5	2.8
AVS-SEM		70.4	40.6	43.5	58.9	26.1	25.3	22.5	18.1	111.2
Percent Gravel		7	9.3	2.3	2.5	1.1	0.5	1.8	11.2	13.6
Percent Sand		24.2	25	23.1	24.2	43.9	76.8	67.1	42.4	30.4
Percent Silt		28.8	28.6	37.4	39.8	29.4	16.3	20.7	30.8	35.2
Percent Clay		40	37.1	37.2	33.5	25.6	6.4	10.4	15.6	20.8