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EFFECTS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN SEDIMENTS FROM LAKE WASHINGTON ON FRESHWATER BIOASSAY ORGANISMS AND BENTHIC MACROINVERTEBRATES

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ABSTRACT

To support ongoing efforts to develop freshwater sediment quality criteria, sediments from Lake Washington, which are contaminated with high concentrations of polycyclic aromatic hydrocarbons (PAHs), were tested against several bioassays.

Eleven sediment samples were collected at eight sites adjacent to Quendall Terminals and the J.H. Baxter site in Lake Washington for chemical analyses, bioassays, and benthic macroinvertebrate identification and enumeration. Laboratory bioassays performed on the sediments included *Daphnia magna, Ceriodaphnia dubia, Hyalella azteca, Chironomus tentans, Hexagenia limbata*, ostracods, and Microtox®. Benthic invertebrate samples were identified to the genus and species level where possible.

Total PAH concentrations ranged from 3.6 to 33,000 mg/kg dry weight. *Hyalella azteca* showed statistically significant reduction in survival at the four most contaminated sites. One Microtox® series showed impairment correlated with increasing PAH concentrations. However, a second series run at a different lab and using a different procedure, showed no such correlation. No other bioassay showed a significant reduction in survival that corresponded to contaminant levels. With one exception, diversity or abundance of benthic macroinvertebrate communities showed no clear relationship to concentrations of contaminants.

PAH concentrations were normalized to total organic carbon (TOC) and compared to the Provincial Sediment Quality Guidelines established by the Ontario Ministry of the Environment (OMOE). Significantly reduced survival of *Hyalella azteca* occurred at all four sites which exceeded the severe effect level of 11,000 mg PAH/kg TOC. Microtox® was used at three of these sites and indicated toxicity at all of them. Benthic diversity was examined at two of these sites and was shown to be reduced at one of them.

INTRODUCTION

The Washington State Department of Ecology (Ecology) is currently in the process of developing sediment chemical criteria and selecting bioassays to evaluate the toxicity of freshwater sediments to aquatic biota. When adopted, these criteria will be used to regulate the discharge of pollutants and guide sediment cleanup activities in freshwater systems statewide. The focus of this study was to evaluate the toxicity of sediments contaminated with polynuclear aromatic hydrocarbons (PAH). As the major component of creosote, PAHs are considered the most important class of hydrocarbons in creosote contaminated sediments because of their toxicity, persistence, and potential linkage to carcinogenicity and mutagenicity in susceptible organisms (Waterways Experiment Station, 1990).

Two approaches are available to evaluate sediment toxicity. The triad approach (Long and Chapman, 1985), which measures bioassay response, benthic community structure, and contaminant concentrations, was used at a contaminated site in Lake Union (Yake *et al.*, 1986). This approach found toxicity associated with PAH. Sediments are often tested with several bioassays, using what is termed the "battery-of-tests" approach, because various organisms may respond differently to types and levels of contaminants. These two approaches were employed in this study to determine the effects of PAH on the aquatic environment.

PAH contaminated sediments in Lake Washington adjacent to Quendall Terminals and the J.H. Baxter site were selected for use in this study. Quendall Terminals and the J.H. Baxter site are adjoining parcels of land located along the southeast shoreline of Lake Washington near the City of Renton (Figure 1). Between 1917 and 1970, Quendall Terminals was the site of a small refinery that produced creosote and other distillates from various tars. The J.H. Baxter property was the site of a wood treating facility. Substantial contamination of ground water, on-site soils, and Lake Washington sediments adjacent to these sites has been documented in previous studies (Woodward-Clyde, 1989; USEPA, 1984; and Norton, 1991 and 1992).

The primary objectives of the present study were to:

- Analyze sediments at Quendall Terminals and the J.H. Baxter site for PAH and ancillary parameters,
- evaluate PAH toxicity through the use of several bioassays,
- determine what effects PAH have on the distribution and numbers of benthic organisms, and
- apply these data to help determine PAH criteria for freshwater sediments in Washington State.

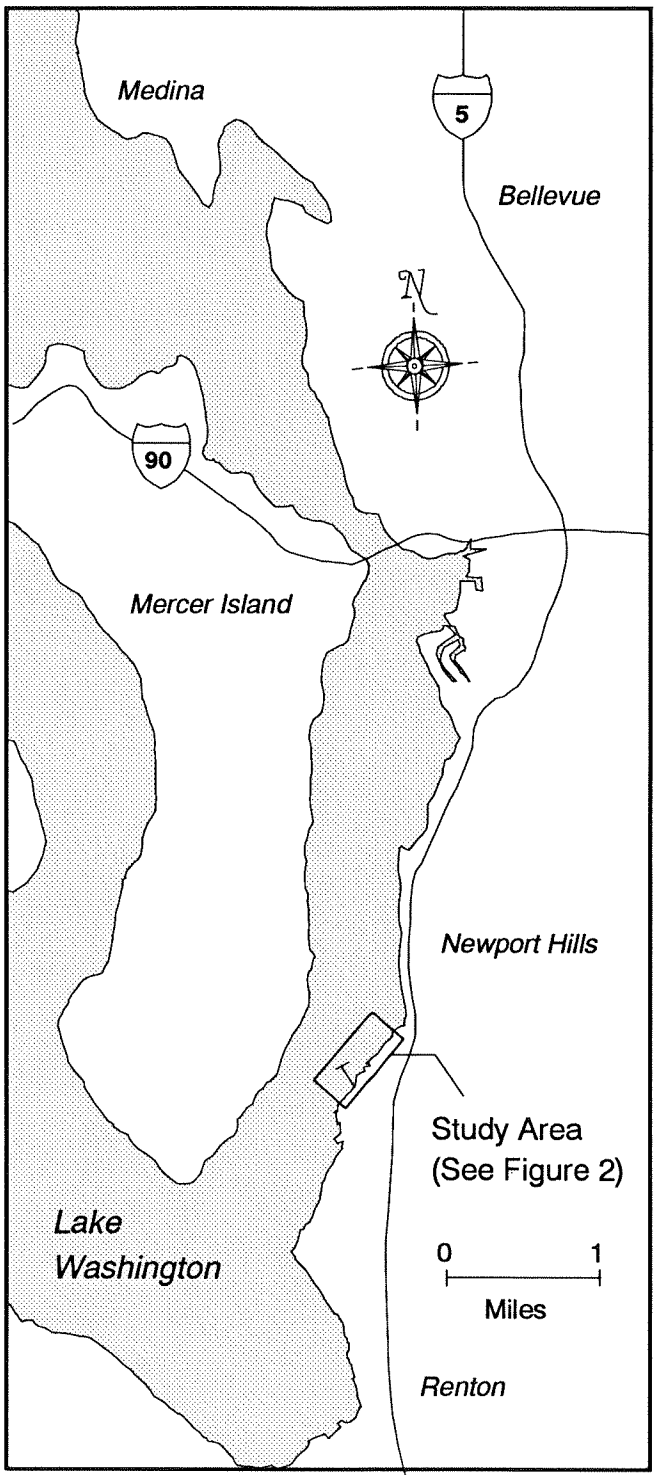

Figure 1: Vicinity Map of Quendall Terminals and the J.H. Baxter Site.

METHODS

Study Design

Sediments adjacent to Quendall Terminals and the J.H. Baxter site were selected for use in this study for the following reasons:

- Sediment contamination was limited primarily to PAHs.
- Information was available from previous and ongoing studies at the sites that documented both the spatial extent of contamination and its magnitude.

Sites were selected for chemical and biological sampling to cover a range of anticipated PAH concentrations. Sampling was conducted in three phases, and designed to build on knowledge obtained during previous sampling phases. Sample sites are shown in Figure 2. Descriptive information for each site is also listed in Table 1. All samples were collected by Ecology's Environmental Investigations and Laboratory Services (EILS) Program.

The first set of samples was collected on May 16-17, 1990. Data from four sites sampled for chemistry, bioassay, and benthic macroinvertebrate analyses are used in the present study. Two of the sites (Norton, 1991) were from areas with low contaminant levels, and two were from areas with high contaminant levels.

Phase II sediments were collected on February 27, 1991, from three contaminated sites and one reference site. The reference site was located northeast of the Quendall Terminals and J.H. Baxter site. The contaminated sites were located in the vicinity of the abandoned T-pier (Figure 2).

Phase III sediments were obtained on June 6, 1991, to investigate extremely high contaminant levels in a small cove at the southern end of the J.H. Baxter property.

Sample Collection

Sediment samples for chemical analyses and bioassays were collected with a 0.1 m^2 stainless steel van Veen grab. To minimize contamination between sites, samples were collected in order of anticipated increasing contaminant levels. In addition, the grab sampler was thoroughly rinsed with lake water between stations. For each grab sample, the overlying water was removed. Only the top two centimeters of sediment (not in contact with the sides of the sampler) were retained for analysis. Several grabs were composited into a 4 gallon stainless steel bucket at each station. The contents of the bucket were gently homogenized with a stainless steel spoon and dispensed to glass sample containers (I-Chem Series 300). All other sediment handling equipment was cleaned in Liquinox® detergent and rinsed sequentially in hot tap water, 10 percent nitric acid, distilled water, and pesticide grade acetone. All samples were labeled, refrigerated, and shipped to the laboratories within 48 hours of collection.

Table 1. Descriptions of sampling sites at Quendall Terminals and the J.H. Baxter site.

Site Number	Collection Date	Lab Sample	Time	Depth (feet)	Latitude Deg.	Min.	Sec.	Longitude Deg.	Min.	Sec.
Phase I:										
QB1-13	5/15-16/90	208292	1515	11	47	32	00	122	12	07
QB1-15	"	208296	1030	9	47	32	6	122	12	1
QB1-1 (Ref.)	"	208280	1045	40	47	32	9	122	12	16
QB1-2 (Ref.)	"	208281	1148	40	47	32	17	122	12	27
Phase II:										
QB2-5	2/27/91	098021	1300	20	47	32	04	122	12	10
QB2-12	"	098022	1500	13	47	31	58	122	12	08
QB2-13	"	098023	1700	6	47	32	00	122	12	07
QB2-3 (Ref.)	"	098020	1100	19	47	32	16	122	11	52
Phase III:										
QB3-1	6/6/91	238043	1200	2	47	32	08	122	11	97
QB3-2	"	238042	1135	2	47	32	08	122	11	99
QB3-R (Ref.)	"	238040	1015	40	47	32	17	122	12	27

(Ref.) = Reference site, approximate location.

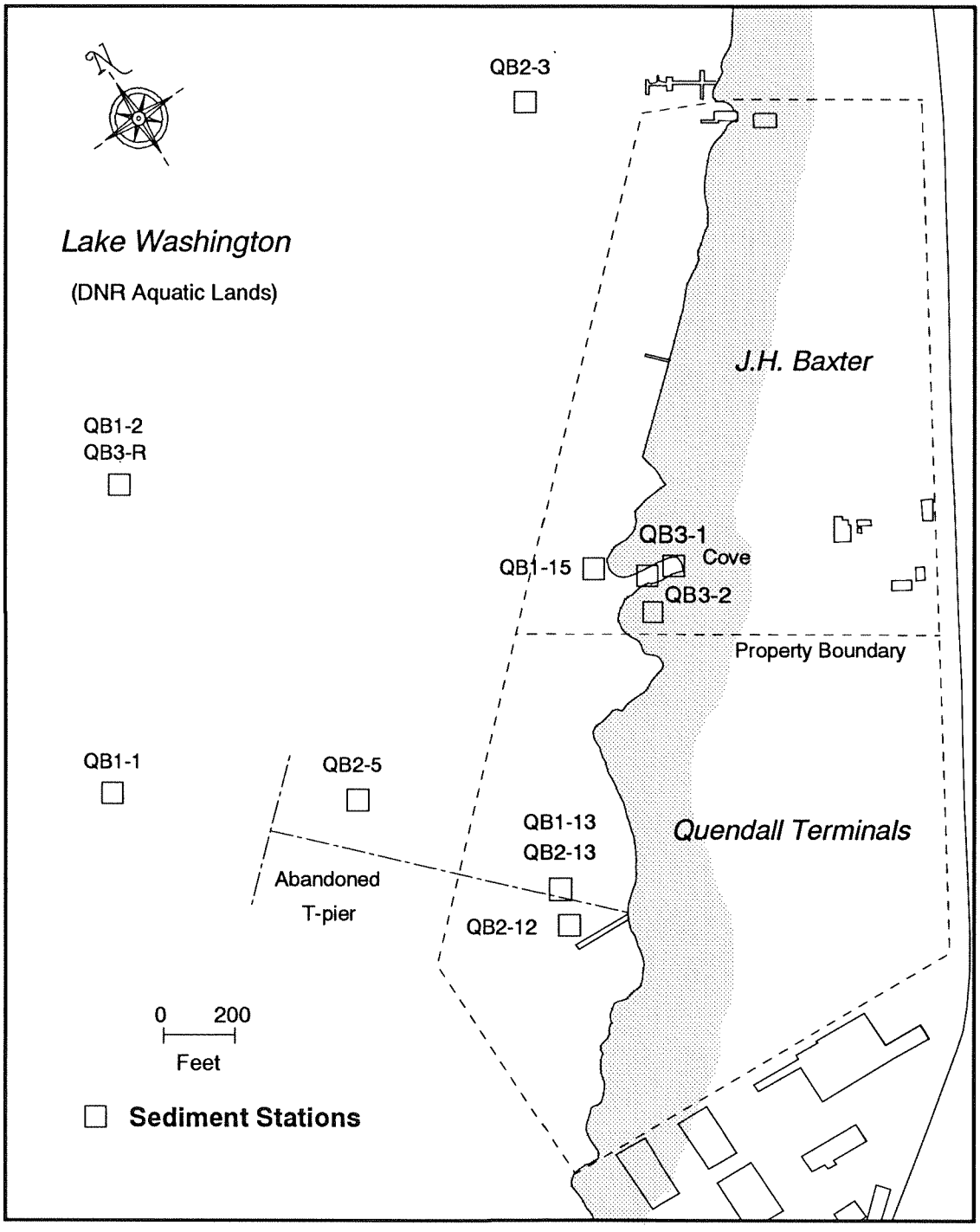

Figure 2: Sediment Sample Locations, Quendall Terminals and the J.H. Baxter Site, Lake Washington.

Benthic macroinvertebrates were obtained with a 0.02 m^2 stainless steel petite Ponar grab. After retrieval, the entire contents of the grab were washed through a 30-mesh (0.589 mm) screen. Retained material from each grab was placed in separate 1-quart glass jars and preserved with 10 percent formalin solution. Four replicate samples were collected at each station. After a minimum of 2 weeks, the formalin solution was replaced with 70 percent ethanol. Samples were submitted to commercial labs for identification and enumeration.

Analytical and Bioassay Procedures

A summary of the analyses performed at each station is listed in Table 2. Analytical methods and laboratories used in this study are shown in Table 3. Besides the chemical analyses, several bioassays were performed to evaluate sediment toxicity. Methods for each bioassay and the benthic macroinvertebrate identifications are described below. All bioassay responses, except Microtox®, were tested for significance with comparison to a laboratory control sample with Dunnett's Test. The Wright State University bioassay report is given in Appendix A (Burton, 1991b). The Minnesota State University bioassay report is given in Appendix B (Henry *et al.*, 1991).

Daphnia magna

D. magna, a cladoceran or "water flea," is a water column organism that feeds on the sediment surface (Burton, 1991a). This organism is widely used in effluent studies, and is now widely used in tests adapted for sediment. The procedure used here is the 48-hour static acute test. Tests followed methods specified by the American Society for Testing and Materials (ASTM, 1990).

Ceriodaphnia dubia

C. dubia, a cladoceran which is closely related to *D. magna*, is widely used to test wastewater effluent and has also been adapted to sediment work. Its susceptibility to toxic effects is dependent on contaminant transfer from sediment to the water column. The chronic test used the three brood, 7-day reproduction procedure which measures the production and survival of young. Tests followed methods in ASTM E1383-90 (ASTM, 1990).

Hyalella azteca

H. azteca is an amphipod which spends time both in the water column and burrowing in upper sediment layers. It is frequently used to determine freshwater sediment toxicity (Nebeker and Miller, 1988). The procedure employed measures 14-day survival and was conducted according to ASTM E1383-90 (ASTM, 1990).

Table 2. Summary of analyses performed on Phase I, II, and III samples at Quendall Terminals and the J.H. Baxter site.

	Phase I				Phase II				Phase III		
Site Number > >	13	15	1 (Ref.)	2 (Ref.)	5	12	13	3 (Ref.)	1	2	3 (Ref.)
Conventionals:											
Grain Size	x	x	x	x	x	x	x	x	x	x	x
Percent Solids	x	x	x	x	x	x	x	x	x	x	x
TOC	x	x	x	x	x	x	x	x	x	x	x
Sulfides	x	x	x	x	x	x	x	x	-	-	-
NH3	-	-	-	-	x	x	x	x	-	-	-
Organics:											
PAH	x	x	x	x	x	x	x	x	x	x	x
Pentachlorophenol	x	x	x	x	x	x	x	x	x	x	x
Others	x	x	x	x	x	x	x	x	x	x	x
Bioassays:											
D. magna (acute)	x	x	x	x	x	x	x	x	-	-	-
C. dubia (chronic)	-	-	-	-	x	x	x	x	-	-	-
H. azteca (acute)	x	x	x	x	x	x	x	x	x	x	x
C. tentans (acute/chronic)	-	-	-	-	x	x	x	x	-	-	-
H. limbata (acute)	-	-	-	-	x	x	x	x	-	-	-
Ostracods	-	-	-	-	x	x	x	x	-	-	-
Microtox	-	-	-	-	x	x	x	x	x	x	x
Benthic Macroinvertebrates:											
Identify, Analyze	x	x	x	x	x	x	x	x	-	-	-

(Ref.) = Reference site.

Table 3. Summary of analytical methods and laboratories used in Quendall Terminals and the J.H. Baxter site studies.

Analysis	Method	Reference	Laboratory
Conventionals:			
Percent Solids	Dry @ 104 C	PSEP, 1987	Soil Technology, Inc.
Grain Size	Sieves and Pipettes	"	"
TOC	Combustion CO2, EPA 415.2	"	Amtest, Inc.
Sulfides	Titrimetric, EPA 376.1	"	"
Ammonia	Colorimetric, EPA 350.1	"	"
Organics:			
PAH	GC/MS, EPA 8270	USEPA, 1986	Manchester Environmental Lab
Pentachlorophenol	GC/ECD, EPA 8150	"	"
Biology:			"
Daphnia magna	Solid phase, 48-hour acute	ASTM, 1990	"
Hyalella azteca	Solid phase, 14-day acute	"	"
Ceriodaphnia dubia	Solid phase, 7-day chronic	"	Wright State University
Hexagenia limbata	Solid phase, 10-day acute	Nebeker et al., 1984 and Fremling and Mauck, 1980	"
Chironomus tentans	ASTM: E 729-88a	Mosher et al., 1982 and ASTM, 1989	Univ. of Minnesota
Microtox	Standard Assay	PSEP, 1987	"
Microtox	100% Assay Procedure	Microbics, Inc., 1989	"
Ostracods	Acute	Woodward et al. (In Draft)	Oak Ridge National Labs
Benthic Infauna:			
Identify, enumerate	Enumerate and Identify	-	Western Aquatic Institute

Chironomus tentans

The larva of *C. tentans*, a true fly, is a benthic organism frequently used for sediment toxicity tests because of its burrowing characteristics. The procedures used here, which consisted of 10-day survival (acute) and percent weight reduction (chronic) tests, can be found in Henry *et al.*, (1991).

Hexagenia limbata

H. limbata is a burrowing mayfly nymph (Order Ephemeroptera). *Hexagenia* is unavailable during May and June and has not been successfully raised in the laboratory. Contaminant sensitivity can vary depending on organism age (Morse, Personal Communication). Henry *et al.* (1991) reports procedures for the 10-day survival test used here.

Ostracods

Ostracods (seed shrimps) have been used in sediment bioassays. The Ostracod used here, *Cyprinotus incongruens*, is primarily a surface feeder. However, it also feeds just below the sediment surface by means of shallow burrowing. Therefore, this organism is potentially exposed to contaminants through both direct contact and ingestion. Test procedures followed the methods of Woodward *et al.* (1992, In Draft).

Microtox®

Microtox® bioassays were originally designed for marine water samples, but have been adapted to freshwater sediment studies through the use of liquid extraction techniques. The Microtox® procedure is based on the reduction in the level of light produced by the bioluminescent marine bacterium, *Photobacterium phosphoreum*, caused by enzyme inhibition following exposure to a toxicant. Results are reported as Effective Concentrations 50 percent (EC_{50}) defined as the percent of test material which, when mixed with a control, reduces the light output by 50 percent. EC_{50} values $\geq 100\%$ indicate very low toxicity.

Manchester Environmental Laboratory used the Standard Assay method outlined in the Puget Sound Protocols (Puget Sound Estuary Program, 1987). This procedure is designed for samples with high toxicity, and the initial analysis uses a sample concentration below 50 percent. The University of Minnesota used a variation, the 100 percent Assay Procedure, outlined in the Microtox® analysis manual (Microbics, Inc., 1989). This procedure is designed for use with samples with low or unknown toxicity, and the initial analysis uses a sample concentration between 50 and 100 percent.

Quality Assurance/Quality Control

Data quality was assessed by analysis of method blanks, internal standards, surrogate spikes, duplicate matrix spikes, and blind field duplicates. Overall data precision was estimated based on the relative percent difference (RPD) between duplicate analyses. Data from all three phases were checked prior to inclusion in this report and, except where noted, were considered acceptable.

Analytical results for Phase I were considered acceptable with the exception of sulfides and, to a lesser extent, grain size and mercury analyses. Quality assurance/quality control data for Phase I samples were reviewed in detail by Norton (1991).

For Phase II, matrix spike recovery data for PAH and other organics are given in Table 4. Only benzo(g,h,i)perylene fell outside the 50 to 150 percent control limits for spike recovery recommended by PSEP. RPD values were as follows: organics ($\pm$24%), NH_3 ($\pm$15%), sulfide ($\pm$9.5%), total organic carbon (TOC) ($\pm$24%), total solids ($\pm$1.3%), and grain size ($\pm$29%). These values indicate that there were no major problems encountered in the analysis of these samples. For bioassay data, a definitive statement regarding the *Ceriodaphnia dubia* young production test cannot be made because of high variances. The *Daphnia magna* response to the reference toxicant was nonlinear and could not be used to determine an lethal concentration-50 (LC_{50}). Unless noted, all other data are acceptable for use without limitation.

Phase III data were considered acceptable, although precision for the chlorophenols was relatively low. Again, a complete review of quality assurance data is presented in Norton (1992).

RESULTS

Conventionals

Results of conventional analyses of sediments from Phase I, II, and III are given in Table 5. Grain size distribution of the Quendall Terminals and J.H. Baxter site sediments is given in Figure 3. Sediments from the reference areas, usually located furthest off-shore, were predominantly silt. Sediments from the areas of the T-pier and the cove contained larger percentages of sand than the reference samples. Most samples were low in clay ($\leq$21%), although one sample from the cove was somewhat higher with 36 percent clay.

In Phase I samples, TOC ranged from 7.7 to 15 percent for the contaminated sites and from 3.6 to 6.4 percent for the reference sites. Sulfide was detected only at the site near the T-pier, which had a value of 45 mg/kg dry weight. Ammonia was not measured for these samples. Lower TOC values were measured during Phase II, averaging about 2 percent. The reason for this considerable difference from the Phase I TOC values is unexplained. Sulfide at the

Table 4. Results of matrix spike recovery tests for organics from Quendall Terminals and the J.H. Baxter site.

	Spike Recovery Accuracy and Precision		
Type > >	Sediment	Sediment	
Lab No. > >	098020	098020	RPD
LPAH			
Acenaphthene	72%	65%	10.2%
Acenaphthylene	72%	67%	7.2%
Naphthalene	64%	60%	6.5%
Fluorene	75%	73%	2.7%
Anthracene	64%	62%	3.2%
Phenanthrene	66%	67%	1.5%
HPAH			
Fluoranthene	71%	67%	5.8%
Benzo(a)anthracene	93%	85%	9.0%
Chrysene	81%	81%	0%
Pyrene	77%	69%	11%
Benzo(b)fluoranthene	74%	67%	9.9%
Benzo(k)fluoranthene	55%	64%	15%
Benzo(a)pyrene	57%	58%	1.7%
Dibenzo(a,h)anthracene	65%	66%	1.5%
Indeno(1,2,3-cd)pyrene	66%	58%	13%
Benzo(g,h,i)perylene	14%	11%	24%
OTHER			
Carbazole	NAR	NAR	-
Dibenzofuran	75%	68%	9.8%
1-Methylnaphthalene	NAR	NAR	-
2-Methylnaphthalene	53%	49%	7.8%
2-Chloronaphthalene	72%	68%	5.7%
Pentachlorophenol	68%	65%	4.5%

RPD = Relative percent difference = (S1-S2)/((S1+S2)/2)*100.
NAR = No analytical result.

Table 5. Results of conventionals analyses of Phase I, II, and III sediments from Lake Washington.

Site Number	Percent Solids	TOC Percent	Sulfide mg/kg dry	NH3 mg/kg dry	Percent Gravel	Percent Sand	Percent Silt	Percent Clay	Description
Phase I:									
QB1-13	29	7.7	45	-	0	47	35	18	Sandy, silty sediment with some clay, oily sheen present
QB1-15	21	15	4.1 U	-	2	49	30	19	Sandy, silty sediment with some clay
QB1-1 (Ref.)	27	3.6	2.1 U	-	0	11	68	21	Silty sediment, some sand and clay
QB1-2 (Ref.)	26	6.4	6.5 U	-	1	25	59	15	Silty sediment, some sand and clay
Phase II:									
QB2-5	18	1.7	290	230	5	31	54	10	Thin, black, some organic matter
QB2-12	26	2.2	69	300	0	46	46	8	Thin, dark brown, sandy- oily sheen at surface
QB2-13	31	1.3	26	230	1	65	28	6	Thin, dark brown, clumpy- organic matter and oily sheen
QB2-3 (Ref.)	19	2.0	110	350	0	22	57	21	Dark brown, medium coarse
Phase III:									
QB3-1	27	8.3	-	-	8	38	38	16	Dark brown sediment with large pieces organic matter and oily sheen
QB3-2	24	10	-	-	2	12	50	36	Dark brown sediment with some organic matter
QB3-R (Ref.)	22	5.7	-	-	0	24	59	17	Uniform, sandy, light brown sediment

Gravel = >2mm, Sand = 2mm-62um, Silt = 62-4um, Clay = <4um.

(-) Not analyzed.

U = Not detected at detection limit shown.

(Ref.) = Reference site.

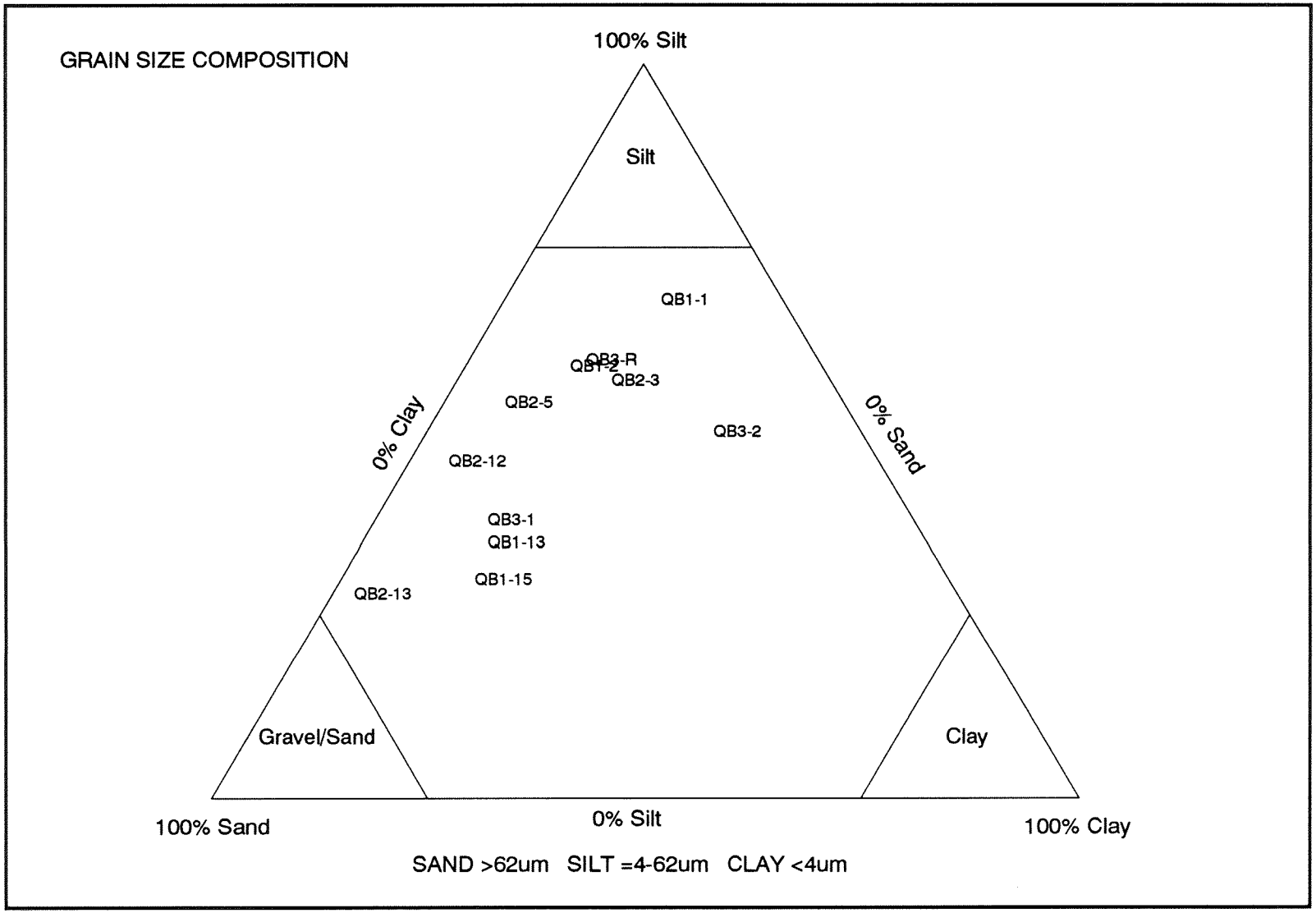

Figure 3: Grain Size Composition of Sediments from Quendall Terminals and the J.H. Baxter Site.

contaminated sites was quite variable (26-290 mg/kg dry weight), while the reference sediment was intermediate at 110 mg/kg dry weight. Ammonia (as NH_3), a potential toxicant, was high at all sites and ranged from 230-350 mg/kg dry weight. TOC averaged about 9 percent in the cove and 5.7 percent at the reference site in Phase III samples.

Organics

The results of organics analyses of sediments from Phase I, II, and III are summarized in Tables 6, 7, and 8. The spacial extent of sediment contamination in Lake Washington adjacent to Quendall Terminals and the J.H. Baxter site is discussed in detail by Norton (1991 and 1992). Since the primary focus of the present study is to evaluate bioassay data in relation to PAH concentrations, only a brief discussion of the organics results will be presented here.

In Phase I, the highest total PAH concentrations were measured near the abandoned T-pier with a maximum of 1200 mg/kg dry weight. The reference sites were both low in PAH, the minimum being 4.5 mg/kg dry weight. In Phase II, areas along the abandoned T-pier had the highest concentrations of total PAH with a maximum of 350 mg/kg dry weight. Concentrations of total PAH at the reference site were much lower with 7.0 mg/kg dry weight. Sediments within the J.H. Baxter cove contained the highest total PAH concentrations measured near the Quendall Terminals and J.H. Baxter site. Phase III total PAH concentrations ranged from 3.6 mg/kg dry weight at the reference site to 33,000 mg/kg dry weight in the cove.

Besides PAH, another compound found consistently at all sites was retene, a naturally occurring resin acid-derived compound (Prahl and Carpenter, 1984). Retene in these sediments probably has its source in the log raft operations at the site. Retene concentrations ranged from 0.52 to 810 mg/kg dry weight. Low levels of pentachlorophenol (PCP) were also detected (0.02-16 mg/kg dry weight).

Bioassays

Bioassay results, along with sediment PAH concentrations, are shown in Table 9. Results for individual bioassays are discussed below.

Daphnia magna and *Ceriodaphnia dubia*

No samples produced significant reduction in survival of *D. magna*. *C. dubia* showed neither significant reduction in survival, nor significant reduction in production of young at any site tested.

Hyalella azteca

H. azteca showed significant toxicity at the four sites with the highest total PAH concentrations. Two of those sites, with 48 and 32 percent survival, were from the vicinity of the abandoned T-pier.

Table 6. Summary of organics analyses of Phase I sediments, Quendall Terminals and the J.H. Baxter site (mg/kg dry weight).

Station No.	QB1-13	QB1-15	QB1-1 (Ref.)	QB1-2 (Ref.)
Sample No. 20-	8292	8296	8280	8281
Naphthalene	92 J	1.3 J	0.1 J	0.1 J
Acenaphthylene	0.84 J	2.8 UJ	0.02 J	0.42 U
Acenaphthene	26	2.8 UJ	0.04 J	0.02 J
Fluorene	14 J	2.8 UJ	0.04 J	0.03 J
Phenanthrene	52 J	1.9 J	0.29 J	0.21 J
Anthracene	7.9 J	2.8 UJ	0.06 J	0.42 U
Sum LPAH	190 J	3.2 J	0.55 J	0.36 J
Fluoranthene	35 J	3.1 J	0.72	0.46
Pyrene	68 J	3.5 J	0.75	0.56
Benzo(a)anthracene	63 J	1.8 J	0.57	0.28 J
Chrysene	110 J	3.7 J	0.85	0.57
Benzo(b)fluoranthene	280 J	5.3 J	1.7	1.1
Benzo(k)fluoranthene	2.1 U	2.8 UJ	0.38 U	0.42 U
Benzo(a)pyrene	140 J	2.8 J	0.74	0.49
Indeno(1,2,3-cd)pyrene	84 J	1.7 J	0.45	0.31 J
Dibenzo(a,h)anthracene	39 J	0.78 J	0.26 J	0.42 U
Benzo(g,h,i)perylene	170 J	2.5 J	0.7	0.51
Sum HPAH	990 J	25 J	6.7 J	4.3 J
Total PAH	1200 J	28 J	7.3 J	4.7 J
2-Methylnaphthalene	42 J	2.8 UJ	0.02 J	0.02 J
1-Methylnaphthalene	64 J	2.8 UJ	0.04 J	0.03 J
2-Chloronaphthalene	2.1 UJ	2.8 UJ	0.38 U	0.42 U
Dibenzofuran	7.9 J	2.8 UJ	0.38 U	0.42 U
Carbazole	2.1 UJ	2.8 UJ	0.38 UJ	0.42 UJ
Retene	27 J	810	8.7	1.3
Pentachlorophenol	0.02	0.41	0.02	0.02

J = Estimated concentration.

U = Not detected at detection limit shown.

UJ = Estimated detection limit.

(Ref.) = Reference site.

Table 7. Summary of organics analyses of Phase II sediments, Quendall Terminals and the J.H. Baxter site (mg/kg dry weight).

Station No.	QB2-5	QB2-12	QB2-13	QB2-3 (Ref.)
Sample No. 09-	8021	8022	8023	8020
Naphthalene	0.49	4.4	18	0.12 J
Acenaphthylene	0.03 J	0.15 J	0.25 J	0.01 J
Acenaphthene	0.17 J	2.8	7.7	0.03 J
Fluorene	0.2 J	1.8	5.4	0.04 J
Phenanthrene	0.94	5.4	18	0.34 J
Anthracene	0.35 J	1.4	5.2	0.07 J
Sum LPAH	2.2 J	16 J	55 J	0.6 J
Fluoranthene	2.1	6.3	19	0.42
Pyrene	2.9	10	29	0.76
Benzo(a)anthracene	2.3	9.3	31	0.38 J
Chrysene	3.4	19	71	0.88
Benzo(b)fluoranthene	6.4	29	65	1.4
Benzo(k)fluoranthene	1.6	3.3	13	0.036 J
Benzo(a)pyrene	4.5	20	31	0.77
Indeno(1,2,3-cd)pyrene	2.7	8.3	18	0.66
Dibenzo(a,h)anthracene	0.16 U	3.5	8.9	0.2 J
Benzo(g,h,i)perylene	1.5	3.4	3.9	0.59
Sum HPAH	27 J	110 J	290 J	6.4 J
Total PAH	29 J	130 J	350 J	7 J
2-Methylnaphthalene	0.16 J	1.5	13	0.03 J
1-Methylnaphthalene	0.16 J	2.2	13	0.04 J
2-Chloronaphthalene	0.38 U	0.23 U	1 U	0.4 U
Dibenzofuran	0.12 J	0.55	2.4	0.03 J
Carbazole	2 U	1.2 U	5.4 U	2 U
Retene	50	45	18	0.52
Pentachlorophenol	0.08	0.03	0.03	0.03

J = Estimated concentration.

U = Not detected at detection limit shown.

(Ref.) = Reference site.

Table 8. Summary of organics analyses of Phase III sediments, Quendall Terminals and the J.H. Baxter site (mg/kg dry weight).

Station No.	QB3-1		QB3-2		QB3-R (Ref.)	
Sample No. 23-	8043		8042		8040	
Naphthalene	600		2300		1.5	U
Acenaphthylene	3.5	J	11	J	1.5	U
Acenaphthene	980		3900		1.5	U
Fluorene	930		3200		1.5	U
Phenanthrene	260		9500		0.19	J
Anthracene	680		890		0.06	J
Sum LPAH	5800	J	20000	J	0.25	J
Fluoranthene	1600		5200		0.41	J
Pyrene	1100		3900		0.41	J
Benzo(a)anthracene	280		890		0.35	J
Chrysene	300		950	J	0.51	J
Benzo(b)fluoranthene	120		420		0.53	J
Benzo(k)fluoranthene	43	J	140	J	0.15	J
Benzo(a)pyrene	66	J	250		0.28	J
Indeno(1,2,3-cd)pyrene	29	J	88	J	0.33	J
Dibenzo(a,h)anthracene	320	U	710	J	3.9	U
Benzo(g,h,i)perylene	22	J	310	J	0.32	J
Sum HPAH	3600	J	13000	J	3.3	J
Total PAH	9400	J	33000	J	3.6	J
2-Methylnaphthalene	460		1600		1.5	U
1-Methylnaphthalene	470		1700		1.5	U
2-Chloronaphthalene	130	U	360		1.5	U
Dibenzofuran	580		2200		1.5	U
Carbazole	450		480		7.9	U
Retene	130	U	360		35	
Pentachlorophenol	16		0.39	J	0.03	U

J = Estimated concentration.

U = Not detected at detection limit shown.

(Ref.) = Reference site.

Table 9. Bioassay results from Phase I, II, and III sampling. Acute bioassays list percent survival. For chronic bioassays, see notes.

Site Number	Sediment LPAH, mg/kg Dry	Sediment LPAH, mg/kg TOC	Sediment HPAH, mg/kg Dry	Sediment HPAH, mg/kg TOC	*D. magna* (acute)	*C. dubia* (chronic)		*H. azteca* (acute)	*C. tentans* (chronic)	*C. tentans* (acute)	*H. limbata* (acute)	Microtox* EC50		Microtox** EC50	
Phase I:															
QB1-13	190	2,600	990	13,000	98	–	–	48	–	–	–	–	–	–	–
QB1-15	3.2	13	25	100	97	–	–	88	–	–	–	–	–	–	–
QB1-1 (Ref.)	0.6	15	7	190	98	–	–	70	–	–	–	–	–	–	–
QB1-2 (Ref.)	0.4	6	4	70	94	–	–	88	–	–	–	–	–	–	–
Phase II:															
QB2-5	2.2	130	27	1,600	95	90	18.5 (7.5)	75	50	53	100	19	21	84	77
QB2-12	16	730	110	5,100	85	90	14.5 (6.2)	75	8	73	80	30	29	63	62
QB2-13	55	4,200	290	22,000	77	70	13.2 (10.5)	32	23	80	90	66	77	38	43
QB2-3 (Ref.)	0.6	30	6.4	320	90	80	14.4 (8.8)	80	23	80	100	25	21	100	100
Lab Control	–	–	–	–	80	90	17.7 (7.6)	92	0	80	85 avg.	a	a	–	–
Phase III:															
QB3-1	5,800	70,000	3,600	43,000	–	–	–	0	–	–	–	1.0	0.9	–	–
QB3-2	20,000	200,000	13,000	130,000	–	–	–	0	–	–	–	0.1	0.3	–	–
QB3-R (Ref.)	0.25	4	3.3	58	–	–	–	50	–	–	–	33	24	–	–
Lab Control	–	–	–	–	–	–	–	92	–	–	–	–	–	–	–

(Ref.) = Reference site.

Dry = mg PAH/kg dry weight.

TOC = mg PAH/kg total organic carbon.

(–) No analysis or bioassay conducted.

Ceriodaphnia dubia (chronic) – results given as percent survival and production of young, respectively. Values in parentheses are standard deviations.

Chironomus tentans (chronic) – results given as percent weight loss.

EC50 – Ratio of sediment which gives a 50% light reduction.

*Microtox – Manchester Environmental Laboratory. EC50 values at 5 and 15 minutes.

a = negative gammas, indicating low toxicity. PSEP (1987).

**Microtox – University of Minnesota. EC50 values at 5 and 15 minutes. 100% Assay Procedure (Microbics, Inc., 1989).

Indicates survival significantly lower than control; in the case of Microtox, EC50 less than 100%.

Exceeds Provincial Sediment Quality Guidelines, severe effect level for total PAH, 11,000 mg/kg organic carbon. (See Discussion).

The other two sites, from the J.H. Baxter Cove, had no survival. Survival of only 50 percent at the Phase III reference site is unexplained. *H. azteca* did not show significant toxicity at any other site in this study.

Chironomus tentans

C. tentans showed no statistically significant lack of survival at any site. The organism did show unexplained chronic toxicity, with a 50 percent weight loss, at one of the lesser contaminated sites near the abandoned T-pier.

Hexagenia limbata

No significant toxicity to *Hexagenia* was observed at any Phase I, II, or III sites.

Ostracods

Unacceptably low control survival invalidated the results of this test.

Microtox®

Microtox® for Phase II samples was done by two different methods. Contradictory results were obtained. Analyses using the 100 percent Assay Procedure gave the lowest EC_{50} values with the highest levels of contamination, a result which is consistent with increased concentrations producing increased toxicity. Analyses with the PSEP method gave the highest EC_{50} values for the highest contaminant levels, a result inverse to the expected result.

Phase III sediments from the Baxter cove were toxic to Microtox® as shown by the very low EC_{50} values (all less than or equal to 1 percent). The reference sediment showed fairly low values of 33 and 24 percent for the five and 15 minute tests, respectively. This indication of toxicity is consistent with the *Hyalella* results above which also showed toxicity at the Phase III reference site, although, as noted earlier, the cause of this apparent toxicity is unknown.

Benthic Macroinvertebrate Identification and Analysis

Table 10 reviews the benthic invertebrates found at the study sites in Phase I. A total of 28 taxa were identified in sediments. The large number of porifera (sponges) at sites 13 and 15 reduce the overall diversity measures at those sites (Norton, 1991). Subsequent analysis of animals from adjacent sites (Phase II) by a different taxonomist has suggested that these porifera may be misidentified blue-green algae. Table 10 shows several community measures, calculated without the porifera, for these four sites. Site QB1-13, the site with the highest concentrations of PAH, had the lowest number of taxa, the lowest diversity measure (Shannon's H'), and the lowest overall density of invertebrates. This site was the only site in Phase I that caused significant *Hyalella* mortality. The biotic index, a measure of the pollution tolerance of the

Table 10. Review of benthic macroinvertebrate analysis from Phase I sample and the J.H. Baxter site.

Taxa	Tolerance*	Average number of organisms/square meter QB1-1	QB1-2	QB1-13	QB1-15
Nematoda		10			
Annelida					
Hirudinea	10	10	20		
Oligochaeta					
Tubificidae	8	130	80		
Naididae	8	630	310	200	560
Mollusca					
Gastropoda					
Planorbella	8				40
Bivalvia					
Pisidium	8	800	630	40	240
Porifera (See Note 1)		450	220	232000	172000
Arachnida					
Piona				40	
Crustacea					
Brachiopoda		30	30		
Copepoda		160	10		240
Ostracoda		10	10		
Amphipoda					
Hyalella azteca	4				80
Isopoda					
Caecidotea	8				80
Mysidacea					
Taphromysis	6		10		
Insecta					
Plecoptera					
Perlodidae	2		10		
Coleoptera					
Psephenus	4		10		
Diptera					
Chironomidae					
Macropelopia	6	20	20	120	80
Eukiefferiella	6	10	70		
Heterotrissocladius	6	100	150		
Parakiefferiella	6		110		40
Chironomus	8		20	40	
Cladopelama	8				240
Cryptochironomus	8		10		
Dicrotendipes	8	30	60		
Einfeldia	8		30		
Phaenopsectra	8	40	30	40	
Polypedilum	8				80
Tanytarsus	6	30	190		80
Note 1: "Porifera" not included in calculations. See text.					
Abundance Estimates					
Total organisms/square m		2010	1810	480	1760
Number of Taxa		14	20	6	11
Diversity Indices		(Higher number = more diverse)			
Shannon's H' (Washington, 1984)		2.42	3.15	2.22	2.96
J (H'/H'max)		50.8%	66.3%	46.7%	62.3%
Swartz's index (Swartz et al., 1985)		3	5	3	5
Biotic Index		(Higher number = more pollution tolerant)			
Hilsenhoff Index (Hilsenhoff, 1987)		7.8	7.3	7.5	7.5

*Tolerance: Index assigned to taxa to calculate Hilsenhoff Index.
Ranges from 10 (highly pollution tolerant) to 1.

endemic organisms, varied little between sites but in all cases implied a pollution-tolerant fauna. Biological community measures at the other three sites showed little relationship to the differences in PAH concentrations. The benthic organism report by Taxon Aquatic Monitoring Service is given in Appendix C (Taxon, 1990).

Table 11 reviews the invertebrates found during Phase II. Due to budget constraints, these samples were not identified to the same low taxonomic level as Phase I. In contrast to the Phase I samples, these samples showed greater abundance and diversity of animals at the sites with the higher concentrations of PAH. Higher population abundance could result from the contribution of nutrients and habitat structure provided by log debris at the sites with high PAH concentrations near the abandoned T-pier. The dominant organisms at these two sites are highly pollution tolerant. The Phase II reference site had a low level of taxa diversity and abundance. The reason for this lack of benthic life is unknown. The benthic report by Western Aquatic Institute is shown in Appendix D (Wisseman, 1991).

DISCUSSION

Conventionals

Sulfide may be responsible for some of the toxicity seen at several sites. The high sulfide level in the vicinity of the abandoned T-pier may have caused the chronic toxicity in *Chironomus tentans*. Increased sulfide concentrations in some Phase II samples correspond to sediments with decreased Microtox® EC_{50} values, a sign of toxicity. Sulfide is probably not directly responsible for these Microtox® responses because the samples become aerated during processing, thereby removing the sulfide through oxidation. All Phase II sediments exceed 200 ppm ammonia on a dry weight basis. This concentration classifies them as heavily polluted according to EPA Region V Guidelines for the Pollutional Classification of Harbor Sediments, which are used for the disposal of dredged material (Bahnick *et al.*, 1981; Bennett and Cubbage, 1991). Ammonia is probably not responsible for the toxicity observed, since the higher ammonia concentrations do not correspond to either the low levels of *Hyalella azteca* survival or Microtox® toxicity indications.

Bioassays

Daphnia magna and *Ceriodaphnia dubia*

Neither *Daphnia* nor *Ceriodaphnia* showed any acute effects despite high levels of PAHs in the samples. These results contradict those found by many researchers who consider both organisms to be highly sensitive indicators of toxicity (Burton, 1991a). Creosote has a low solubility in water and thus may not affect organisms that reside solely in the water column.

Table 11. Review of benthic macroinvertebrate analysis from Phase II samples at Quendall Terminals and the J.H. Baxter site.

	Average number of organisms/square meter			
Taxa	QB2-3	QB2-5	QB2-12	QB2-13
Nematoda		225	25	50
Annelida				
Hirudinea		100		25
Oligochaeta	100	163	2000	9850
Mollusca				
Planorbidae				2625
Sphaeriidae		63	75	325
Other	13		13	225
Arthropoda				
Arachnida				
Acari			63	50
Crustacea				
Amphipoda				100
Asellidae		25	13	
Insecta				
Ephemeridae	25			
Chironomidae	38	800	1050	2950
Ceratopogonidae		13	38	
Turbellaria				25

Hyalella azteca

H. azteca was the only bioassay organism tested at each site in all three phases. It was also the only bioassay to show significant toxicity at the most contaminated site(s) in each of the three sampling groups. With the exception of the Phase III reference site, this organism has shown the most consistent response to PAH contamination. In a review of several bioassay organisms exposed to PAHs, *Hyalella* had extremely consistent responses (Bennett and Cubbage, 1992).

Chironomus tentans and *Hexagenia limbata*

Chironomus and *Hexagenia* survival were both unaffected by varying PAH concentrations. *Chironomus* growth was affected only at a site with comparatively low PAH concentrations. We do not know the reasons for this lack of consistent responses.

Microtox®

The 100 percent Assay Procedure results show more inhibition with increased contamination. The PSEP (1987) procedure (Manchester Lab) showed the opposite results with less inhibition at increased contamination levels. The reason for these contradictory results is unknown, although procedural differences could have been responsible (Microbics, Inc., Personal Communication). This problem should be resolved before a Microtox® procedure is recommended for freshwater sediment toxicity testing. The "Solid Phase Test", recently published by Microbics, Inc., should be examined.

Microtox® results for the Phase III sediments indicated high toxicity with high contaminant levels. This is consistent with the *Hyalella* bioassay. The reference site in this phase showed some toxicity by both the *Hyalella* and Microtox® tests. Because the PAH concentrations were comparatively low, some other unidentified chemical is likely the cause of the toxicity.

Criteria and Guidelines

Some government agencies have created sediment criteria or guidelines to predict concentrations of organic chemicals that will harm freshwater biota (Bennett and Cubbage, 1991). The Provincial Sediment Quality Guidelines, published by the Ontario Ministry of the Environment (OMOE), established a "Severe Effect Level" at 11,000 mg PAH/kg TOC (Persaud *et al.*, 1991). This level has been deduced from analysis of *in situ* benthic communities, and is presumed to be the total PAH concentration above which benthic communities will be severely affected.

In Phase I, a site near the abandoned T-pier shows concentrations of 16,000 mg PAH/kg TOC, thereby exceeding the Severe Effect Level set by OMOE. This is the only site during Phase I that was toxic to *Hyalella*. The Phase II sample from the same site showed PAH at 27,000 mg PAH/kg TOC. This site was also above the Provincial guidelines and again it was the sole site

with significantly reduced *Hyalella* survival. The Baxter Cove sites sampled in Phase III had TOC normalized concentrations of 110,000 mg PAH/kg TOC and 330,000mg PAH/kg TOC, respectively. No *Hyalella* survived at either of these two sites. With the exception of the reference site during Phase III, which had somewhat reduced *Hyalella* survival, the Provincial Guideline for total PAH was an accurate predictor of whether sediments would be toxic to *Hyalella*.

Benthic Interpretation

For Phase II samples, Western Aquatic Institute reports that sediments from the most contaminated sites showed the highest taxa richness and abundance. The Phase I results, reported in Norton (1991), show greater overall diversity at roughly the same sites and show a slight trend towards more diversity at the less contaminated sites. The two data sets were analyzed by different laboratories. The lab for Phase II suggests that they are not directly comparable because of: 1) different levels of taxonomic effort, 2) different seasons, and 3) Station 13 is the only one sampled during both projects.

SUMMARY

Results of the chemical analyses, bioassay tests, and benthic invertebrate studies performed on Quendall Terminals and the J.H. Baxter site sediments demonstrate that several of these tests are useful as indicators of PAH toxicity. *Hyalella azteca* was the most consistent by showing a significant decrease in survival at all four sites where PAH contamination exceeded the OMOE, Severe Effect Level guideline. Microtox® also indicated toxicity at the most contaminated sites, although differences in sediment extract dilutions were probably responsible for contradictory results at the Phase II sites. A drop in benthic diversity and number of taxa corresponded with increased PAH contamination at the Phase I sites.

CONCLUSIONS

1. On a TOC normalized basis, total PAH concentrations in sediments from the study area ranged from 62 mg PAH/kg TOC to 330,000 mg PAH/kg TOC.

2. *Hyalella azteca* bioassays showed significant toxicity at sites with high concentrations of PAH. Significant reduction in survival occurred at all sites which exceeded the Provincial Sediment Quality Guidelines, Severe Effect Level for total PAH (11,000 mg PAH/kg TOC).

3. Microtox® showed toxicity at all sites. One of two Microtox® tests also showed toxicity at the reference for Phase II. More consistent responses were obtained with the 100 percent Assay Procedure than the PSEP procedure.

4. Acute tests with *Daphnia magna, Ceriodaphnia dubia, Hexagenia limbata,* and *Chironomus tentans* did not show significant toxicity at any site. A chronic *Chironomus tentans* test showed toxicity only at a relatively uncontaminated (based on pollutants measured in this study) site.

5. In Phase I, benthic community structure analysis showed reasonably good agreement among reduced benthic diversity, reduced abundance, elevated contaminant levels, and low survival of *Hyalella azteca*. Phase II showed no clear relationship.

RECOMMENDATIONS

1. Use *Hyalella azteca* to indicate toxicity of PAH contaminated sediments.

2. Use Microtox® in conjunction with other valid toxicity indicators (a "battery of tests"). We recommend that different Microtox® procedures (Standard Assay, 100 percent Assay, Solid Phase Test) be evaluated for their ability to indicate sediment PAH toxicity.

3. Due to apparent insensitivity, we do not recommend *Daphnia magna, Ceriodaphnia dubia, Hexagenia limbata,* or *Chironomus tentans* for testing of PAH contaminated freshwater sediments.

4. Additional studies, or a new site, will be needed to determine the impact of PAH on benthic macroinvertebrates.

5. Future studies involving benthic macroinvertebrate work should identify organisms to the genus and species level, if possible.

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APPENDIX A

Sediment Toxicity Assessment at Quendall Terminals and the J.H. Baxter Site, Lake Washington

Sediment Toxicity Assessment

at the

Quendall/Baxter Site, Lake Washington

by

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Background

The study site is known as Quendall/Baxter, located on the southeast shoreline of Lake Washington, near Renton, Washington (Figures 1 and 2). The site was contaminated from at least one spill of creosote during barge off-loading. This spill released thousands of gallons of creosote at station QB2-13. Four sites were selected based on earlier Washington State Department of Ecology studies by Dale Norton et al. Sites (Table 1) were thought to possess similar grain size and total organic carbon, and increasing levels of polycyclic aromatic hydrocarbons (PAHs). Study participants included the Dept. of Ecology's Manchester Lab, University of Minnesota, Oak Ridge National Lab, USEPA-Duluth, and Wright State University.

Sample Collection and Processing

Samples were collected, composited, and shipped by Department of Ecology personnel. Samples were collected on February 27, shipped Feb. 28, and received on ice on March 1, 1991 at Wright State University.

Test Procedures

Methods followed previously published protocols (2-6) and adhered to proper quality assurance procedures as defined by the USEPA (5), the laboratory's standard operating procedures and Quality Assurance Project Plan (7).

Samples were maintained at 4°C until testing. Sediments were mixed for 3 minutes with a hand paddle prior to assay initiation. Chemical and physical parameters included: alkalinity, hardness, conductivity, pH, temperature, and dissolved oxygen. All parameters were measured in overlying waters of the test

beakers. Sediment dry weight determinations were conducted in triplicate with each assay.

Short-term chronic toxicity was determined in whole sediment exposures following draft ASTM methods (4) for cladocerans (see Appendix A). The test species was Ceriodaphnia dubia. Briefly, ten replicate 30 ml beakers were used, containing a 1:4 ratio of sediment to water. One neonate (< 24 hr old) was randomly placed in each beaker. Dissolved oxygen, pH, and temperature were monitored daily, before overlying waters were replaced. Alkalinity, hardness, and conductivity were measured at test initiation and termination.

Results and Preliminary* Discussion

Summary toxicity test results are provided in Table 2. Slightly inhibitory results were observed in the short-term chronic exposure of C. dubia to sample QB2-13. Survival was reduced to 70% and mean young production was 13.2 neonates/3 broods as compared to 90% survival and 17.7 young in the controls. The high variance noted (standard deviations of 6.2 to 10.5), however, precludes the use of confident statements concerning significant differences.

It should be noted that PAHs are relatively volatile compounds. The high sediment oxygen demand observed initially in these sediments required that each test beaker undergo daily aeration to prevent dissolved oxygen sags below 40% saturation. This may have decreased organism exposure to PAHs.

In addition, PAHs are known to react with ultraviolet wavelengths to produce extremely phototoxic forms (8,9). Exposure to aquatic organisms in situ,

* A more complete discussion of the results and their significance may be provided if chemical and biological data from co-investigators is made available in the future.

therefore, may be more hazardous in the presence of natural light and PAHs.

Recommendations

Based on the observation of oily sediments, slight effects on C. dubia, and previous findings in creosote contaminated sediments (9), assays should be conducted again under natural light (lab and/or in situ) to better assess in situ conditions. In addition, the usefulness of indigenous microbial activity in field assessments (1,3) of contaminated sediments suggests they should be incorporated in all ecosystem impact evaluations.

Table 1. Study Site Station Descriptions

Site QB2-3:	Uncontaminated reference site slightly north of the Quendall/Baxter property. Moderate residential and pleasure boat use. Depth about 19 feet. LAB #098020
Site QB2-5:	North side of abandoned T-pier. Slight appearance of oil in sediment. Expect moderately low level of contamination. Depth about 20 feet. LAB #098021
Site QB2-12:	South side of T-pier. Oil apparent in sediment but not severe. Expect moderately high level of contamination. Depth about 13 feet. LAB #098022
Site QB2-13:	Adjacent to north side of T-pier in approximate area of creosote spill. Sediment shows high concentration of oil. Sheen appeared on water surface after dredging. Expect very high level of contamination. Depth about six feet. LAB #098023

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Table 2. Data Summary for the Quendall/Baxter Study

Assays:	Ceriodaphnia dubia 3 brood survival/ reproduction, whole sediment assay
Test phase:	Whole sediment
Sample date:	27, February, 1991
Sample stations:	QB2-3 (098020), QB2-5 (098021), QB2-12 (098022), QB2-13 (098023)

Assay Responses (x ± SD)

Sample	C. dubia[a] Survival (%)	Young
Control	90	17.7 (7.6)
QB2-3	80	14.4 (8.8)
QB2-5	90	18.5 (7.5)
QB2-12	90	14.5 (6.2)
QB-213	70	13.2 (10.5)

[a] Mean young produced per female and standard deviation (SD) with 10 replicates. Survival is not a mean value, n=10.

APPENDIX B

Bioassessment Analysis of Lake Washington Sediments

BIOASSESSMENT ANALYSIS OF

LAKE WASHINGTON SEDIMENTS

Mary G. Henry, Leader

Sylvia Morse and Donald Jaschke
University of Minnesota

INTRODUCTION

Petroleum products and by-products such as creosote are complex mixtures of organic compounds including polycyclic aromatic hydrocarbons (PAHs). The majority of PAHs are released to the environment as a result of anthropogenic activity. Seepage and spillage of petroleum products are two important sources of PAHs. Creosote from submerged treated wooden structures has also been shown to induce a significant localized effect on marine invertebrates.

Many PAHs are highly persistent in the environment and the biological effects of these compounds are not completely understood. Due to their nonpolar, hydrophobic nature, PAHs are not very water soluble (Rand and Petrocelli, 1985). Alkyl PAHs, such as creosote, show even lower solubilities. PAHs are readily adsorbed onto suspended particulate matter and are often eventually deposited in the sediment. Sediment mixing, as a result of human activity, can significantly affect the PAH distribution in the sediments. PAHs adsorbed onto sediment material have limited bioavailability to aquatic organisms. Some degradation of PAHs occurs due to metabolism by benthic and microbial aquatic organisms, however, certain metabolic intermediates have been shown to be highly carcinogenic, mutagenic and/or teratogenic and the more aliphatic compounds can be acutely toxic. Biomagnification appears to occur to a limited extent.

Sediment samples were collected from and adjacent to the Quendall Baxter (private ownership) property located on the southeast shore of Lake Washington, Seattle, WA.. The site has had a history of spill events associated with leakage of creosote from docking facilities used by barges. Concern over the toxicity of creosote to aquatic organisms in this area has prompted this bioassessment evaluation.

STUDY OBJECTIVES

1. to conduct a series of toxicity test evaluations on sediment samples from several locations in Lake Washington to determine the biological impacts of creosote levels in sediment on two benthic invertebrates species

2. to characterize the magnitude of the contamination at each site by using the results of the toxicity tests

3. to provide a report describing the results and interpretation of the toxicity evaluations

TEST PROCEDURE

Two toxicity tests were performed to assess the presence of potentially toxic levels of creosote in sediment samples. These test procedures were developed to determine toxicity under controlled laboratory conditions of sediment-bound contaminants using representative species from the aquatic benthic community. Bulk-sediment toxicity was assessed using a two week (14-d) chronic *Chironomus tentans* toxicity test (Mosher et al., 1984 and ASTM, 1989) and a 10-d static *Hexagenia limbata* toxicity test (Nebecker et al., 1984 and Fremling, 1989). *Chironomus tentans* and *Hexagenia limbata* are important indicators of ecosystem health and have demonstrated sensitivities to environmental contaminants (Cairns, et al., 1984). Tests were run using field collected sediment samples which were replicated (N=15 for midge, N=10 for mayflies). If no appreciable mortality was evident using this full strength field collected sediment, a definitive dilution series was judged unnecessary. Well water was used as the overlying water in both toxicity tests. Well water at the University of Minnesota is used routinely in the culture of *Chironomus tentans* and *Hexagenia limbata* as well as other aquatic organisms and has produced healthy and robust cultures, justifying its use as the aqueous overlay phase in these tests.

MICROTOX®

The MICROTOX® toxicity test uses rehydrated lyophilized cells of the bacterium *Photobacterium phosphoreum* and measures the phosphorescence of these bacteria before and after exposure to a contaminated medium. Light production by this organism is a normal byproduct of metabolism. The toxicity test is based on detecting and quantifying any inhibition of light production in the presence of a sample thereby indicating the presence of a toxic substance (or substances) in that sample. The 100% Assay Procedure, as outlined by Microbics, Inc., was followed. This assay, as opposed to the Standard Assay procedure (Microbics, Inc.), seems most appropriate for screening environmental samples of unknown toxicity.

Chironomus tentans

Second instar *Chironomus tentans* larvae were used to evaluate bulk-sediment toxicity. The experimental design consisted of an initial, full strength test with sediment from each collection site. Egg cases were placed in artificial substrate and allowed to develop to the second instar, 14-16 days post-hatch. The test chambers were individual 50 mL polypropylene centrifuge tubes. Each test chamber contained 7.5 g of

sediment. A culture media control of digested paper towel substratum as well as clean reference sediment were used to check organism health in the absence of contaminants thereby serving as a set of controls. Test chambers were filled to the 50 mL mark with well water. One second instar larva was placed in each assay tube. Each sediment collected was replicated 15 times, one larva per chamber. Larvae were fed 0.1 mL of food daily and continual aeration was supplied to each test chamber. Mortality was recorded daily. The test was terminated after 14 days and larvae were placed in aluminum ashing pans and dried in an 80° C oven for 24 hours and weighed to a tenth of a milligram. Measured endpoints were mortality and percent reduction in weight relative to the control group.

Hexagenia limbata

Hexagenia limbata nymphs were field collected and acclimated to test water in our laboratory 48 hours prior to testing. Full-strength sediment samples were evaluated for toxicity. Test chambers consisted of individual 4 oz. acid washed, acetone rinsed, straight-walled jars. Each chamber contained 50g of contaminated or control sediment. Well water was used as overlying water. One nymph was placed in each test chamber. Nymphs were fed 0.2 mL of a prepared diet every other day. All test chambers were fitted with stoppers which were wrapped in acetone rinsed aluminum foil. Chambers were gently aerated using pasteur pipets embedded in the stoppers. Each sediment treatment group consisted of 10 replicate test chambers. Chambers were held under low light conditions in a flow-through water bath at 18° C. Mortality and molting frequency were recorded daily. The test was terminated after 10 days. Lethality was the measured endpoint.

RESULTS and DISCUSSION

MICROTOX®

Pore-water associated with bulk sediment was tested as an indicator of the potential toxicity of the sediment samples from Lake Washington. The MICROTOX® toxicity test assessing pore-water extracted from bulk sediment showed toxic responses at three of the four sites tested. The sites showing a toxic response are QB2-5, QB2-12 and QB2-13 and the EC_{50} values are displayed in Fig. 1. Site QB2-3 gave no toxic response when analyzed using the MICROTOX® toxicity test.

Chironomus tentans

A 14 day *C. tentans* partial life-cycle toxicity test was conducted to assess the potential toxicity and determine the biological impact of creosote contaminated sediments from Lake Washington. Mortality and reduction in body weight were the measured endpoints of the test. Percent mortality is shown in Fig. 2 for all sites and the percent weight reduction relative to the control is shown in Fig. 3. For statistical purposes a dead midge was recorded as having 100% reduction in body weight (Giesy et al., 1990). Mortality patterns indicate that site QB2-5 and QB2-12 induced mortality at levels exceeding 20% (an acceptable background mortality level). A one tailed t-test comparing the control with the treatment sites showed one site, QB2-5, to induce statistically significant ($P=0.10$) weight loss in exposed midge. The percent mean reduction in weight for site QB2-5 was 50.01%. The other three sites were not significantly different from the control.

Hexagenia limbata

Hexagenia limbata, a burrowing mayfly, was incorporated into a toxicity test to assess the potential toxicity of bulk-sediment taken from Lake Washington. The 10-d *H. limbata* toxicity test was conducted and daily observations of molting frequency and mortality were noted. The toxicity test revealed no *H. limbata* mortality over 20% during the 10 day exposure and molting did not occur during the test.

CONCLUSION

The MICROTOX® analysis of pore-water toxicity associated with bulk sediment revealed three sites, QB2-5, QB2-12 and QB2-13, to posess biologically detectable levels of contaminants. *C. tentans* exposure to Site QB2-5 sediment produced statistically significant weight reduction when compared to the control. *H. limbata* mortality was insignificant at all four sites. Although no consistent toxicity induced mortality pattern was shown across all tests, the MICROTOX® and *C. tentans* toxicity tests were able to detect sublethal toxic responses to a contaminant(s). In conclusion, there seems to exist a contaminant related concern associated with sediments from the QB2-5, QB2-12, and QB2-13 collection sites. The most consistent concern exists for QB2-5 sediments and chemical characterization of that sediment is warranted to confirm the presence of creosote and its concentration.

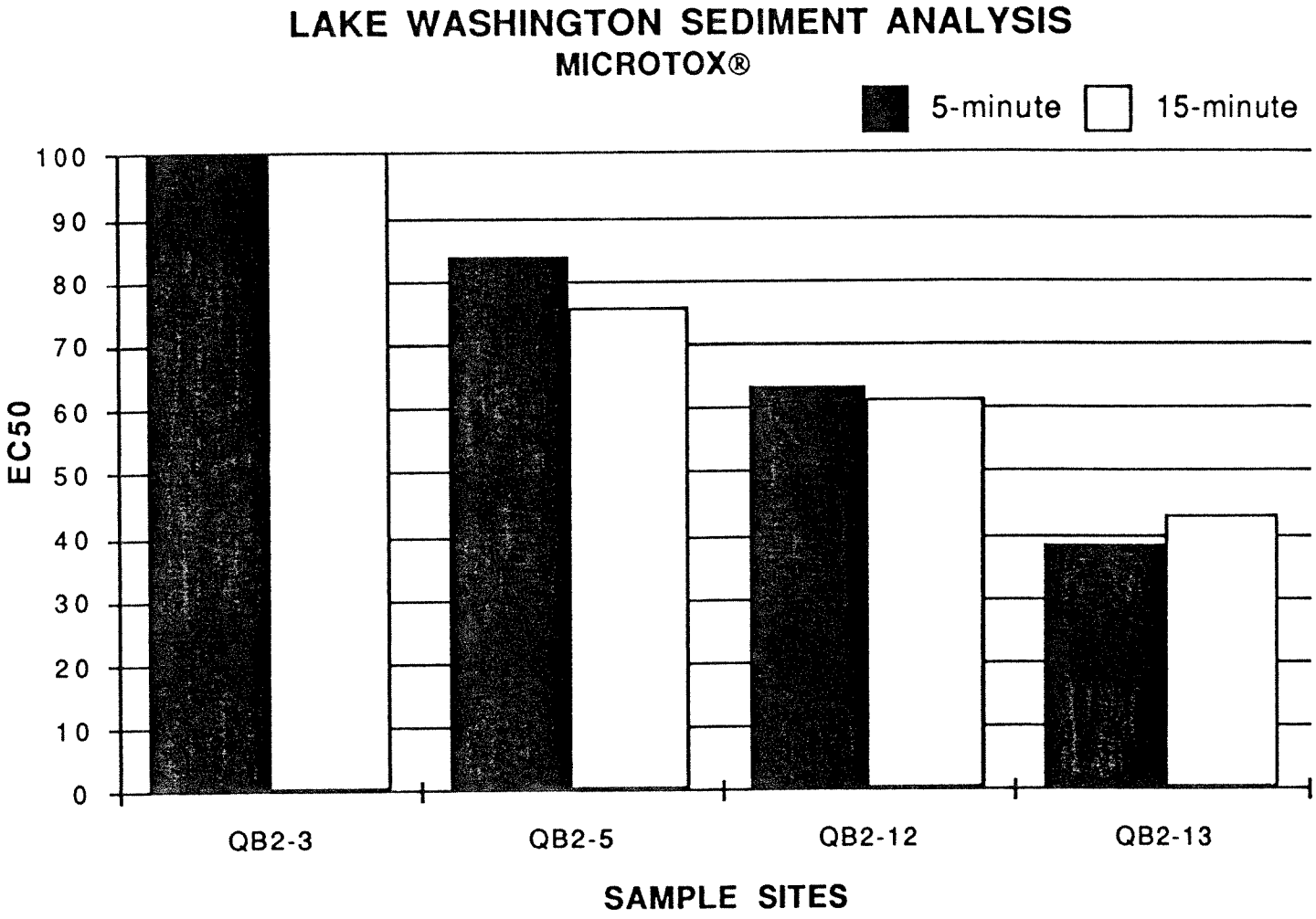

FIG. 1. EC50 values obtained from the MICROTOX® toxicity test.

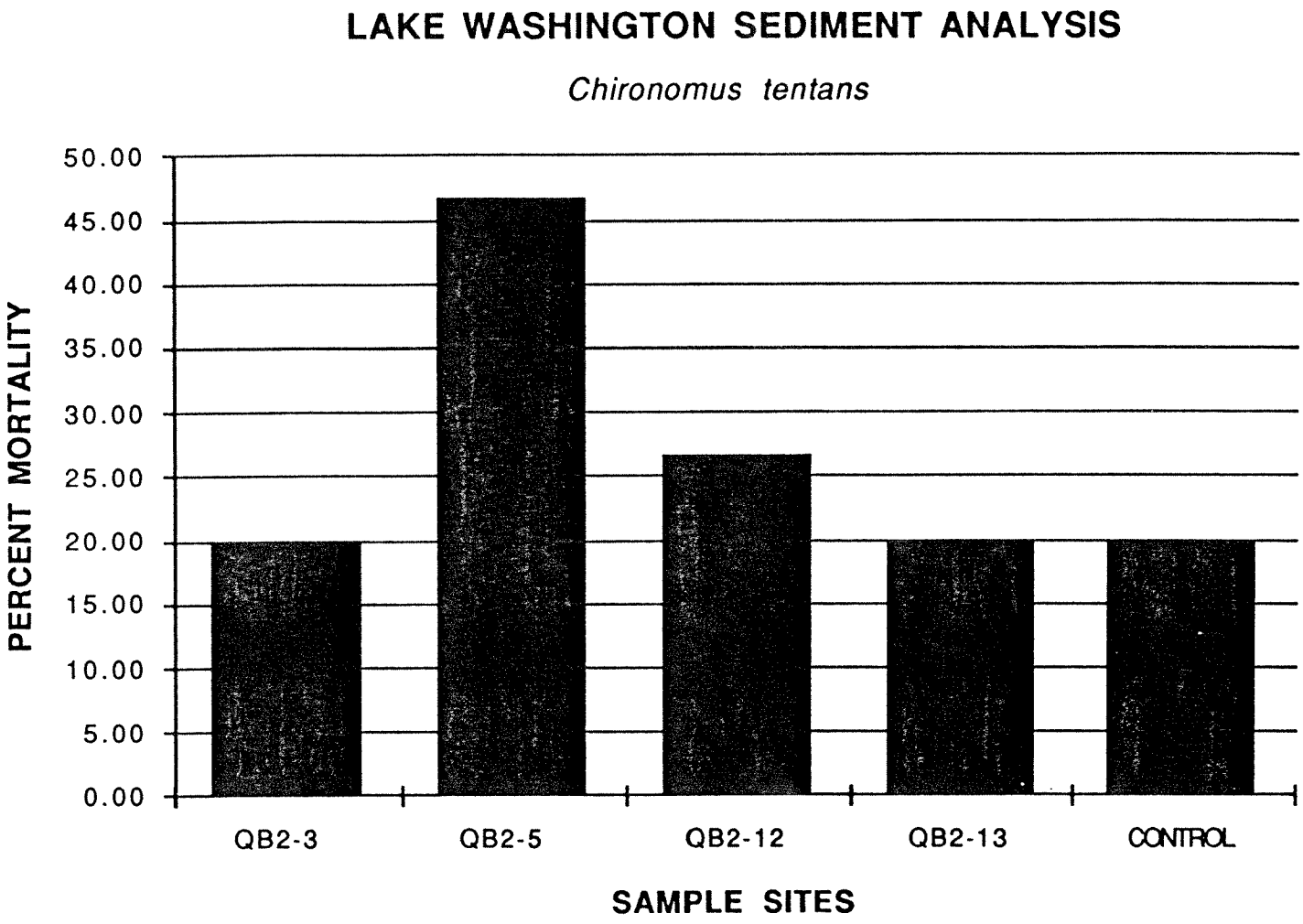

FIG. 2. *Chironomus tentans* percent mortality resulting from a 14 day exposure to sediments.

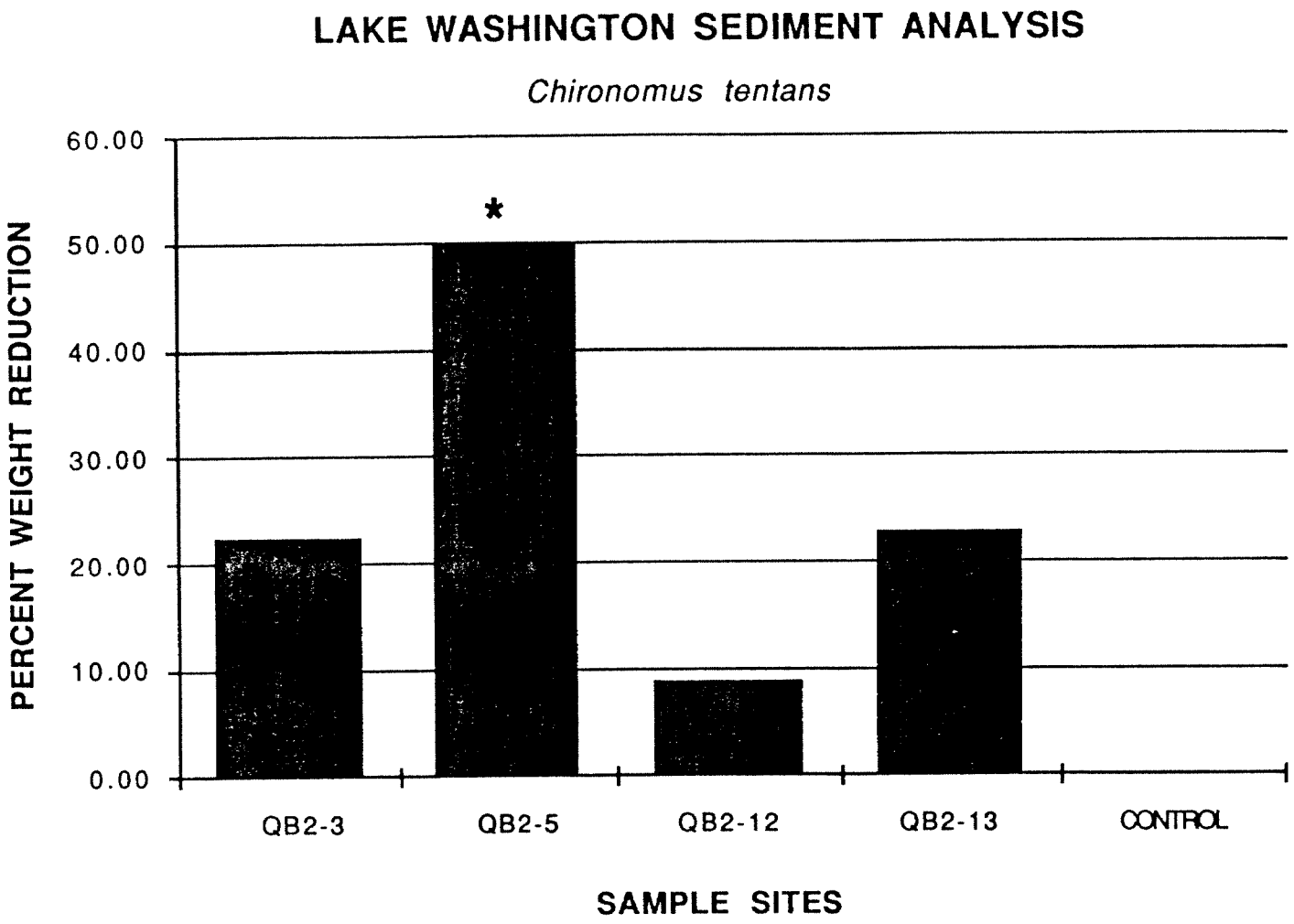

FIG. 3. *Chironomus tentans* percent weight reduction as compared to the control (* shows statistical significance).

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APPENDIX C

Taxon Aquatic Monitoring Service Report on the Benthic Infauna of Quendall Terminals and the J.H. Baxter Site

Jon

DATA REVIEW

BY: Margaret Stinson mcs

FOR: Quendall/Baxter, Sample Nos. 20-8280, -81, -92, -96

DATE: July 23, 1990

Taxon Aquatic Monitoring Service has submitted the attached report on infaunal benthic identification of samples from the Quendall/Baxter site. This laboratory hasn't the experience to evaluate these data, so the results are being forwarded to the requesting field staff without data review. These arrangements were approved by the field staff before the samples were submitted for analysis.

	QB-1A	QB-1B	QB-1C	QB-1D	QB-1E
Nematoda				1	
Annelida (segmented worms)					
Hirudinea (leeches)			1		
Oligochaeta (segmented worms)					
Tubificidae	2	5	2	2	2
Naididae	13	8	12	20	10
Mollusca					
Gastropoda (snails)					
Planorbella					
Bivalvia (clams)					
Pisidium	12	12	21	16	19
Porifera (sponge gemmules)	15	16		2	12
Arachnida					
Piona (mites)					
Crustacea					
Brachiopoda (daphnia)	2				1
Copepoda	1		6	2	7
Ostracoda (seed shripms)				1	
Amphipoda (scuds)					
Hyalella azteca					
Isopoda (aquatic sow bugs)					
Caecidotea					
Mysidacea (opossum shrimps)					
Taphromysis					
Insecta					
Plecoptera (stoneflies)					
Perlodidae					
Coleoptera (beetles)					
Psephenus					
Diptera (flies)					
Chironomidae (midges)					
Macropelopia			2		
Eukiefferiella					1
Heterotrissocladius	9		1		
Parakiefferiella					
Chironomus					
Cladopelma					
Cryptochironomus					
Dicrotendipes			1		2
Einfeldia					
Phaenopsectra			1	2	1
Polypedilum					
Tanytarsus			2		1

	QB-2A	QB-2B	QB-2C	QB-2D	QB-2E
Nematoda					
Annelida (segmented worms)					
Hirudinea (leeches)			1	1	
Oligochaeta (segmented worms)					
Tubificidae	1	5	1	1	
Naididae	12		4	10	5
Mollusca					
Gastropoda (snails)					
Planorbella					
Bilvalvia (clams)					
Pisidium	22	10	7	12	12
Porifera (sponge gemmules)		6	3	8	5
Arachnida					
Piona (mites)					
Crustacea					
Brachiopoda (daphnia)					3
Copepoda					1
Ostracoda (seed shripms)					1
Amphipoda (scuds)					
Hyalella azteca					
Isopoda (aquatic sow bugs)					
Caecidotea					
Mysidacea (opossum shrimps)					
Taphromysis					1
Insecta					
Plecoptera (stoneflies)					
Perlodidae	1				
Coleoptera (beetles)					
Psephenus					1
Diptera (flies)					
Chironomidae (midges)					
Macropelopia		1		1	
Eukiefferiella	1				6
Heterotrissocladius		6		8	1
Parakiefferiella	2			3	6
Chironomus			1	1	
Cladopelma					
Cryptochironomus					1
Dicrotendipes				4	2
Einfeldia				3	
Phaenopsectra	1			1	1
Polypedilum					
Tanytarsus	2		7	8	2

	QB-13A	QB-13B	QB-13C	QB-13D	QB-13E
Nematoda					
Annelida (segmented worms)					
Hirudinea (leeches)					
Oligochaeta (segmented worms)					
Tubificidae					
Naididae			4	12	4
Mollusca					
Gastropoda (snails)					
Planorbella					
Bilvalvia (clams)					
Pisidium				4	
Porifera (sponge gemmules)	6000	6480	3000	4320	3360
Arachnida					
Piona (mites)			4		
Crustacea					
Brachiopoda (daphnia)					
Copepoda					
Ostracoda (seed shripms)					
Amphipoda (scuds)					
Hyalella azteca					
Isopoda (aquatic sow bugs)					
Caecidotea					
Mysidacea (opossum shrimps)					
Taphromysis					
Insecta					
Plecoptera (stoneflies)					
Perlodidae					
Coleoptera (beetles)					
Psephenus					
Diptera (flies)					
Chironomidae (midges)					
Macropelopia			4		8
Eukiefferiella					
Heterotrissocladius					
Parakiefferiella					
Chironomus			4		
Cladopelma					
Cryptochironomus					
Dicrotendipes					
Einfeldia					
Phaenopsectra				4	
Polypedilum					
Tanytarsus					

	QB-15A	QB-15B	QB-15C	QB-15D	QB-15E
Annelida (segmented worms)					
Hirudinea (leeches)					
Oligochaeta (segmented worms)					
Tubificidae					
Naididae	8	32		16	
Mollusca					
Gastropoda (snails)					
Planorbella				4	
Bivalvia (clams)					
Pisidium		8	16		
Porifera (sponge gemmules)	2608	4080	5600	1520	4960
Arachnida					
Piona (mites)					
Crustacea					
Brachiopoda (daphnia)					
Copepoda	8	8		8	
Ostracoda (seed shripms)					
Amphipoda (scuds)					
Hyalella azteca					8
Isopoda (aquatic sow bugs)					
Caecidotea			8		
Mysidacea (opossum shrimps)					
Taphromysis					
Insecta					
Plecoptera (stoneflies)					
Perlodidae					
Coleoptera (beetles)					
Psephenus					
Diptera (flies)					
Chironomidae (midges)					
Macropelopia	8				
Eukiefferiella					
Heterotrissocladius					
Parakiefferiella				4	
Chironomus					
Cladopelma	24				
Cryptochironomus					
Dicrotendipes					
Einfeldia					
Phaenopsectra					
Polypedilum	8				
Tanytarsus	8				

APPENDIX D

Benthic Macroinvertebrate Analysis - Lake Washington, Quendall Terminals and the J.H. Baxter Site Sediments

BENTHIC INFAUNAL ANALYSIS LAKE WASHINGTON
Quendall Terminals/ J.H. Baxter sediments.
February 1991 Samples. Coarse level identifications.

Western Aquatic Institute, Robert W. Wisseman, Corvallis, Oregon, (503) 752-1568.

This report provides coarse level identifications (Phase I) of benthic macroinvertebrates from the Quendall Terminal site. Abundances have not been adjusted to a square meter basis. Samples were processed using a 500 micron sieve. Until a finer level of identification (Phase II I.D.) is completed, it is not possible to provide much of a discussion regarding the infaunal communities present at the four stations. Notes on the samples and the data are provided below.

Stations 12 and 13 displayed the highest taxa richness and abundance, despite the presence of high amounts of creosote residues. The dominant organisms at these two stations appear to be highly tolerant forms, though a finer level of identification (Phase II I.D.) is required to confirm this.

Samples from station 13 contained remains from aquatic macrophytes, indicating that submerged plant beds were present. A higher richness and abundance of organisms at this station may be partially explained by the presence of macrophyte beds, since some taxa will be associated solely with this habitat (e.g. planorbid snails).

Samples from stations 5, 12 and 13 contained a high amount of coarse particulate organic matter (CPOM), mostly as bark chips. Station 3 (control) samples consisted primarily of fine particulate organic matter (FPOM).

I cannot explain the paucity of organisms at Station 3 without having more knowledge of the substrates and habitat conditions. If the influence of toxins or a severe depression of dissolved oxygen is ruled out, then it may be that these sediments are particularly nutrient poor and unable to attract and maintain much of an infaunal community, or the surface sediments may have been recently disturbed or deposited. Station 3 does not appear to be a suitable control site.

Results from the Phase I analysis of the February 1991 samples are not directly comparable to the analysis done by Taxon Aquatic Monitoring Services on a previous sample set, since: 1. levels of taxonomic effort differ, 2. there is a difference in seasons, and 3. sites differ except for station 13.

At station 13, the February 1991 community is considerably more diverse and densities are significantly higher than was found in the ?Fall (Taxon) sample set. Total organism density will

translate to ca. 10,000/organisms per square meter. Species richness and abundance is highest in the winter in many temperate zone lakes (Timms 1985).

The organism identified as Porifera by Taxon is believed to be a benthic, blue-green algae that was in bloom at the shallower stations (13 & 15) at the time those samples were taken. This algae was not present in February, with the exception of several senescent colonies found in several samples.

I would recommend that the organisms present in the February 1991 samples from stations 5, 12 and 13 be identified to a finer taxonomic level (Phase II I.D.) to determine whether the communities are dominated by highly tolerant forms, and to enable calculation of various indices for comparative purposes.

It is probable that organic debris from the log storage facilities contributes nutrients and habitat structure to the sediments beneath these floating structures, which allows more tolerant organisms to attain high relative abundances, particularly in the winter months when cooler temperatures and higher levels of dissolved oxygen prevail. It is also possible that creosote itself may provide nutrient enrichment and an energy source for the infaunal organisms, especially if the more toxic components of creosote are water soluble and have been leached out.

The results from the two sampling periods are ambiguous and not readily interpretable. I'm enclosing a publication by Timms (1985) which discusses sampling strategies for lake benthos. I would suggest that the sampling should be stratified in space and time.

I would recommend that samples either be taken on a 4 season basis, or that a winter and late summer sampling schedule be followed. Species richness and abundance will probably be highest in the winter during optimal environmental conditions, and before the emergence of insect taxa in the spring. Stress in the benthic infaunal community may be most evident in the late summer or early fall (before the fall turn-over) if sediments have stagnated over the summer.

Before any recommendations on how to stratify the sampling according to habitat type and depth, it would be necessary to have more information on the area. It is probable that the littoral zone area that you are trying to monitor is a mosaic of habitat types, particularly since human activities have probably compounded the natural substrate heterogeneity.

You may want to consider running a transect through the impacted sites and along an approximately similar depth contour, rather than run transects from the shore out into the lake along rapidly changing depth contours.

I realize that sample processing costs multiply rapidly when numerous stations are established along a transect(s), with replicate samples taken at each station. It may be advisable to establish many stations in a pilot study, so that spatial heterogeneity can be ascertained, and suitable reference sites chosen. For this type of study, a fraction (e.g. one-half) of each replicate can be initially pooled and treated as a single sample, to cut down on costs. Once the results from these composite samples are reviewed, final station selection can be made and the remaining fraction of each replicate processed separately.

It will be important to keep careful notes on habitat characteristics at the stations (e.g. depth, presence and density of macrophyte beds, presence and abundance of CPOM and FPOM, etc.).

In some monitoring situations, such as beneath log boom areas, it may be impossible to find control sites that are matched with the impact areas in all habitat characteristics. CPOM debris will have altered the sediment habitat structure and chemistry, and infaunal communities will be different from natural substrates because of this. Under these circumstances, it would be impossible to separate out whether a specific toxin such as creosote caused differences in community structure.

Most indices used to describe benthic community structure require only relative abundance estimates of organisms present. Thus, it is more economic to pool several samples from a station and process as a single composite sample. Since a quantitative sampler is used (e.g. petite ponar dredge), estimates of population density can still be made for each organism present.

I would recommend that a more extensive (increased stations) sampling program be undertaken initially, rather than concentrate on a more intensive (numerous replicates) program at a few stations. The number of samples and cost of processing can remain about the same. The extensive approach would provide a better picture of spatial variation in infaunal community composition.

Perhaps the next time I'm in your area, we can discuss study objectives, site characteristics, and sampling strategies. I travel to the Olympia and Seattle area occasionally.

Benthic Infaunal Analysis Lake Washington
Quendall Terminals/J.H. Baxter sediments.

February 1991 R.W. Wisseman Coarse identifications.
Abundances have not been adjusted to a square meter basis.

TAXON	R1	R2	R3	R4	MEAN	STDEV
Station QB2-3	50	100	100	50	% sample	sorted
Oligochaeta	6	0	1	2	2.25	2.28
?Mollusca	0	0	1	0	0.25	0.43
Ephemeridae	0	0	0	2	0.5	0.87
Chironomidae	0	0	1	2	0.75	0.83
TOTAL	6	0	3	6	3.75	2.49
Station QB2-5	100	100	100	100	% sample	sorted
Nematoda	1	11	1	5	4.5	4.09
Oligochaeta	7	0	4	2	3.25	2.59
Hirudinea	3	1	1	3	2	1.00
Sphaeriidae	4	1	0	0	1.25	1.64
Asellidae	1	0	1	0	0.5	0.50
Ceratopogonidae	1	0	0	0	0.25	0.43
Chironomidae	27	6	20	11	16	8.09
TOTAL	44	19	27	21	27.75	9.83
Station QB-12	100	100	100	50	% sample	sorted
Nematoda	0	0	0	2	0.5	0.87
Oligochaeta	10	70	54	26	40	23.41
Sphaeriidae	0	0	4	2	1.5	1.66
?Mollusca	0	0	1	0	0.25	0.43
Asellidae	0	0	1	0	0.25	0.43
Acari	0	1	1	0	0.5	0.50
Ceratopogonidae	0	1	2	0	0.75	0.83
Chironomidae	14	32	24	14	21	7.55
TOTAL	24	104	87	44	64.75	32.12
Station QB-13	50	50	50	50	% sample	sorted
Turbellaria	0	0	2	0	0.5	0.87
Nematoda	0	4	0	0	1	1.73
Oligochaeta	204	82	292	210	197	74.95
Hirudinea	0	0	2	0	0.5	0.87
Sphaeriidae	6	2	6	12	6.5	3.57
Planorbidae	46	24	52	88	52.5	22.99
?Mollusca	2	2	10	4	4.5	3.28
Amphipoda	0	0	8	0	2	3.46
Acari	2	0	2	0	1	1.00
Chironomidae	22	36	88	90	59	30.41
TOTAL	282	150	462	404	324.5	119.88