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COPPER IN SEDIMENTS FROM STEILACOOM LAKE, PIERCE COUNTY, WASHINGTON

by

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Prepared for

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ABSTRACT

The Washington State Department of Ecology is developing criteria and testing procedures for several freshwater sediment contaminants. Copper, a metal highly toxic to aquatic life, is the focus of this study. Sediment copper levels exceed 1,000 mg/kg dry weight in Steilacoom Lake, a shallow, eutrophic lake in Pierce County. The primary source of the metal in the sediments is many years of application of the algicide, copper sulfate. Three separate approaches were tested to determine the effects of copper on Steilacoom Lake; triad, acid volatile sulfide; and "battery-of-tests".

Sediment samples were collected in Steilacoom Lake for metals analyses, bioassays, and benthic macroinvertebrate identification and enumeration. Bioassays included *Daphnia magna*, *Hyalella azteca*, *Hexagenia limbata*, *Chironomus tentans*, Microtox®, microbial enzymes, *Ceriodaphnia dubia* and ostracods. Benthic samples were identified to the genus and, if possible, species level. Black Lake, with little history of copper treatment, provided reference sediment.

Sediment copper levels in Steilacoom Lake ranged from 180 to 1100 mg/kg dry weight. *Hyalella* (acute) and *Hexagenia* (acute) bioassays showed significant response. Microbial enzyme tests showed reduced activity with increasing contaminant concentrations. Interpretation of benthic invertebrate data was inconclusive due to variation among replicate samples. The benthic organisms in Steilacoom Lake are all pollution-tolerant. Acid volatile sulfide, a possible normalizing factor for metal toxicity, was not a controlling factor for copper. Black Lake was a poor reference because of a dearth of benthic diversity and the presence of unidentified toxic compounds in the sediment.

INTRODUCTION

Numerical criteria and standard test procedures are often used to identify levels of contamination toxic to aquatic biota in freshwater sediments. These criteria and tests may eventually be incorporated into regulations that govern discharges of pollutants and cleanup of contaminated sediments. As part of an effort to define these standards and criteria, the Department of Ecology is evaluating several methods of assessing toxicity in freshwater sediments in Washington State. Most of this recent work has been conducted in areas of high metals or PAH concentrations.

Several methods of evaluating toxicity have been studied. 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 polycyclic aromatic hydrocarbons. The acid volatile sulfide (AVS) approach normalizes metals concentrations in sediment to AVS to determine bioavailability of metals and thus toxicity (DiToro *et al.*, 1990). To determine toxicity, sediments are often tested with several bioassays. This approach is termed the "battery-of-tests" approach, and is used because various organisms may respond differently to types and levels of contaminants. With greater toxicity, more organisms will respond adversely. These three approaches were tested in this study to determine the effects of copper on the aquatic environment.

Copper is a contaminant of concern in Washington State because it is frequently dispersed in freshwater lakes to kill algae. As a precipitate, it accumulates in sediments which can then become toxic to aquatic organisms. Hanson and Stefan (1984) report that short-term effects of copper treatment include: a) the intended temporary killing of algae; b) dissolved oxygen depletion by decomposition of dead algae; c) accelerated phosphorous recycling and rapid algal recovery; and d) occasional fish kills from oxygen depletion and/or copper toxicity. Long-term effects include: a) copper accumulation in sediments; b) tolerance adjustment of certain algal species to higher copper sulfate dosages; c) shift of species from green to blue-green algae and from game fish to non-game fish; d) disappearance of macrophytes; and e) reductions in benthic macroinvertebrates. Therefore, while copper sulfate is very effective at killing algae, its use can produce a number of both immediate and cumulative side effects which may be harmful to aquatic life.

Steilacoom Lake, located in Pierce County near Lakewood, Washington, was chosen as a site to study the possible effects of copper on sediment biota, because copper sulfate has been added to the lake for over 25 years in an effort to kill algae. Precipitated copper has accumulated in the sediment, and in at least one location exceeds 1000 mg/kg dry weight (Johnson and Norton, 1990). The lake, which covers approximately 320 acres, was created about 50 years ago by impoundment, and today is surrounded by single family homes. Maximum depth is 20 feet. This typical urban lake is classified eutrophic (Brower and Kendra, 1990).

The objectives of this study were to:

Phase I

- Characterize the levels of copper and AVS in Steilacoom Lake sediments.

Phase II

- Determine the toxicity of selected sediments through the use of several bioassays.
- Evaluate the effects of sediment copper on the distribution and numbers of benthic organisms.
- Apply these data toward determining freshwater sediment criteria for copper.

METHODS

Reference Site

Black Lake was chosen as the source of reference sediment because, like Steilacoom Lake, it is eutrophic and at least partially surrounded by development. It differs from Steilacoom Lake in that it has not been treated regularly with copper and has low concentrations of copper in its sediment (27.6 mg/kg dry weight) (Johnson and Norton, 1990). The locations of these lakes are shown in Figure 1.

Sediment Sampling

Sediments were collected in two phases. The first phase was designed to verify earlier studies and to characterize copper concentrations throughout Steilacoom Lake. The second phase focused on conducting several bioassays and benthic community measures at a few sites selected to vary widely in Cu/AVS, based on Phase I results.

Phase I samples from 12 sites in Steilacoom Lake and two samples in Black Lake were collected on September 17 and 18, 1990. For Phase II, on November 26 and 27, three sites (4, 11, and 12) with Cu/AVS ratios that ranged from 0.6 to 27 were sampled in Steilacoom Lake. One sample was taken from Black Lake during Phase II. Steilacoom Lake sample sites are shown in Figure 2. Black Lake sample sites are shown in Figure 3. Descriptions for sampling sites in Steilacoom and Black Lakes are also listed in Tables 1 and 2, respectively.

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

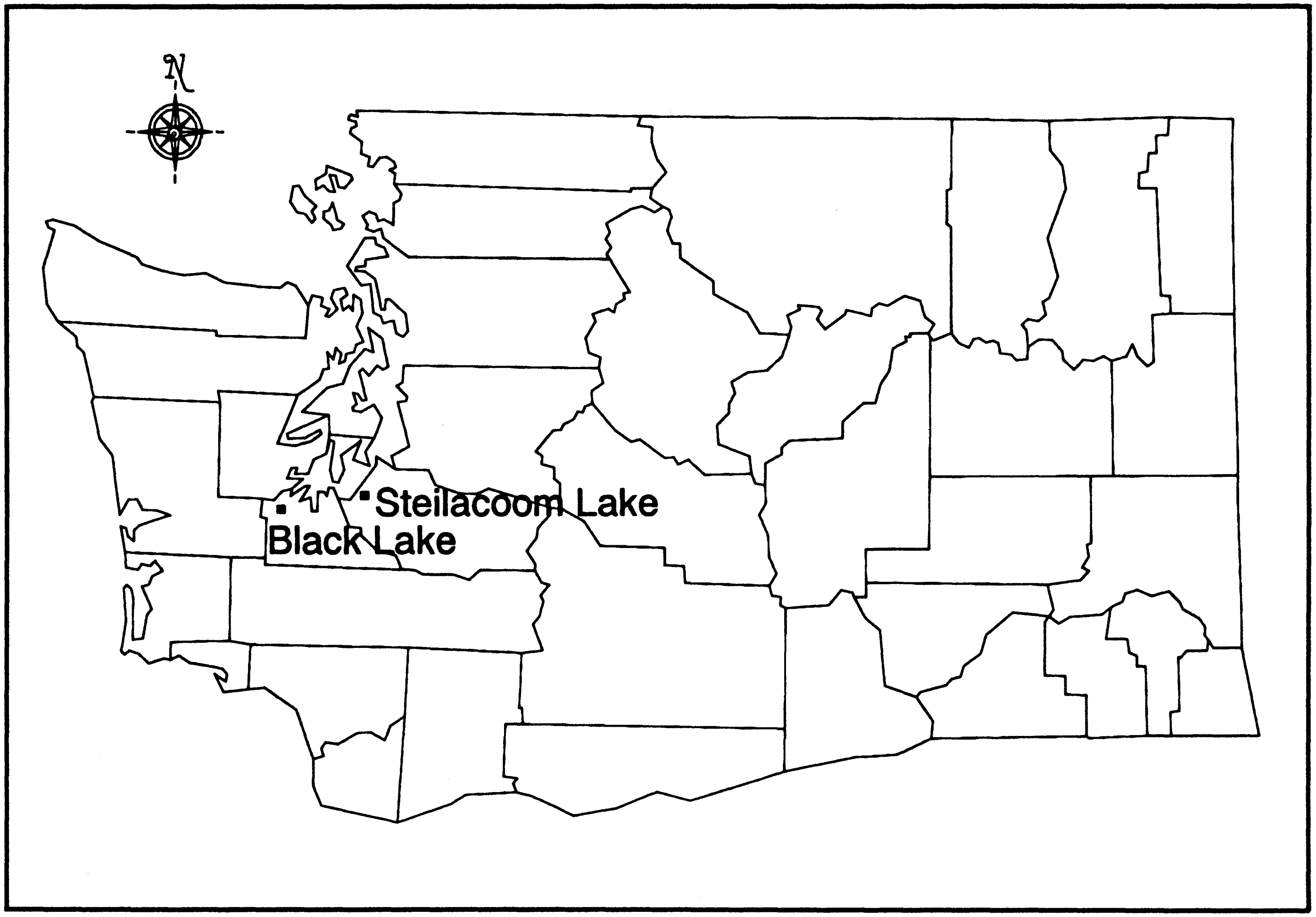

Figure 1. Locations of Steilacoom Lake and Black Lake.

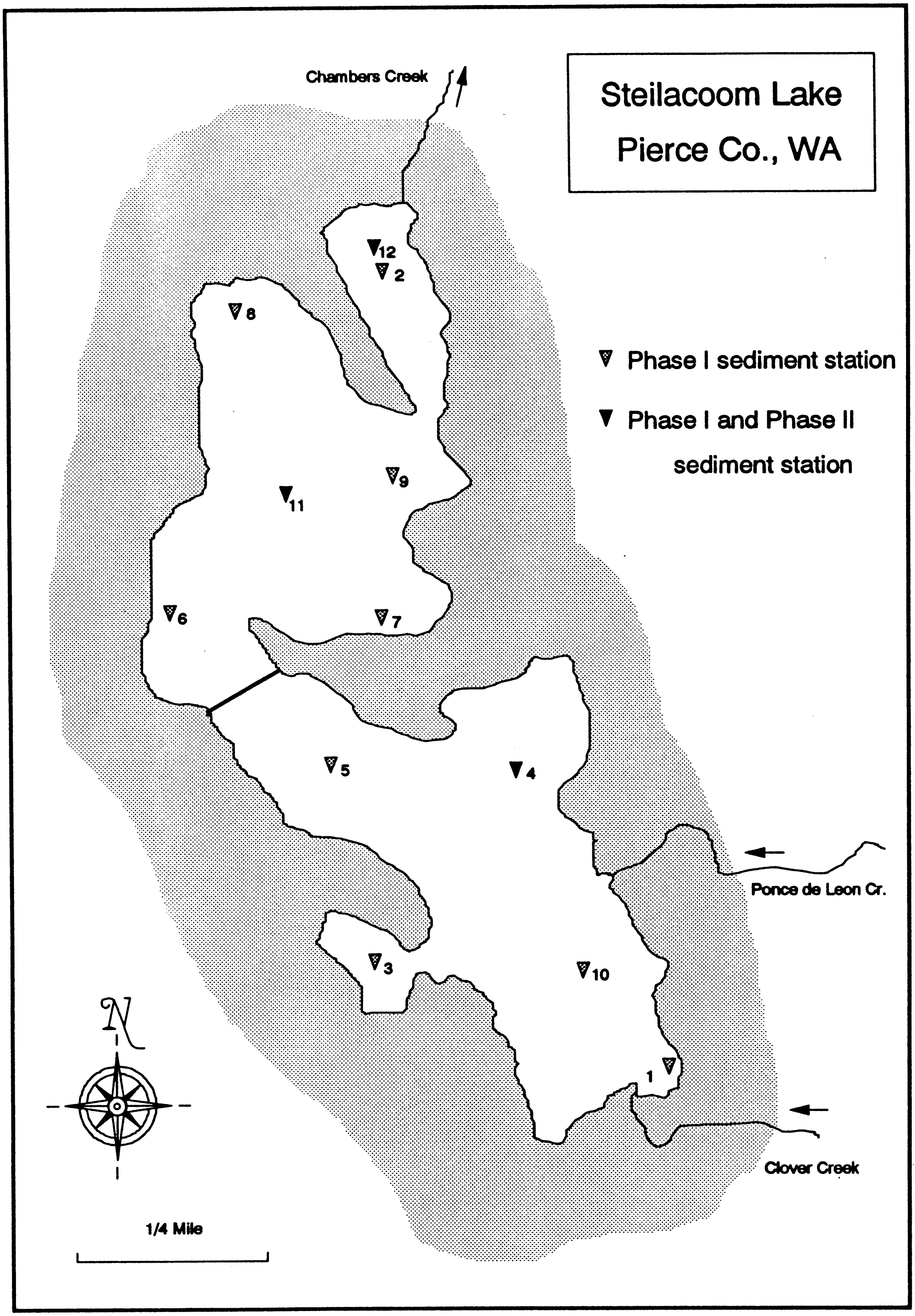

Figure 2. Steilacoom Lake sediment sampling sites.

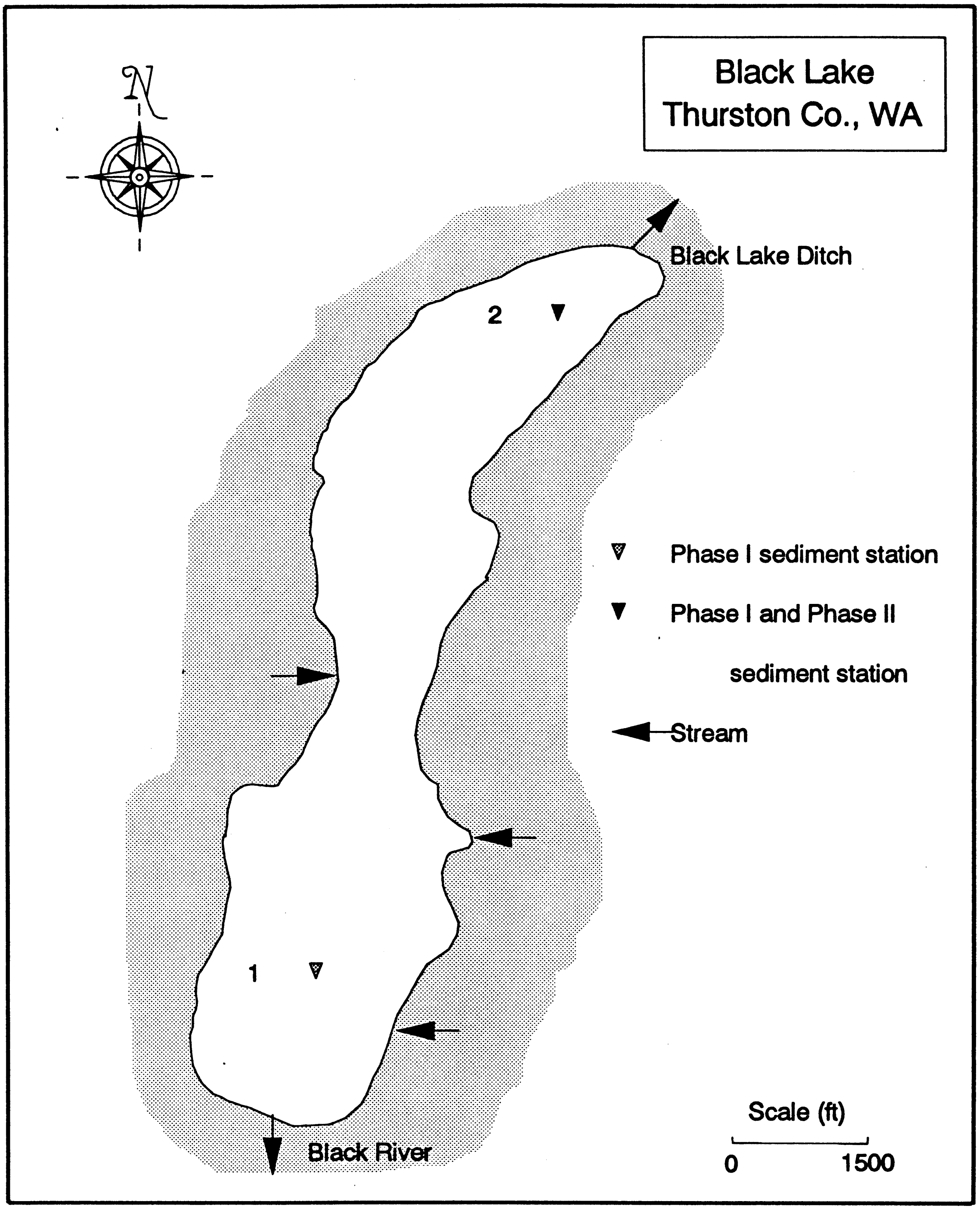

Figure 3. Black Lake sediment sampling sites.

Table 1. Steilacoom Lake and Black Lake – Phase I sample times and locations.

Site	Date	Lab		Depth	Latitude			Longitude		
Number	Sept.	Sample	Time	(feet)	Deg.	Min.	Sec.	Deg.	Min.	Sec.
ST1	17	388130	1245	12	47	9	29	122	31	36
ST2	17	388131	1200	12	47	10	34	122	32	6
ST3	17	388132	1315	7	47	9	37	122	32	8
ST4	17	388133	1330	17	47	9	53	122	31	52
ST5	17	388134	1345	16	47	9	53	122	32	13
ST6	17	388135	1400	10	47	10	6	122	32	31
ST7	17	388136	1430	7.5	47	10	5	122	32	7
ST8	17	388136	1445	10	47	10	31	122	32	23
ST9	17	388138	1500	12	47	10	17	122	32	6
ST10	17	388141	1300	18	47	9	36	122	31	45
ST11	17	388142	1415	11	47	10	16	122	32	17
ST12	17	388143	1515	12	47	10	34	122	32	6
BL1	18	388139	1100	15	46	58	49	122	58	48
BL2	18	388144	1130	16	47	0	29	122	58	5

ST = Steilacoom Lake.

BL = Black Lake reference.

Table 2. Steilacoom Lake and Black Lake – Phase II sample times and locations.

Site	Date	Lab		Depth	Latitude			Longitude		
Number	Nov.	Sample	Time	(feet)	deg.	min.	sec.	deg.	min.	sec.
ST4	26	488155	1100	20	47	9	53	122	31	52
ST11	26	488156	1230	16	47	10	16	122	32	17
ST12	26	488157	1400	12	47	10	34	122	32	6
BL2	27	488158	1100	22	47	0	29	122	58	5

ST = Steilacoom Lake.

BL = Black Lake reference.

Benthic macroinvertebrates were obtained with a 0.02 m^2 stainless steel Petite Ponar dredge. Four replicates (individual samples collected at the same location) were collected at each benthic site. The entire contents of the grab were washed through a 30-mesh (0.589 mm) screen. Retained material from each individual sample was placed into separate one-quart glass jars and preserved with 10% formalin solution. Contents of the jars were transferred to 70% ethanol after a minimum of 14 days in the formalin fixative.

Analytical and Bioassay Procedures

Table 3 presents a summary of analyses performed at each site. Analytical methods and laboratories used in this study are shown in Table 4. Analyses were performed for seven metals, besides copper, to determine their concentrations in lake sediment and pore water. Besides the chemical analyses, several bioassays were performed to test for sediment toxicity. Methods for each bioassay and the benthic macroinvertebrate identifications are described below.

Sediment Pore Water Extraction

Sediment pore water was extracted based on a procedure described by Ankley *et al.* (1990). An aliquot of sediment was placed into a 250 mL round bottom centrifuge tube and centrifuged at 3000-4000 Gs for 30 minutes. The supernatant liquid was then decanted and digested. Because of insufficient volume (<100 ml), the pore water was not filtered prior to digestion.

Daphnia magna

D. magna, a cladoceran or "water flea", is a water column organism that feeds on the sediment surface (Burton, Personal Communication). This organism was originally used in effluent studies, and is now widely used in tests adapted for sediment. The procedure used here for testing for acute toxicity was the 48-hour survival test. The chronic test was the three brood, seven-day reproduction test which measures the production and survival of young. 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 waste water effluent and has been adapted to sediment work. Its susceptibility to toxic effects is dependent on contaminant transfer from sediment to the water column. As with *Daphnia*, the acute test was based on 48-hour survival. The chronic test used the three brood, seven-day reproduction procedure which measures the production and survival of young. Tests followed methods in ASTM E1383-90 (ASTM, 1990).

Hyalella azteca

H. azteca, an amphipod, 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 used measured 14-day survival and was conducted according to ASTM E1383-90 (ASTM, 1990).

Table 3. Steilacoom Lake and Black Lake, Phase I and Phase II - analyses performed at each site.

	Phase I Sites														Phase II Sites			
Site Number >>	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10	ST11	ST12	BL1	BL2	ST4	ST11	ST12	BL2
Metals:																		
Sediment Metals (except Cu)	-	-	-	-	-	-	-	-	-	x	x	x	-	x	x	x	x	x
Sediment Copper	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Sediment Cu/AVS	x	x	x	x	x	x	x	x	x	x	x	x	-	-	-	-	-	-
Pore Water Metals (except Hg)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
Conventionals:																		
Sediment pH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
TOC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
Ammonia (NH3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
Total Sulfide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
Grain Size	-	-	-	-	-	-	-	-	-	x	x	x	-	x	x	x	x	x
Percent Solids	-	-	-	-	-	-	-	-	-	x	x	x	-	x	x	x	x	x
Surface Water Hardness	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-
Pore Water Ammonia (NH3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
Mixed/Aerated Sediments	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
Bioassays:																		
D. magna (chronic/acute)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
C. dubia (chronic/acute)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
H. azteca (acute)	x	x	x	x	x	x	x	x	x	x	x	x	-	-	x	x	x	x
C. tentans (chronic/acute)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
H. limbata (acute)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
Ostracods	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
Microtox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
Microbial Enzymes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x
Benthic Invertebrates:																		
Identify, Enumerate, Analyze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x

Metals analyzed: arsenic, cadmium, chromium, copper, lead, mercury, nickel, zinc.

Sediments digested as totals (except Cu/AVS), pore waters digested as total recoverables.

ST = Steilacoom Lake.

BL = Black Lake reference.

X = Analysis performed.

Table 4. Steilacoom Lake and Black Lake – analyses, methods, references, and laboratories.

PHASE I

Analysis	Method	Reference	Laboratory
Sediment – Total Metals:		USEPA, 1986b	Manchester Environmental
Digestion	EPA 3005	"	(Ecology/EPA)
Arsenic	ICP – 200.7/6010	"	"
Cadmium	"	"	"
Chromium	"	"	"
Copper	"	"	"
Lead	"	"	"
Mercury	Cold Vapor – 245.1,245.5	"	"
Nickel	ICP – 200.7/6010	"	"
Zinc	"	"	"
Cu/AVS	Gravimetric	Ankley et al., 1991, In Press	EPA – Duluth
H. azteca (acute)	–	Phipps & Ankley, 1990	"

PHASE II

Analysis	Method	Reference	Laboratory
Sediment – Total Metals: see Phase I			Manchester Environmental
Pore Water – Metals	Total Recoverable Digestion	USEPA, 1986b	(Ecology/EPA)
Sediment pH	SW 846–9045	"	"
Surface water pH	EPA 150.1	"	"
Hardness	EPA 130.2	"	"
TOC	EPA 415.2	"	Amtest, Inc.
NH3	EPA 350.1	"	"
Total Sulfides	EPA 376.1	"	"
Grain Size	Sieves and Pipettes	PSEP, 1987	Soil Technology, Inc.
Percent Solids	Dry @ 104 C	"	"
H. azteca (acute)	ASTM E 1383	ASTM, 1990	Manchester Environmental
D. magna (acute)	"	Nebeker et al., 1984	"
D. magna (chronic/acute)	ASTM E1383, ANNEX X4	ASTM, 1990	Wright State University
C. dubia (chronic/acute)	"	"	"
Microbial Enzymes	-	Burton, 1991	"
H. limbata	-	Nebeker et al., 1984 and	University of Minnesota
		Fremling and Mauck, 1980	"
C. tentans	ASTM: E 729–88a	Mosher et al., 1982 and	"
		ASTM, 1989	"
Microtox	Puget Sound Protocols	PSEP, 1987	Manchester Environmental
Microtox	100% Assay Procedure	Microbics, Inc., 1989	University of Minnesota
Ostracods	-	Woodward et al., In Draft	Oak Ridge National Labs
Benthic Identification	Lowest Practical Level	–	Western Aquatic Institute

Chironomus tentans

The larvae of *C. tentans*, a true fly, is a benthic organism frequently used for sediment toxicity tests because of its burrowing characteristics. Wentsel *et al.* (1978) demonstrated its capacity to develop a degree of resistance to metal pollution. The procedures used here, which consisted of ten-day survival (acute) and percent weight reduction (chronic) tests, can be found in Henry *et al.* (1991).

Hexagenia limbata

H. limbata, a burrowing mayfly nymph (Order Ephemeroptera) is a benthic dweller used in sediment toxicity tests. *Hexagenia* is unavailable during May and June and has not been successfully raised in the laboratory. Contaminant sensitivity may vary depending on organism age (Morse, Personal Communication). Henry *et al.* (1991) report procedures for the ten-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.* (In Draft).

Microtox®

Microtox® bioassays are based on the reduction in the level of light produced by the bioluminescent marine microorganism, *Photobacterium phosphoreum*, caused by enzyme inhibition following exposure to a toxicant. Results are reported as Effective Concentrations 50% (EC_{50}) defined as the percent of test material which, when mixed with a control, reduces the light output by 50%. EC_{50} values $\geq 100\%$ indicate very low toxicity.

Ecology/EPA Manchester Environmental Laboratory used the method outlined in the Puget Sound Protocols (Puget Sound Estuary Program, 1987). The University of Minnesota analyzed pore water using the 100% Assay Procedure, outlined in the Microtox® manual (Microbics, Inc., 1989). Although originally designed for marine water samples, Microtox® has been adapted to freshwater sediment studies using pore water, elutriate solutions, and solid phase.

Microbial Enzymes

Microbial enzyme activity tests, which may act as early-warning indicators of chronic contamination, use the ability of indigenous sediment bacteria to metabolize a nitrophenol-linked enzyme substrate. Test results, reported as activity, are defined as micrograms (μg) of product formed per gram dry weight of sediment. The four enzyme systems tested were: alkaline phosphatases (APA); dehydrogenases, or electron transport system activity (DHA); b-galactosidase(GAL); and b-glucosidase (GLU). One would expect increased contamination to result in decreased metabolism. Test procedures can be found in Burton (1991).

Benthic Macroinvertebrate Analyses

Samples were submitted to Mr. Bob Wisseman, Western Aquatic Institute, Corvallis, Oregon, for identification and enumeration. Animals were identified to the lowest practical (usually genus) level. Qualitative observations were also made by the laboratory.

Quality Assurance/Quality Control

Sediment samples for metals analyses were digested by the total method using nitric acid and hydrogen peroxide. Sediment pore water for metals analyses was digested by the total recoverable method using nitric acid only. Data quality for metals analyses were assessed through method blanks, laboratory duplicates, and matrix spikes. In general, the data are considered acceptable in terms of accuracy and precision except for the problems noted below. In each data table, the accompanying qualifiers are noted where appropriate.

QA/QC data are given in Table 5. Matrix spike recoveries for Phase I were not available for copper. High concentrations of copper in samples overwhelmed the small amounts of copper used in spike measurements. Recoveries of other metals ranged from 91% for nickel to 108% for zinc. Copper data for Phase I Black Lake reference sediments may be elevated because of a contaminated lab blank. Duplicate analyses were not run.

For Phase II samples, recovery from one matrix spike test of copper in sediment was 77.6%. Matrix spikes for other metals, except mercury, ranged from 87.5% for zinc to 100% for chromium. Spike recovery for mercury (68% and 52%) was low. With the exception of the duplicate spikes reported for mercury, duplicate analyses for sediment metals were not conducted.

Spike recovery for pore water copper was 104%. Pore water cadmium was not reported because of laboratory blank contamination. Zinc contamination of a lab blank also caused pore water zinc data from three of the four sites to be flagged.

All bioassays were tested with benign sediments (control) and reference toxicants. Data from the first of two *H. limbata* bioassays were discarded due to unacceptably low survival using the control sediment (Morse, Personal Communication).

Due to high variations between replicates of benthic invertebrate samples, numerical measures of correlation between benthic communities and copper concentrations were not possible. Several indices were computed.

Table 5. Results of laboratory accuracy and precision tests for metals in Steilacoom Lake and Black Lake sediments.

	Spike Recoveries								Lab Duplicates		
	Phase I			Phase II					Phase II		
Type >>	Sediment			Sediment				Water	Water (ug/L)		
Lab No. >>	388143			488156	488158			488158	488155		
Metal	Spike #1	Spike #2	RPD	Spike #1	Spike #1	Spike #2	RPD	Spike #1	Result	Dup. #1	RPD
Arsenic	-	-	-	NAR	-	-	-	95%	9.3	8.8	5.5%
Cadmium	-	-	-	93.4%	-	-	-	103%	2.1 JB	2.6 JB	21% JB
Copper	-	-	-	77.6%	-	-	-	104%	160	160	0.0%
Chromium	100%	101%	1%	100%	-	-	-	99%	5.0 U	5.0 U	-
Lead	-	-	-	NAR	-	-	-	109%	120	110	8.7%
Mercury	-	-	-	-	68%	52%	27%	-	-	-	-
Nickel	91%	93%	2%	96.8%	-	-	-	97%	14	14	-
Zinc	108%	102%	6%	87.5%	-	-	-	98%	32 B	47	38% B

RPD = Relative percent difference = (S1-S2)/((S1+S2)/2)*100.

B = Possible blank contamination.

JB = Estimated value, not accurate - possible blank contamination.

NAR = No analytical result.

U = Not detected at detection limit shown.

(-) = Analysis not done.

RESULTS

Conventionals

Results of conventionals analyses of Phase I and Phase II sediment samples are given in Table 6.

All Steilacoom Lake and Black Lake sediments were slightly acidic with pH values ranging from 6.3 to 6.5 and probably results from decomposing vegetation. Surface water hardness, which was only measured at the north basin Steilacoom Lake site, was 44 mg/L as $CaCO_3$ which is relatively soft. This is an important factor since the toxicity of many metals to aquatic organisms increases with decreasing hardness. TOC (dry weight) for north basin Steilacoom Lake sediment was 7.2%, which is similar to the Black Lake site, 7.8%. In contrast, much higher TOC values were present in the south basin (13%) and near the outlet (14%) of Steilacoom Lake. Higher values for the latter two sites probably reflect their greater proximity to land and sources of organic material. Ammonia in the Steilacoom Lake sediments ranged from 700 to 960 mg/kg dry weight, and Black Lake reference sediment ammonia was 750 mg/kg dry weight. All sediments exceeded the 200 mg/kg dry weight threshold set by the Great Lakes Harbors Classification of Sediments for being heavily polluted with ammonia (Bahnick *et al.*, 1981). Ammonia is a product of the biodegradation of plant and animal matter, but it can also come from sewage and fertilizer runoff. The classification of heavy ammonia pollution in the Steilacoom Lake sediments indicates there may be anthropogenic sources. Total sulfide in Steilacoom Lake sediments was less than 20 mg/kg wet weight at all sites, and about 12 mg/kg wet weight for the Black Lake reference sediment.

Grain size distributions for Steilacoom Lake showed the sediment to be predominantly silt with lesser and equal amounts of sand and clay. Black Lake sediment had a slightly greater percentage of silt and a lower percentage of clay. These subtle differences probably reflect the fact that Steilacoom Lake is an artificial impoundment built on a former wetland, while Black Lake is the product of glacial activity which included the deposition, sorting, and scouring of transported material.

Sediment Metals

Sediment metals concentrations in Steilacoom Lake and Black Lake are listed in Tables 7 (Phase I) and 8 (Phase II).

Copper

Copper data for Steilacoom Lake during Phase I and II are shown in Figures 4 and 5, respectively. Sediment copper concentrations in Steilacoom Lake show a steady increase from an average of about 300 mg/kg at the south end of the lake, where Clover and Ponce de Leon Creeks enter, to the outlet at the north end where Chambers Creek originates with over 1000 mg/kg. This pattern is probably the result of copper precipitate being distributed by the prevailing current. Areas of elevated copper concentrations, such as the small cove in the

Table 6. Results of conventionals analyses of Phase I and Phase II sediment samples from Steilacoom Lake and Black Lake.

Phase I sediment samples.

Site	Solids	Grain Size (%)				Visual
Number	Percent	Gravel	Sand	Silt	Clay	Description
ST1	-	-	-	-	-	Dark brown sandy mud
ST2	-	-	-	-	-	Dark brown mud
ST3	-	-	-	-	-	Dark brown mud
ST4	-	-	-	-	-	Brown mud
ST5	-	-	-	-	-	Greenish-brown mud
ST6	-	-	-	-	-	Brown mud
ST7	-	-	-	-	-	Dark brown mud
ST8	-	-	-	-	-	Dark brown mud
ST9	-	-	-	-	-	Dark green mud
ST10	9	1	17	50	32	Dark green-brown mud
ST11	6	0	33	39	28	Green-brown mud
ST12	8	0	34	39	27	Dark brown mud
BL1	-	-	-	-	-	Dark brown mud
BL2	12	0	39	53	8	Dark brown mud

Gravel = >2 mm, Sand = 2 mm-62 um, Silt = 62 um-4 um, Clay = <4 um, (-) = Analysis not done.
ST = Steilacoom Lake. BL = Black Lake reference.

Phase II sediment samples.

Site	Solids	Grain Size (%)				pH	TOC	NH3	Sulfide	Visual
Number	Percent	Gravel	Sand	Silt	Clay	Std. Units	Percent	mg/kg dry	mg/kg wet	Description
ST4	8	0	18	46	36	6.3	13	930	<21	Brown, fine, evenly-textured
ST11	9	0	36	32	32	6.5	7.2	700	<20	Brown, fine, evenly-textured
ST12	8	0	23	57	20	6.5	14	960	<20	Dark brown, evenly-textured, medium-coarse
BL2	16	0	33	57	10	6.3	7.8	750	12 J	Brown, evenly-textured, sandy

Gravel = >2 mm, Sand = 2 mm-62 um, Silt = 62 um-4 um, Clay = <4 um, J = estimate.
ST = Steilacoom Lake. BL = Black Lake reference.

Table 7. Total metals (mg/kg dry weight) and Cu/AVS in Phase I sediment samples from Steilacoom Lake and Black Lake.

Site Number	Arsenic	Cadmium	Chromium	Copper	Lead	Mercury	Nickel	Zinc	Cu/AVS Ratio
ST1	-	-	-	180	-	-	-	-	0.3
ST2	-	-	-	1100	-	-	-	-	0.7
ST3	-	-	-	890	-	-	-	-	29
ST4	-	-	-	440	-	-	-	-	6.5
ST5	-	-	-	520	-	-	-	-	7.2
ST6	-	-	-	770	-	-	-	-	1.1
ST7	-	-	-	890	-	-	-	-	0.7
ST8	-	-	-	870	-	-	-	-	1.5
ST9	-	-	-	790	-	-	-	-	59
ST10	15	2.6	39	340	260	0.05	22	180	1.7
ST11	14	1.7	23	750	140	0.05 J	16	140	27
ST12	16	2.4	23	1000	160	0.63	18	190	0.6
BL1	-	-	-	31 B	-	-	-	-	-
BL2	9.4	0.2 U	49	32 B	37	0.02 J	33	58	-

▨ Exceeds Provincial Sediment Quality Guidelines, severe effect level. (See Discussion).

B = Possible blank contamination.

J = Estimated value.

U = Not detected at detection limit shown.

ST = Steilacoom Lake.

BL = Black Lake reference.

(-) = Analysis not done.

Table 8. Total metals (mg/kg dry weight) in Phase II sediment samples from Steilacoom Lake and Black Lake.

Site Number	Arsenic	Cadmium	Chromium	Copper	Lead	Mercury	Nickel	Zinc
ST4	15	2.0	28	400	260	0.05 J	16	140
ST11	10	1.9	14	840	160	0.04 J	10	170
ST12	40	2.5	20	890	200	0.06	15	240
BL2	6.3	0.2 U	37	24	NAR	0.05 J	22	54

▨ Exceeds Provincial Sediment Quality Guidelines, severe effect level, mg/kg dry weight. (See Discussion).

J = Estimated value.

NAR = No Analytical Result.

U = Not detected at detection limit shown.

ST = Steilacoom Lake.

BL = Black Lake reference.

Table 9. Steilacoom Lake and Black Lake Phase II sediment - pore water total recoverable metals (ug/L).

Site Number	Arsenic	Chromium	Copper	Lead	Nickel	Zinc
ST4	9.3	5.0 U	160	120	14 J	32 B
ST11	14	5.0 U	240	40	10 U	34 B
ST12	27	5.0 U	440	92	12 J	85
BL2	11	5.0 U	11	10	10 U	10 JB

B = Blank contamination found at 4.6 ug/L.

J = Estimated value.

JB = Estimated value, not accurate - possible blank contamination.

U = Not detected at detection limit shown.

ST = Steilacoom Lake.

BL = Black Lake reference.

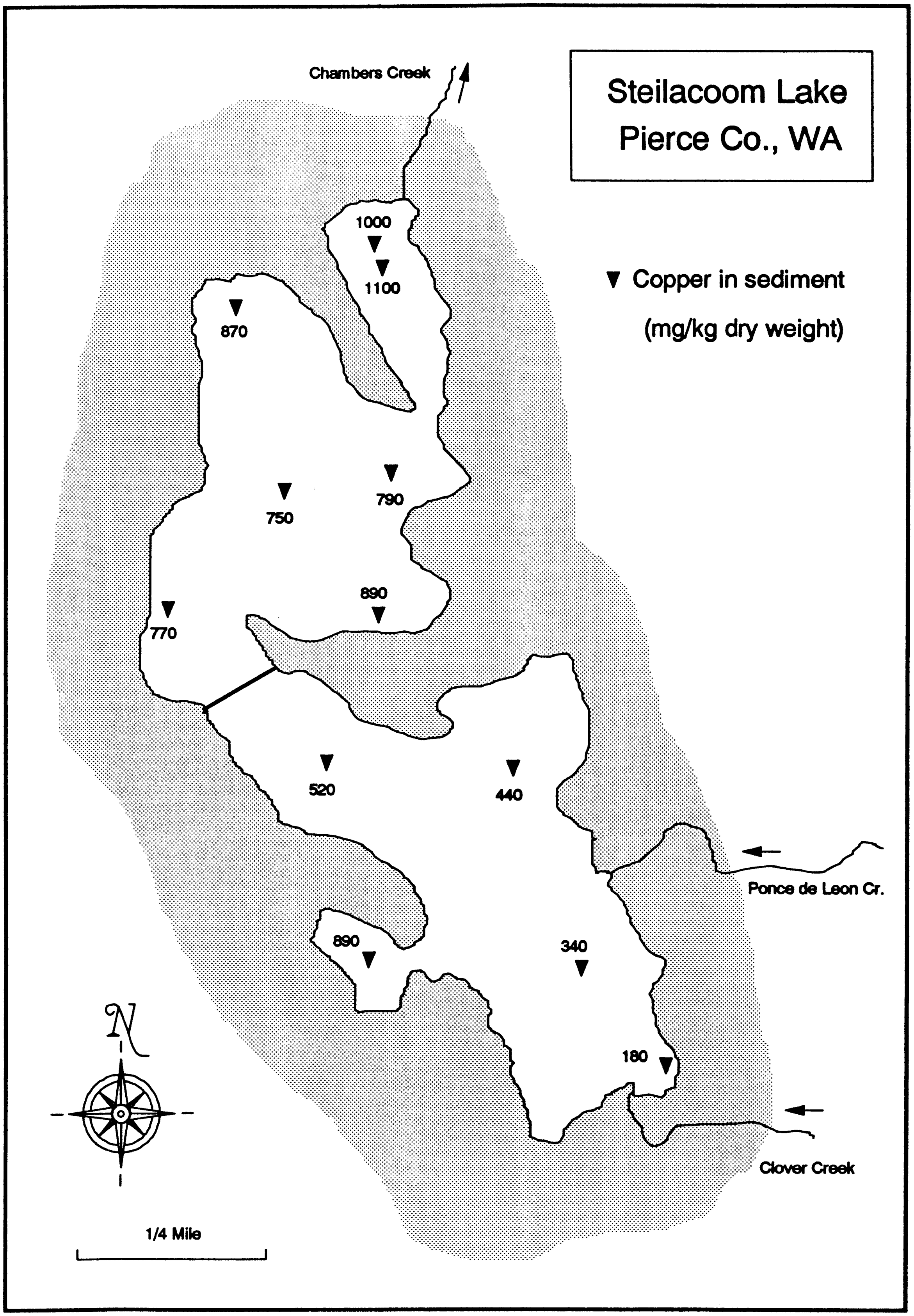

Figure 4. Copper in sediment at Phase I sites.

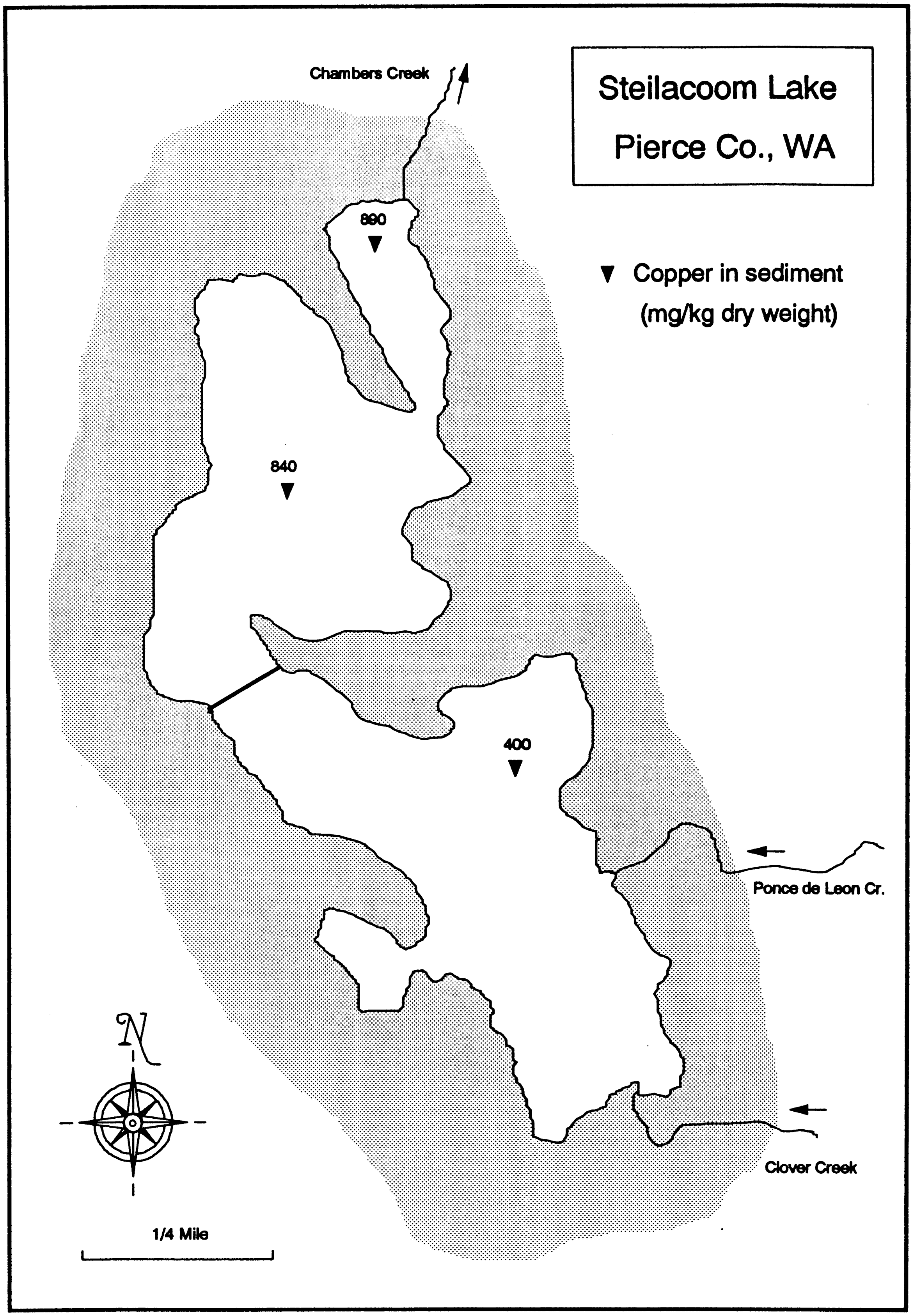

Figure 5. Copper in sediment at Phase II sites.

western part of the south basin with 890 mg/kg, may result from a low flushing rate to the main body of the lake. A comparison of Phase I and Phase II copper data shows agreement within approximately 20%. This agreement indicates a consistent pattern of copper distribution in the lake, as well as comparable sampling and analytical procedures. The low concentration of copper in Black Lake sediment, averaging 28 mg/kg, reflects the minimal level of copper sulfate treatment.

Other Metals

High concentrations of lead in Steilacoom Lake sediments in the south basin, at 260 mg/kg, reflect input by Clover and Ponce de Leon Creeks, probably from automobile exhaust, industrial, military, and other anthropogenic sources. Lower sediment lead concentrations in the north basin, averaging 150 mg/kg, and near the outlet, averaging 180 mg/kg, result from more localized contaminant sources such as storm sewers (no major stream inputs). The elevated lead levels of Steilacoom Lake, in its urban setting, contrast with the much lower levels of lead in rural Black Lake, 37 mg/kg. Zinc in Steilacoom Lake sediments averaged about 150 mg/kg in both the south and north basins, and averaged 215 mg/kg near the lake outlet. These values also reflect input from human activities. The zinc concentration in Black Lake sediment was 56 mg/kg.

Ratio of Copper to Acid Volatile Sulfides (Cu/AVS)

Cu/AVS ratios in sediments collected during Phase I were calculated from data supplied by EPA-Duluth. The ratios ranged from 0.3 to 59. The same data also showed that Cu/AVS ratios can vary significantly with storage. One sample changed from a ratio of approximately 5 to over 130 within 10 days. This low stability in Cu/AVS ratios may render any single measurement meaningless. The Duluth-EPA lab did not analyze the Phase II sediments for Cu/AVS, though these were presumed to be similar to the Phase I values (Ankley, Personal Communication).

Pore Water Metals

Data for pore water copper and other metals are given in Table 9.

Copper

Pore water copper concentrations near the outlet of Steilacoom Lake (440 μg/L) were nearly twice the concentrations in the north basin (240 μg/L) and almost three times higher than in the south basin (160 μg/L). These concentrations may, in part, reflect changes in the environmental conditions controlling copper distribution. They contrast with equivalent concentrations of total copper in sediment from both the north basin and outlet area; although differences between south and north basin copper concentrations are reflected in both total and pore water copper concentrations. The pore water copper concentration in the Black Lake reference sediment was 11 μg/L.

Other Metals

Lead in the sediment pore water was nearly three times higher (120 μg/L) in the south basin than in the north basin (40 μg/L), and was 92 μg/L near the outlet. These concentrations tend to parallel the sediment lead data and are also indicative of anthropogenic sources. Black Lake reference sediment pore water concentrations for lead (10 μg/L) reflect its rural setting.

Pore water chromium was below the detection limit for both lakes. Pore water arsenic, nickel, and zinc were present in low concentrations and are not considered a source of significant toxicity.

Bioassays

Steilacoom Lake and Black Lake bioassay results from samples collected during Phase II are summarized in Table 10. The results, by test, are described below.

Daphnia magna and *Ceriodaphnia dubia*

Daphnia magna bioassays performed on Steilacoom Lake sediments and Black Lake reference sediment showed no significant decrease in survival when compared to control sediment (Dunnett's Test).

Bioassays performed by Burton showed that only reference sediment from Black Lake was significantly toxic to *C. dubia* and *D. magna*. In an attempt to determine the effects of aeration on toxicity, sediment samples were mixed vigorously for one hour. Using mixed sediments, survival of *C. dubia* increased from 90% to 100% for Steilacoom Lake sediment from both the south basin and the north basin, and from 30% to 100% for Black Lake reference sediment. Mixing and aeration apparently removed the unidentified source of sediment toxicity. Survival was 100% in both aerated and nonaerated Steilacoom Lake sediment from the outlet area. Aerated sediment tests were not performed using *D. magna*.

In chronic tests, *D. magna* and *C. dubia* showed a significant decrease in the production of young only when tested on Black Lake reference sediment. Production of *C. dubia* young increased by nearly a factor of five when the sediment was aerated. Data are given in Appendix A (Burton, 1991).

Hyalella azteca

When exposed to sediment from the outlet area of Steilacoom Lake, 70% of the *Hyalella azteca* survived. The survival rate was 67% at the Black Lake reference site. These survival results were both significantly lower ($p<0.05$) than the control (Dunnett's Test), which averaged 90% survival. Survival was 80% at the south basin of Steilacoom Lake and 82% at the north basin, neither one being significantly lower than the control.

Table 10. Results – Steilacoom Lake and Black Lake Phase II sediment bioassays.

Phase II bioassays – percent survival (acute), production of young or percent weight reduction (chronic).

	Copper		*Daphnia*		*Daphnia*	*Ceriodaphnia*	*Ceriodaphnia*	*Hyalella*	*Chironomus*	*Hexagenia*	Microtox EC50			
Site	Sediment	Pore Water	*magna*		*Magna*	*dubia*	*dubia*	*azteca*	*tentans*	*limbata*	Manchester		Univ. of Minn	
Number	mg/kg dry	ug/L	(acute)		(chronic)	(acute)	(chronic)	(acute)	(chronic/acute)	(acute)	5 min.	15 min.	5 min.	5 min
ST4	400	160	90	97	44	90	18	80	6, 15	90	>100	>100	a	a
ST11	840	240	100	95	52	90	17	82	17, 14	100	a	a	a	a
ST12	890	440	100	98	48	100	17	$\boxed{70}$	14, 15	$\boxed{50}$	a	a	a	a
BL2	24	11	$\boxed{60}$	97	$\boxed{37}$	$\boxed{30}$	$\boxed{5.3}$	$\boxed{67}$	8, 15	100	$\boxed{73}$	$\boxed{63}$	>100	>100
Control	-	-	100	100	62	100	23	90	0, 14	90	b	b	–	–

Note 1: Sediments digested as total metals, pore waters digested as total recoverable metals. (–) = Analysis not done.

Note 2: Steilacoom Lake Phase I bioassays for Hyalella azteca were negative at all sites. Sediment copper range: 183–1100 mg/kg dry.

Note 3: Daphnia magna (acute) data from Wright State University and Manchester Environmental Laboratory, respectively.

Note 4: Daphnia magna (chronic) and Ceriodaphnia dubia (chronic) results given as production of young.

Note 5: Chironomus tentans – Chronic: percent weight reduction. Acute: number of survivors of 15 replicates.

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

Microtox – EC50 values at 5 and 15 minutes.

Microtox – (a) negative gammas (increased light output) which indicate low toxicity, (b) – no EC50 calculable from data.

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

ST = Steilacoom Lake.

BL = Black Lake reference.

Phase II microbial enzyme activities.

	Copper		Relative Enzyme Activity			
Site	Sediment	Pore Water				
Number	mg/kg dry	ug/L	APA	DHA	GA	GLU
ST4	400	160	110	52	8	110
ST11	840	240	69	25	4	62
ST12	890	440	52	6	1	18
BL2	24	11	98	30	5	66

Note 1: Sediments digested as total metals, pore waters digested as total recoverable metals.

APA = alkaline phosphatases; DHA = dehydrogenases (or electron transport system activity); GAL = b-galactosidase; GLU = b-glucosidase.

ST = Steilacoom Lake.

BL = Black Lake reference.

Phase I sediments sent to EPA-Duluth for Cu/AVS analysis were also tested with *H. azteca* in an effort to determine the validity of the Cu/AVS toxicity theory. The survival rate of *H. azteca* in Steilacoom Lake sediments collected during Phase I ranged from 85% to 100% for the 12 sites. None of these tests showed statistically significant reduction in survival (Ankley *et al.*, In Draft).

Chironomus tentans

C. tentans bioassays showed no significant decrease in survival or percent reduction in body weight with any sediment. The University of Minnesota report is given in Appendix B (Henry, 1991).

Hexagenia limbata

Bioassays using *Hexagenia limbata* had 50% survival with Steilacoom Lake sediment from the outlet. This result compares to 90% survival with both control and south basin sediments. Neither north basin sediment nor Black Lake reference sediment produced any reduction in *Hexagenia* survival. The University of Minnesota report is given in Appendix B (Henry, 1991).

Ostracods

Ostracod bioassays performed at Oak Ridge National Labs were invalid because of inconsistent and variable survival of the test organisms at successive sediment dilutions, exclusive of the controls. A modification of the test procedure may improve results (Stewart, Personal Communication).

Microtox®

Microtox® bioassays performed on sediment extracts at Manchester Laboratory showed evidence of toxicity in only the Black Lake reference sediment which produced a five minute EC_{50} of 73% and a 15 minute EC_{50} of 63%. Microtox® bioassays performed at the University of Minnesota showed no toxicity (results were either $EC_{50} > 100\%$ or negative gammas) with extracts from any sediment.

Microbial Enzymes

Microbial enzyme activity for alkaline phosphatases (APA), dehyrogenases (DHA), b-galactosidase (GAL) and b-glucosidase (GLU) decreased with increasing copper in Steilacoom Lake sediments (Figure 6) and in Steilacoom Lake pore water (Figure 7). Black Lake is not included in these figures because enzyme response was inhibited by some toxicant other than copper. No relationship was found with microbial enzyme activity and the Steilacoom Lake Cu/AVS ratios. The enzyme activity response of the reference sediment from Black Lake was intermediate between the south basin of Steilacoom Lake, which was always the highest, and north basin sediment, which was next highest.

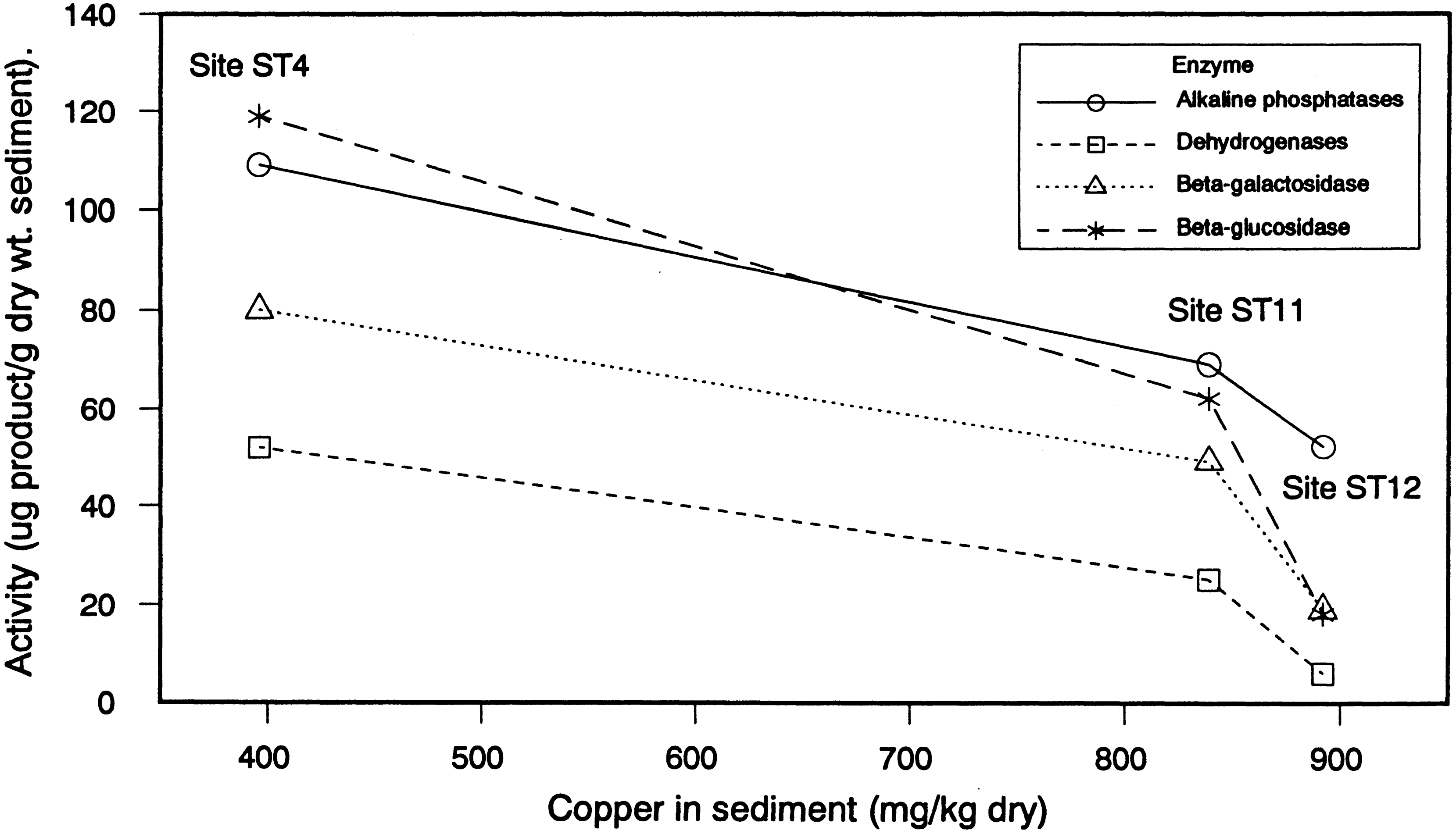

Figure 6. Bacterial enzyme activity vs. sediment copper concentrations.

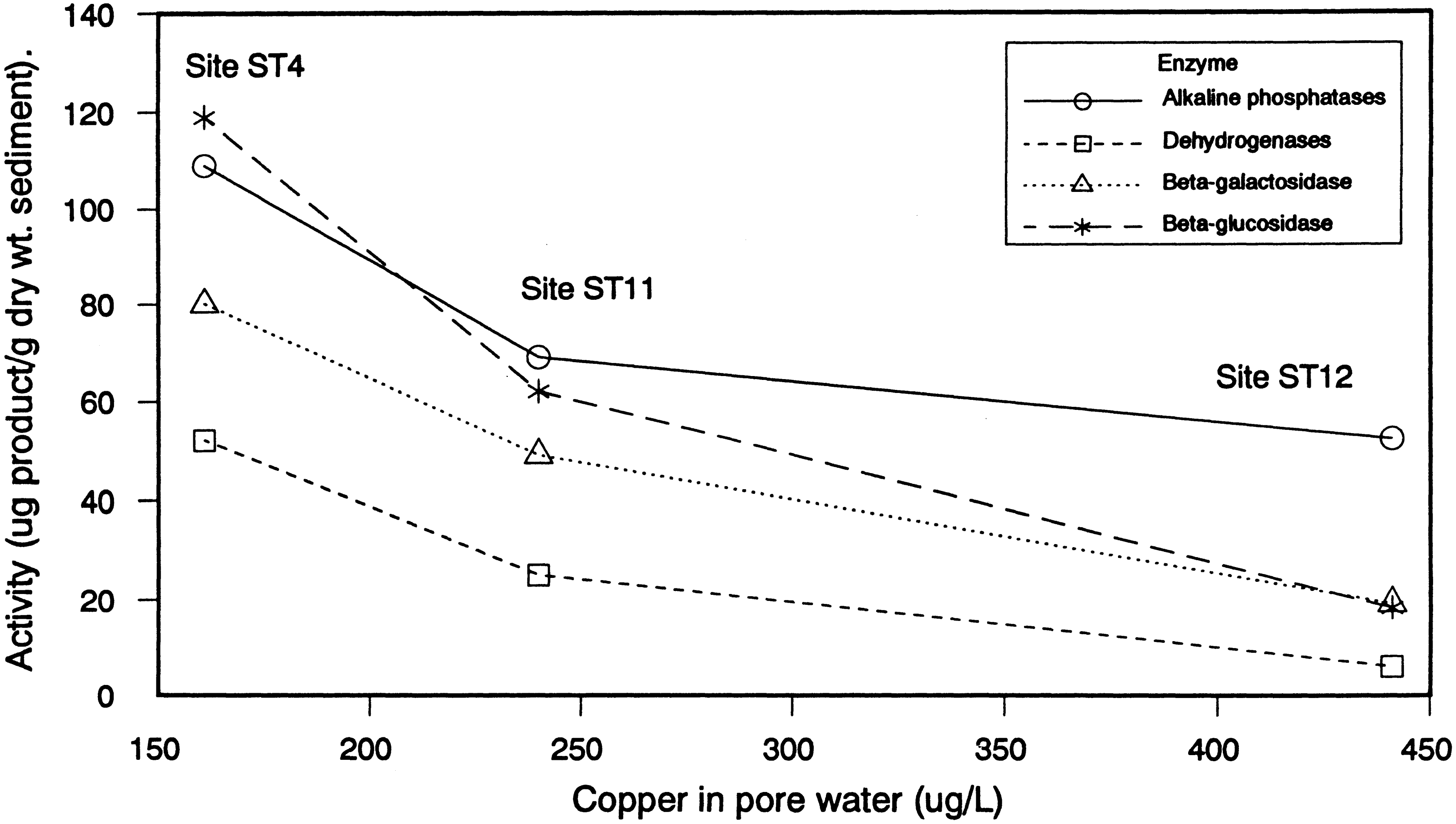

Figure 7. Bacterial enzyme activity vs. pore water copper concentrations.

Benthic Macroinvertebrate Identification and Analysis

Benthic macroinvertebrate samples from Steilacoom Lake and the reference, Black Lake, were identified at least to the genus level and to the species level where possible. A total of 33 taxa were identified including 12 annelid worms, nematodes, 8 crustaceans, 3 mollusks, 3 mites (Acari), and 7 insect taxa. Taxa richness ranged from eight to 18 per station. Taxa abundance ranged from 1366-5733 organisms per square meter. The Hilsenhoff Biotic Index (Hilsenhoff, 1987) used in the analysis assigns a tolerance from 0 (pollution intolerant) to 10 (pollution tolerant) for each taxa. The index sums the individual tolerance values of each taxa (multiplied by the log relative abundance) and divides by the number of taxa present. Hilsenhoff Biotic Index values for the Steilacoom Lake and Black Lake benthic samples ranged from 6.9 at the north basin site to 9.3 at the south basin site. Most taxa had indices of 8-10, which represents a highly tolerant range.

The Hilsenhoff Biotic Index was developed for lotic (running water) environments. It is used here because many of the Steilacoom Lake and Black Lake taxa are also found in slow-moving lotic waters. However, care should be used when interpreting these data. Examination of the functional feeding groups (FFG) present at each station indicated a high degree of community stress, especially at the south basin site. Analysis was also done of the taxonomic composition and contribution of the dominant organisms at each station. The dominant north basin organisms, such as *Ophidonais serpentina*, an oligochaete, and *Simocephalus vetulus*, a cladoceran, were among the least pollution tolerant of the organisms found in the lake. The dominant south basin organisms, such as *Chironomus* and *Bothrioneurum vejdovskyanum*, an oligochaete, were among the most tolerant.

The laboratory which identified the samples concluded that benthic communities at both Steilacoom Lake and Black Lake were highly stressed and indicative of significant water quality problems. It also noted both low taxa density and richness, and that many taxa were pollution-tolerant forms. The Western Aquatic Institute report is given in Appendix C (Wisseman, 1991).

DISCUSSION

Sediment Metals Compared to Available Guidelines

Copper

All Steilacoom Lake sediment copper levels exceed the Provincial Sediment Quality Guidelines severe effect level of 110 mg/kg (Persaud *et al.*, 1991). This severe effect level has been deduced from analysis of *in-situ* benthic communities and is presumed to be the level above which benthic communities would always be severely affected. They also exceed the 50 mg/kg guideline for copper in heavily polluted harbor sediments as defined by the USEPA, Region V (Bahnick *et al.*, 1981). These guidelines were originally developed for classifying Great Lakes harbor sediments, but were used mainly for determining the suitability of dredged material for open water disposal (Bennett and Cubbage, 1991).

Other Metals

Steilacoom Lake sediment lead levels in the south basin slightly exceed the Provincial Sediment Quality Guidelines, severe effect level of 250 mg/kg. All Steilacoom Lake sediments exceed the EPA Region V heavily polluted value for lead of 60 mg/kg. For zinc, all Steilacoom Lake sediment exceeds the Region V value of 90 mg/kg for moderate pollution. Only sediment near the outlet exceeds the Region V heavy pollution value for zinc of 200 mg/kg.

Ratio of Copper to Acid Volatile Sulfides (Cu/AVS)

Lack of significant toxicity to *H. azteca* from Steilacoom Lake sediment indicates the AVS theory might not apply to copper, at least in certain settings. In other studies of AVS theory, AVS provided reliable normalization of cadmium toxicity (DiToro *et al.*, 1990).

If we assume that Cu/AVS ratios were similar for both Phase I and Phase II samples from the same site, sediment from the north basin, with a ratio of 26, should have been the most toxic. However, this sample showed potentially toxic effects on microbial enzymes only. Sediment from near the outlet, with the lowest assumed Cu/AVS of 0.6, produced the greatest number of significant effects (*Hyalella*, *Hexagenia*, microbial enzymes), although these effects are not conclusively linked to copper. These tests show that a high Cu/AVS ratio does not necessarily indicate toxic conditions. One possible explanation is that copper may be bound in various phases, and may not always be free copper (Cu^{2+}). The lack of toxicity, despite Cu/AVS values >1, suggests the theory may not apply to copper in sediments. Ankley (Personal Communication) suggests that binding phases other than AVS may control copper availability in freshwater sediments. These phases have not yet been identified.

Pore Water Copper and Lead (Total Recoverable) Compared to EPA Criteria

All pore water copper concentrations in Steilacoom Lake exceed the EPA acute freshwater criteria of 18 μg/L (based on hardness of 100 mg/L as $CaCO_3$, see subsequent discussion). Pore water copper concentrations at the south basin site exceed the EPA criteria by a factor of 9, at the north basin site by a factor of 13, and at the outlet site by a factor of 25.

Lead in pore water from both the south basin and the outlet of Steilacoom Lake exceed the EPA acute freshwater criteria of 82 μg/L. Pore water from both the north basin of Steilacoom Lake and Black Lake exceed the EPA chronic freshwater criteria for lead of 3.2 μg/L (USEPA, 1986a). Note that these criteria are hardness ($CaCO_3$) dependent and are based on a hardness of 100 mg/L. The hardness of the pore water itself was not determined because of insufficient sample volume. Therefore, these criteria should be considered approximations of the actual site-specific toxic thresholds.

Bioassays

Low *Hexagenia* survival from sediment near the outlet contrasts with 100% survival from sediment in the north basin. This result indicates toxic conditions in Steilacoom Lake sediment near the outlet. Sediment-bound copper was probably not responsible for the low level of *Hexagenia* survival because both sites had similar copper levels. Ammonia was probably not responsible either, since concentrations in the south basin (with 90% survival) and the outlet were similar. Lead was sometimes present at relatively high levels in both Steilacoom Lake sediment and pore water, although its concentrations were also inconsistent with the bioassay results. However, lead, ammonia, and other toxicants may still be contributing to the lake's overall ecological degradation. *Hexagenia* survival is consistent with variations in pore water copper. A survival rate of only 50% at the outlet site, with 440 μg/L copper, was significant. Ninety percent survival was not significantly different than the control for south basin sediment with 160 μg/L. There was 100% survival at the north basin sediment that had 240 μg/L copper in the pore water. These pore water copper values exceed the LC_{50} for *Hyalella azteca* of 28 μg/L copper reported by EPA-Duluth (Ankley *et al.*, In Draft).

A statistically significant reduction in survival of *H. azteca* was seen only at the outlet site. The reason pore water was not significantly toxic to either *Hexagenia* or *Hyalella* at the other two sites may be related to the chemical forms, as well as concentrations, of the copper at each site. The toxicity of many metals depends in part on speciation, or the chemical form of the metal. Copper associated with complexes and precipitates (often as fine particulate matter) is generally far less toxic than the equivalent amount of ionic copper (Cu^{2+}). A higher concentration of dissolved copper at the outlet than at the other two sites could have caused the increased toxicity. Because pore water samples were not filtered, we could not differentiate the effects of total and dissolved copper. Sample filtration may resolve these questions in future studies. The complexities of copper speciation, toxicity, and analysis are discussed in USEPA (1980).

The inverse correlation of microbial enzyme response to sediment copper and pore water copper concentrations indicates that the high copper concentrations could be causing enzymatic impairment. Microbial enzymes may be more sensitive than other tests used in this study. However, the ecological significance of these data remains equivocal, especially since factors other than copper may have been responsible.

C. dubia, *D. magna*, and *H. azteca* tests on Black Lake reference sediment indicate the presence of one or several toxic components. Toxicity declined following vigorous aeration. Either an unidentified volatile compound or a reducing agent such as H_2S may have been responsible (Burton, 1991).

A detailed discussion and evaluation of the freshwater bioassays used is available through Ecology's Sediment Management Unit (Bennett and Cubbage, 1992).

Benthic Interpretation

Analysis of benthic macroinvertebrates using taxa diversity, taxa richness, the Hilsenhoff Biotic Index, and other parameters indicates significant water quality impairment at both Steilacoom Lake and Black Lake, as well as populations consisting predominantly of pollution tolerant organisms. Unfortunately, these tests cannot determine the actual cause of impairment. Therefore, the degree to which the addition of copper, in relation to other potential pollutants, has influenced the benthic community and overall health of Steilacoom Lake could not be determined from the available data.

Benthic macroinvertebrates were generally sparse and population counts resulted in low levels of agreement between replicate samples. There was one significant problem at the north basin site where a sample with an algal mat had much higher counts than samples without a mat. This probably occurred because the mat inhabitants, which do not represent the infaunal community, were protected from the effects of toxic sediment. During future sampling trips, those samples which are obviously unique should be rejected.

SUMMARY

Copper concentrations in Steilacoom Lake sediment exceed the Provincial Sediment Quality Guidelines, severe effect level of 110 mg/kg dry weight, established by the Ontario Ministry of the Environment. Pore waters in Steilacoom Lake sediment exceed the concentration of 28 μg/L shown by EPA-Duluth to be toxic to *Hyalella azteca*. Low levels of survival for *H. azteca* and *Hexagenia limbata*, and decreasing microbial enzyme activity, correlated with increasing pore water copper concentrations.

Comparisons of Steilacoom Lake bioassay results to the reference lake (Black Lake), which had far lower concentrations of copper, were inconclusive due to an unknown toxicant in Black Lake that caused reduction in survival in some bioassays. The effect of this toxicant appears to decrease with increased sample aeration.

CONCLUSIONS

1) Steilacoom Lake has sediments with high concentrations of copper and other metals.

2) *Hexagenia,* microbial enzyme activity, and *Hyalella azteca* responses were consistent with variations in copper concentrations measured in pore water from sediment samples. Bioassay results were less consistent with total copper concentrations in sediments. Microtox® showed toxicity only at Black Lake.

3) Water column bioassay organisms *Daphnia magna* and *Ceriodaphnia dubia* were not affected by Steilacoom Lake sediments. The benthic organism *Chironomus tentans* also showed no toxic response.

4) Benthic macroinvertebrate indices demonstrated significant water quality impairment and pollution tolerant macroinvertebrate populations at both Steilacoom Lake and Black Lake. However, the cause of these problems could not be conclusively traced to metals concentrations.

5) Benthic organisms are more likely to respond to sediment contaminants than organisms associated with the water column. For this reason, benthic organisms may be more appropriate choices as bioassay organisms to evaluate sediment toxicity.

6) Sediments with high Cu/AVS did not show a higher degree of toxicity as would be predicted by theory. The theory is limited in its ability to predict toxicity of copper.

7) Sediment pore water copper may be a better indicator of sediment toxicity than sediment copper or Cu/AVS.

8) Black Lake reference sediment was unacceptable because it contained unidentified toxic components. These components were removed by vigorous aeration and mixing.

9) High ammonia levels (700 to 960 mg/kg dry weight) were found at all Phase II sites.

RECOMMENDATIONS

1) Use bioassays, such as *Hexagenia limbata,* which maximize sediment exposure to test organisms.

2) With metals contaminated sediments, analyze for both sediment and pore water metals concentrations. Compare variations in metals concentrations to sediment bioassay results.

3) Discontinue use of Cu/AVS analyses until the theory is better refined and validated.

4) Identify and test an alternate reference lake for use in subsequent freshwater sediment studies.

5) Continue to investigate the use of benthic indices for determining the effects of pollutants on aquatic systems. Include protocols for accepting or rejecting benthic samples.

6) Determine the source(s) of high ammonia concentrations in Steilacoom Lake sediments and their impact on lake ecology.

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APPENDIX A

Sediment Toxicity Assessment of Steilacoom Lake

Wright State University

Department of
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Dayton, Ohio 45435
513/873-2655

February 19, 1991

Jon L. Bennett
Washington State Department of Ecology
Bldg. 8, LH-14
7171 Cleanwater Lane
Olympia, WA 98504

Dear Jon:

Enclosed you'll find a copy of my report for the Steilacoom Lake project. We had some interesting results. The source and agent causing Black Lake sediment toxicity should be investigated. The fact that aeration removed toxicity suggests a volatile or reducing compound is causing the problem. AVS is not an important factor at these sites. The microbial community of Steilacoom Lake is being impacted. This may have a significant effect on the community structure and ecosystem functioning of the lake, now or in the future.

I would like for us to publish this study in Environmental Toxicology and Chemistry, using all the chemical and biological data collected. Let me know your thoughts on this. I doubt I'll have time to take the lead until late summer.

I enjoyed working with you and look forward to future studies.

Sincerely,

G. Allen Burton, Jr., Ph.D.
Associate Professor

Enclosures

xc: Craig Smith

Sediment Toxicity Assessment

of

Steilacoom Lake

by

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Submitted to:

State of Washington
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7171 Cleanwater Lane
Building 8, LH-14
Olympia, WA 98504

Project Officer, Jon L. Bennett
Contract D3500

February 19, 1991

Background

The Washington State Department of Ecology designed a study (1) to assess the possible toxicity of Steilacoom Lake sediments which are heavily contaminated with copper. The study design included multiple toxicity assays comprising key trophic levels and chemical and benthic community analyses. Since the U.S. Environmental Protection Agency is considering Acid Volatile Sulfides (AVS) as a sediment criteria normalization tool for metals, this parameter was also measured. The study was coordinated by Jon L. Bennett of the Department of Ecology. Guidance on sample collection and study design was obtained from the collaborating investigators, which included: Allen Burton (WSU), Gary Ankley (USEPA), Art Stewart (Oak Ridge) and Margaret Stinson (State of Washington).

Sample Collection and Processing

Samples were collected, composited and shipped by Department of Ecology personnel. Black Lake (#B2-2) was used as a reference sediment. Three sites were selected on Steilacoom Lake based on historical sediment copper and AVS data, and comprised a gradient of contamination from low to high (#S2-4, S2-11, and S2-12, respectively). Samples were shipped via overnight express, on ice, and were received at WSU cold.

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, dissolved oxygen, total ammonia, and free copper. All parameters were measured in pore water and overlying waters of the test beakers; except NH_3, Cu, and conductivity were only measured in pore water. Overlying waters were sampled at test termination and returned to the Department of Ecology for further analyses.

Short-term chronic toxicity was determined in whole sediment exposures following draft ASTM methods (4) for cladocerans (see Appendix A). Test species were Daphnia magna and 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 copper were measured at test initiation and termination.

Indigenous microbial enzyme activity was measured as follows. Approximately 1 to 2 ml of cold homogenized sediment was placed in triplicate test tubes containing buffer. Enzyme substrate, for example, *p*-nitrophenyl-B-D-glucoside, was added to the tubes, vortexed, and incubated in the dark at 25°C for 30 min to 2 h. Activity was terminated by placing the tubes on ice or adding 1 to 2 ml acetone, vortexing, and centrifuging (4424 X *g*) for 10 min. The colored reaction product in the supernatant was then measured spectrophotometrically. Substrate was added after activity termination for control tests. Absorbance was converted to µg of product formed using a standard curve and activity defined as product formed per milliliter of gram dry weight of sediment per incubation time. Sediment dry weight determinations were conducted in triplicate with each assay. Microbial activity tests included alkaline phosphatase, dehydrogenase (electron transport activity), β-galactosidase, and β-glucosidase.

Results and Preliminary* Discussion

Summary results are provided in Table 1, including toxicity test responses, free copper and total NH_3 data. Free copper is the toxic form of the metal and was not present at toxic levels. Ammonia concentrations were also low and the below neutral pH reduced unionized ammonia formation.

The sediments from Steilacoom Lake were not toxic to the zooplankton test species; however, indigenous microbial activities were significantly depressed and showed a total copper concentration-response relationship. Sediment S2-12, which apparently had the highest total copper concentrations, had the lowest microbial activity. The sensitivity of sediment microbial communities to toxic contaminants has been demonstrated at every site tested by the P.I., in locations throughout the United States (3). They have been recommended as early warning indicators of ecosystem perturbations by Odum (8) and as criteria tools by others (9). Since these enzyme systems and communities are integral components of key biogeochemical cycling processes (10) and aquatic food webs (11), it is essential that their responses be considered in any ecosystem impact assessment.

The reference sample from Black Lake was toxic to the zooplankton test species. Copper and ammonia concentrations did not appear to be the causative agents. It is of interest that intense aeration of the sediment sample removed the toxicity. This indicates that AVS:metal interactions were not important, as removal of AVS should increase toxicity. It is possible that either a volatile compound or reducing agent (such as H_2S or a heavy metal in a reduced valence state) was lost or oxidized to a less toxic and less available complex. Further study will be required to determine the cause of toxicity.

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* 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.

The toxicity levels observed at Black Lake and Steilacoom Lake were significant to different trophic levels and indicates possible ecosystem degradation. In addition, these results add support to the premise that a multi-trophic level assessment approach is essential to ensure impact detection and to better interpret the significance of sediment contamination.

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Table 1. Data Summary for Lake Steilacoom Study

Assays: *Ceriodaphnia dubia* and *Daphnia magna* 3 brood survival/reproduction, indigenous microbial activities (alkaline phosphatases, dehydrogenases, β-galactosidase, β-glucosidase)

Test phase: Whole sediment

Sample date: 26 and 27, November, 1990

Sample stations: B2-2 (488158), S2-4 (488155), S2-11 (488156), S2-12 (488157)

Assay Responses (x ± SD)[a]

	C. dubia		*D. magna*			Microbial activity[b]			Cu++	NH_3
Sample	Survival	Young	Survival	Young	APA	DHA	GAL	GLU	(ug/l)[e]	(total)[e]
Control[c]	100	23.4 (3.8)	100	62.4 (3.1)	NA	NA	NA	NA	0	0
B2-2	30	5.3 (8.6)	60	37.3 (13.1)	97.6 (2.4)	30 (4.2)	54.9 (7.6)	66 (2.6)	1.12	3.02
(Mixed)[d]	100	24.9 (2.9)							8.31	3.26
S2-4	90	18 (4.8)	90	43.5 (7.1)	109.2 (2.9)	52 (6.7)	79.5 (3)	113 (13)	1.76	1.58
(Mixed)	100	16.2 (5.6)							2.52	2.04
S2-11	90	16.9 (4.6)	100	51.7 (7.3)	68.8 (8.2)	24.8 (5)	48.7 (1.2)	61.8 (5.4)	0.36	0.76
(Mixed)	100	22 (4.4)							0.54	1.01
S2-12	100	17.3 (6.1)	100	48.1 (5.9)	51.8 (7.4)	5.8 (1.2)	18.6 (1.8)	17.9 (5.3)	0.39	1.19
(Mixed)	100	19.6 (6.4)							0.51	1.64

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

[b] APA = alkaline phosphatases; DHA = dehydrogenases (or electron transport system activity) using the tetrazolium salt, INT; GAL = β-galactosidase; and GLU = β-glucosidase.

[c] Moderately hard reconstituted water (used also as a culture water for the test organisms).

[d] Sediments aerated vigorously for 1 hr prior to testing.

[e] Free copper and ammonia measured in interstitial water with ion selective electrodes.

APPENDIX B

Bioassessment Analysis of Steilacoom Lake Sediments

BIOASSESSMENT ANALYSIS OF STEILACOOM LAKE SEDIMENTS

MINNESOTA
COOPERATIVE
FISH & WILDLIFE
RESEARCH
UNIT

FOCUS: HUMAN IMPACTS ON THE ENVIRONMENT

Mary G. Henry, Leader

Sylvia Morse and Donald Jaschke
University of Minnesota

INTRODUCTION

The assessment of the adverse effects of contaminated sediment on fish and invertebrate populations exists as a major problem for aquatic toxicologists. Contaminant material generally precipitates, forms various complexes or adsorbs and binds to particulate matter (Giesy et al., 1990). Ultimately, the sediment serves as the final repository for the pollutant. Benthic organisms can be directly impacted via the ingestion of particulate matter or continual re-exposure due to leaching and resuspension of the contaminant material resulting from physical disturbances to the sediment (Giesy et al., 1988). The bioavailability of sediment contaminants depends on many factors including the physical properties of the sediment and the contaminant and the physical and biological properties of the overlying water. Water quality criteria are based on the concentration of a particular substance in solution in the water column. Sediment criteria have only recently begun to be established.

COPPER TOXICITY

Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) is a registered pesticide for use in fish food ponds to control nuisance algal blooms and eradicate protozoan fish parasites (Boyd, 1979). Copper sulfate dissociates in water to give cupric ion, Cu^{2+}, which inhibits both respiration and photosynthesis in algae. Free unchelated copper (Cu^{2+}) is the toxic component to fish. Copper sulfate concentrations which exhibit no short-term effects may pose problems upon chronic exposure. Cupric ions precipitate as malachite ($Cu_2(OH)_2CO_3$) when the pH is below 7 and as tenorite (CuO) above pH 7. The solubility and toxicity of these compounds depends on the pH and alkalinity of the medium. Copper sulfate is more toxic to algae in soft, acidic water than alkaline waters. Within 1-2 weeks after the application of copper sulfate, copper levels in the water returned to pre-treatment levels.

STUDY OBJECTIVES

1. to conduct a series of toxicity test evaluations on sediment samples from several locations in Steilacoom Lake to determine the biological impacts of elevated copper concentrations in sediment with two benthic invertebrates.

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

METHODS AND MATERIALS

STUDY AREA

Steilacoom Lake is a shallow urban lake located three miles east of Steilacoom, Washington. Largely due to the highly developed residential community surrounding the lake, runoff to the lake has a high concentration of nutrients causing unacceptably high levels of aquatic vegetation. The lake has received chemical treatment since 1955 to control aquatic flora including copper sulfate to control nuisance algal blooms.

Black Lake is a shallow glacial lake with a history of infrequent treatments for control of aquatic vegetation. Black Lake was selected as a reference site.

In order to determine potential toxicity to biota, sediment samples were collected for analysis. Three samples were collected from Steilacoom Lake and 1 reference sample from Black Lake. Collection of material followed the protocol described by Jon Bennett, Dept. of Ecology, State of Washington (personal communication, December, 1990). Sediment samples were collected using a Van Veen grab sampler and homogenized in a stainless steel bucket. Subsamples were subsequently removed for various bioassessment and chemical analyses. Samples for toxicity testing using *Chironomus tentans* and *Hexagenia limbata* were placed in coolers immediately after collection and shipped within 24 hours to the Minnesota Cooperative Fish and Wildlife Research Unit, St. Paul, MN. The samples were held at 4°C until bioassessments were completed.

TEST PROCEDURE

Two toxicity tests were performed to assess the presence of potentially toxic levels of copper in sediment samples. These test procedures were developed to determine the toxicity under controlled laboratory conditions of sediment-bound contaminants using representative species from the aquatic benthic community. Bulk-sediment toxicity was assessed using a ten day (10-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* and other aquatic organisms and has produced healthy and robust cultures, justifying its use as the aqueous 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.), is 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 10 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 treatment group consisted of 10 replicate test chambers. Chambers were held under low light conditions in a flow through water bath at 18^{o} 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 Steilacoom Lake. The MICROTOX® toxicity test assessing pore-water extracted from bulk sediment showed no toxic response in any of the sediment samples from Steilacoom Lake or the reference site, Black Lake.

Chironomus tentans

A 10 day *C. tentans*, partial life-cycle toxicity test was conducted to assess the potential toxicity and determine the biological impact of copper contaminated sediments in Steilacoom Lake. Mortality and reduction in body weight were the measured endpoints of the test. Individual midge dry weights are shown in Table 1. For statistical purposes a dead midge was recorded as having 100% reduction in body weight (Giesy et al., 1990). The percent reduction in body weight, shown in Fig. 1, is a measure of growth reduction and mortality. Individual mortality of *C. tentans* was insignificant with only two individuals dying in the 10 day test period. With negligible mortality, percent reduction in body weight is an estimate of the toxic effect the contaminant has on growth of the organism. From Fig. 1 the highest percent reduction in body weight is 17.15 from site S2-11. A one tailed t-test comparing the control and S2-11 showed that the test result is statistically insignificant (P=.10) inducing no appreciable weight loss in exposed midge.

Hexagenia limbata

Hexagenia limbata, a burrowing mayfly, was incorporated into a toxicity test to assess potential toxicity of bulk-sediment taken from Steilacoom Lake. Extremely high concentrations of copper exist in the sediment of Steilacoom Lake due to repeated use of copper sulfate pentahydrate as an algacide in the lake. The 10-d *H. limbata* toxicity test was conducted and daily observations of molting frequency and mortality were noted. The percent mortality by site of *H. limbata* is shown in Figure 2. Site S2-12 revealed 50% mortality of *H. limbata* after exposure to sediment over the 10-d test period.

CONCLUSION

Slight toxicity was detected in samples from Steilacoom Lake. The site showing the highest percent body weight reduction of *C. tentans* was S2-11. This percentage was shown to be statistically insignificant in comparison to the control. No significant mortality of *C. tentans* was observed in the test. The percent mortality of *Hexagenia limbata* for site S2-12 was 50%. This site, while not producing significant mortality in the *C. tentans* toxicity test, has provided evidence that there may be a possible contaminant problem in the vicinity where this sample was collected based on the *H. limbata* mortality. Contaminant induced biological effects from Lake Steilacoom site S2-12 is of concern. The other sampling site(s) on Lake Steilacoom and Black lake are not considered to have biological detectable levels of contaminant related toxicity.

TABLE 1. *C. tentans* individual dry weights and statistics.

REPLICATE	S2-4	S2-11	S2-12	B2-2	C1-Sed.
1	3.2 mg	2.7 mg	3.4 mg	3.3 mg	0.0* mg
2	4.6	1.7	2.8	2.5	5.1
3	3.1	3.6	2.6	4.3	3.3
4	3.7	3.6	2.2	3.5	2.5
5	2.7	2.9	2.4	3.2	4.3
6	3.9	3.0	2.3	2.9	4.3
7	2.5	3.5	3.6	3.0	2.7
8	3.0	2.6	2.2	2.6	4.2
9	3.6	2.3	1.9	2.7	3.4
10	3.0	3.6	3.4	2.9	0.7
11	3.2	1.8	2.9	2.7	3.4
12	2.4	3.4	3.8	2.5	3.5
13	1.9	3.1	2.9	2.4	4.1
14	1.6	2.3	2.4	3.1	1.6
15	3.2	0.0*	2.7	3.1	5.3
MEAN	3.04	2.67	2.77	2.98	3.23
S. DEVIATION	0.7392	0.9405	0.5485	0.4693	1.4654
MAXIMUM	4.6	3.6	3.8	4.3	5.3
MINIMUM	1.6	0.0	1.9	2.4	0.0
VARIANCE	0.5854	0.9478	0.3224	0.2360	2.3007
% REDUCTION	5.79	17.15	14.26	7.64	0.00

* indicates mortality and is recorded as a 100% reduction in body weight.

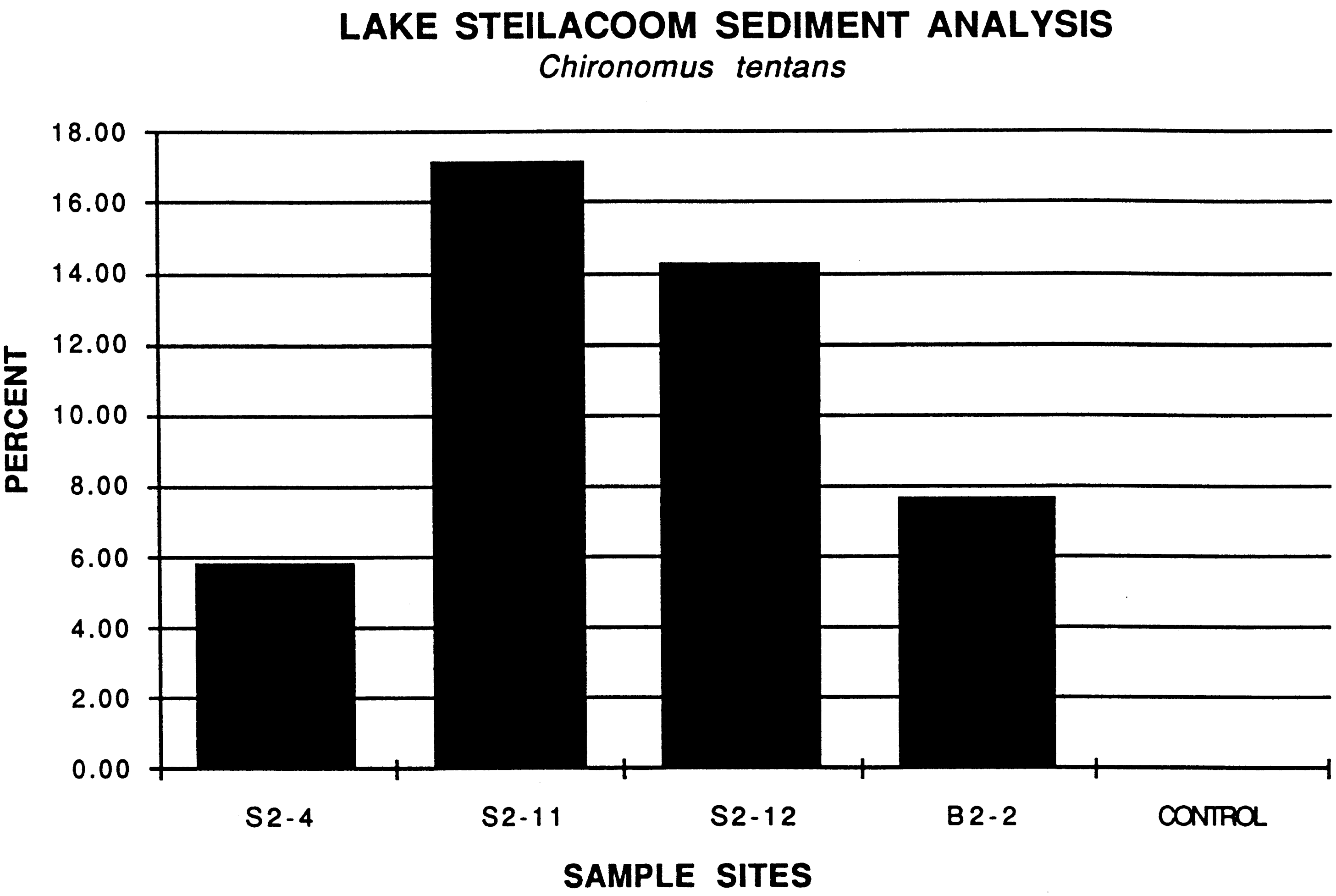

FIG. 1. *Chironomus tentans* percent reduction in body weight as compared with the control.

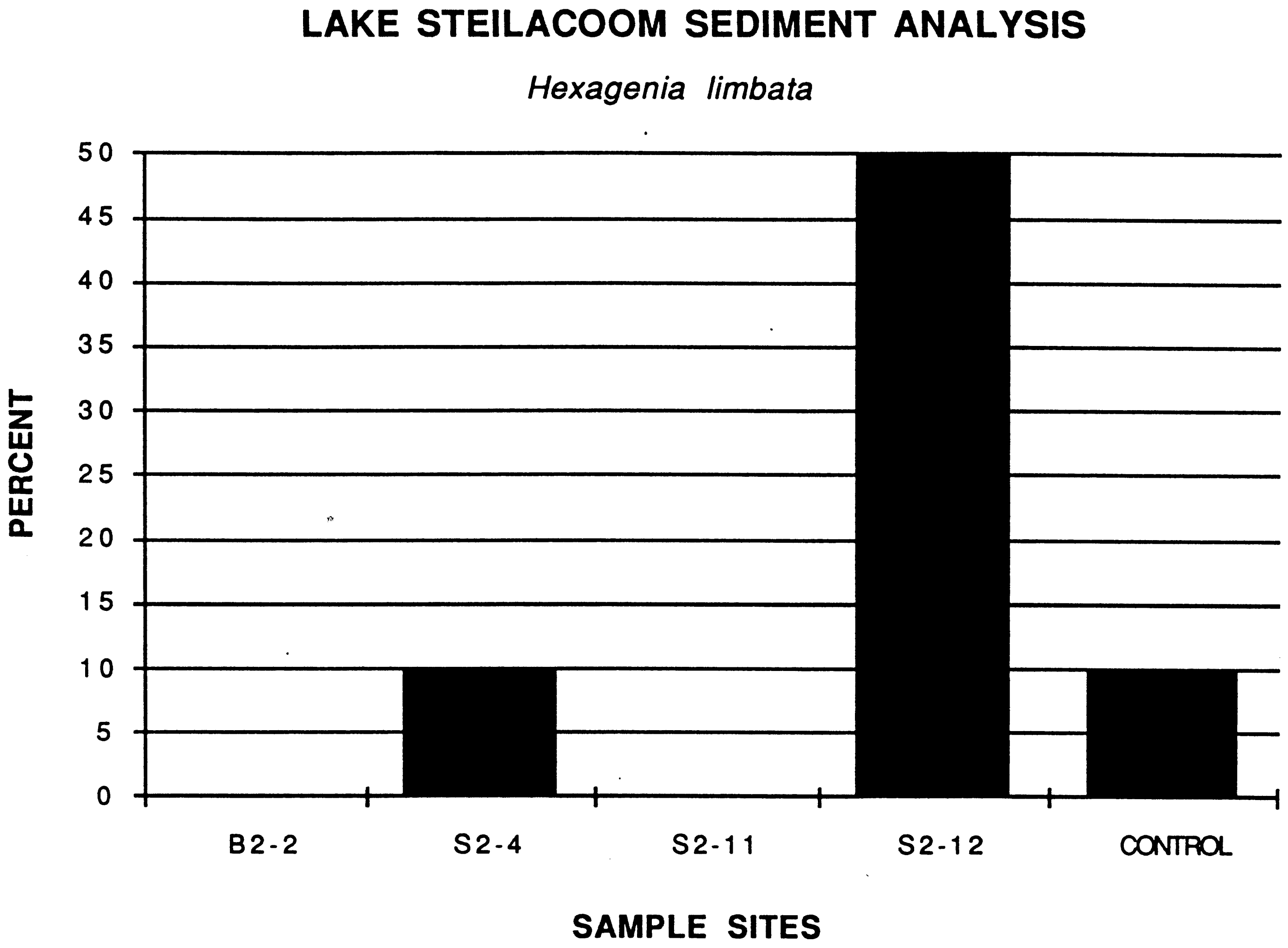

FIG. 2. *Hexagenia limbata* mortality expressed in percent.

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Personal communication: Jon L. Bennett, Environmental Scientist, Toxic Investigations, Department of Ecology, State of Washington, Olympia, WA, memo: December 4, 1990.

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APPENDIX C

Benthic Invertebrate Samples from Black and Steilacoom Lakes

WASHINGTON DEPARTMENT OF ECOLOGY

Benthic Invertebrate Samples from Black and Steilacoom Lakes.
Petite Ponar Dredge Samples taken November 26-27, 1990.

Robert W. Wisseman, Western Aquatic Institute, Corvallis, OR.
503-752-1568. May 1991.

INTRODUCTION AND METHODS

Benthic samples from shallow-water, soft-sediments of Steilacoom and Black Lakes, WA were taken by WA DOE personnel in November 1990 to conduct a pilot study on how benthic macroinvertebrates can be used as a biomonitoring tool in water quality assessment.

A total of 12 samples from Steilacoom (3 stations X 4 replicates/station) and 4 samples from Black (1 station X 4 replicates) were taken with a petite ponar dredge. Sediments were sieved through 500 micron mesh. The sieved Steilacoom Lake samples consisted of ca. 250-500 ml. of fine mineral and organic sediment. Invertebrates from at least a 50 % fraction of these samples were sorted with the aid of a 6X dissecting scope. Sieved sediments from the Black Lake station consisted of 750-1000 ml. of fragmented vascular plant detritus (primarily sedge leaves), fine particulate organic matter (FPOM), and silt. Invertebrates from the entire sample were sorted.

Taxonomic identifications were made to the lowest practical level by a team of specialists under the direction of R.W. Wisseman. Refer to the attached annotated taxa list for persons responsible for identifications.

RESULTS & DISCUSSION

A total of 33 taxa were identified from the benthic samples. These included 12 annelid worms, nematodes, 8 crustaceans, 3 molluscs, 3 mites (Acari), and 7 insect taxa. Taxa abundances for each replicate are recorded in the following data tables. Mean abundance, standard deviation, and percent contribution of taxa for each station is calculated. Note that mean abundances have been adjusted to a square meter basis.

Summary statistics and several indices have been calculated and provided for each set of station data. A brief discussion of the results is provided below.

There is little comparative benthic macroinvertebrate data available from lakes in the Puget Sound Trough Ecoregion. Thus, it is difficult to objectively interpret how the benthic communities present at the Black and Steilacoom Lake stations reflect water quality conditions, since there is no comparative data base developed from an investigation of benthic communities in a range of lake types and impact levels in the region.

It is presumed that the 4 samples per station are fairly representative of shallow, open-water areas in these two lakes, though the spatial heterogeneity of substrate types and faunal communities of the lakes is not yet known.

Taxa richness: The number of taxa present at a station ranged from 8 to 18. This included taxa that are primarily planktonic such as *Daphnia* and *Chaoborus*. From my experience with oligotrophic lakes in the Oregon and Washington Cascades, where taxa richness may range from 50-100+ taxa in shallow water sediments, the fauna of Black and Steilacoom lakes is comparatively depauperate.

The Oligochaeta were the most diverse group present. Drs. Brinkhurst and Kathman will be able to provide a more extensive interpretation for this group.

Only 7 insect genera were recorded from the two lakes and 4 stations. Genera belonged to the chironomid, ceratopogonid and chaoborid midges (Diptera). There were only 5 chironomid midge taxa present in the samples from the 2 lakes, with most stations having only 1 or 2 taxa present. I would expect 15+ taxa to be present in shallow waters of most lakes of good to moderate water quality. The chironomid taxa that did show up in the Black and Steilacoom Lake samples are commonly found in the littoral zone of reservoirs (a disturbed habitat) in the northern hemisphere, and do not appear to occur as commonly in natural lakes of good to fair water quality (Rosenberg et al. 1984, Saether 1979).

Caddisflies (Trichoptera), mayflies (Ephemeroptera), true bugs (Hemiptera), alder flies (Megaloptera), dragon- and damselflies (Odonata), and beetles (Coleoptera) were conspicuously absent. One would expect some representation of these taxa even in soft, shallow, open-water sediments. Mollusc diversity was also low. I suggest some qualitative dip-net samples be taken from littoral zone habitats (including macrophyte beds), to determine if these groups are present in the lakes.

Taxa abundance: Total abundances ranged from 1366-5733 organisms per square meter. As for taxa richness these values are considerably lower when compared with densities found in the littoral zone of Cascade lakes. I do not know what would be a typical density for lowland lakes of the Puget Sound Trough Ecoregion.

Hilsenhoff Biotic Index: This is a saprobic index developed for detecting organic enrichment in lotic environments. Since many of the taxa found at the Black and Steilacoom stations are also found in slow-moving lotic waters, it is instructive to calculate this index. Caution should be taken in interpreting the results, since the index was designed for lotic waters.

Basically, the Hilsenhoff Biotic Index assigns a tolerance value (from 0-10) to each taxa, based on the tolerance that taxa displays towards organic enrichment and depressed DO conditions. Organisms assigned a 0 are highly intolerant, while those assigned a 10 are highly tolerant. The index sums the individual tolerance values of each taxa (multiplied by the log relative abundance) and divides by the number of taxa present. The resultant integrated index can have values from 0-10. Water quality classes are described in the attached table.

For the taxa present at Black and Steilacoom Lakes, tolerance values ranged from 5-10. Most taxa were in the 8-10 range, or highly tolerant. Index values calculated ranged from 6.9 at station S2-11 to 9.3 at station S2-4. For lotic waters (including slow-moving ones), these values indicate poor to severe water quality problems and enriched conditions. Station S2-4 had very poor water quality as indicated by this index. This station also had the lowest taxa richness and densities. Station S2-4 was also dominated by midge and oligochaet taxa that are generally indicative of severely polluted conditions.

Functional feeding group (FFG): The attached data tables provide an analysis of the FFG's present at the stations. Since the samples were taken in soft sediments high in fine particulate organic matter (FPOM), it is no surprise that collector-gatherers were dominant. Station S2-11 had the most diverse array of feeding types, with some indication that macrophyte beds were located in the general vicinity. Only collector-gatherers and predators were present at station S2-4. This is another indication that the community present at this station is severely stressed.

Dominant Taxa: This metric is used to evaluate lotic water quality, and looks at the taxonomic composition and % contribution of the 5 or 10 dominant organisms present. The 5 dominant organisms at all the stations represented primarily highly tolerant taxa. The dominant organisms at station S2-11 were the least tolerant mix to be found in the lakes, while organisms at station S2-4 were all highly tolerant.

Diversity measures: Several measures of diversity are provided in the data tables. I am not particularly fond of these measures, and have a hard time trying to interpret the results from the Black and Steilacoom Lake stations.

Other analysis: Drs. Brinkhurst and Kathman will be more familiar with interpreting lake benthic data in regards to water quality conditions than I will be.

Conclusions: This data is derived from 4 stations, from 2 lakes, from 1 or 2 macrohabitat types, from one season. I have no published data on lakes in your area to evaluate where in the range of habitat conditions and impact levels these benthic communities fit.

After conducting the above analysis, it appears that the benthic communities found at all 4 stations are highly stressed and indicative of significant water quality problems. Taxa richness and densities are low, with taxa present being mostly highly tolerant forms. Also, many of the taxa present have widespread distributions (some worldwide), and are ones that tend to occur in abundance in highly polluted or disturbed environments. In other words, a few common "weed" species dominate the benthic communities at these stations.

I would be interested in receiving any information the WA DOE has on lake benthic macroinvertebrate communities in Washington.

WASHINGTON DEPARTMENT OF ECOLOGY

Benthic Invertebrate Samples from Black and Steilacoom Lakes. Petite Ponar Dredge Samples taken November 26-27, 1990.

ANNOTATED TAXA LIST

Prepared by: Robert W. Wisseman, Western Aquatic Institute, Corvallis, OR. May 1991.

NEMATODA:

Difficult to identify. Rare in samples. Usually common in lake benthos samples.

OLIGOCHAETA: Tubificidae

<u>Immature Tubificidae with capilliform setae</u>: Immature specimens which cannot be identified beyond this level.

<u>Immature Tubificidae without capilliform setae</u>: same.

Aulodrilus limnobius: Widespread. Lentic, silty substrates in mesotrophic waters. Moderately tolerant. Tube-builder.

Aulodrilus piqueti: Widespread. Similar to *A. limnobius* in habitat and tolerance.

Aulodrilus pluriseta: Widespread. Lentic, silty substrates. Tolerant of enriched habitats.

Bothrioneurum vejdovskyanum: Widespread. More common in riverine habitats, but appears in lakes.

Branchiura sowerbyi: Large species with fleshy gill filaments. Widespread. Generally more common in riverine habitats, though found in lakes. Can thrive in heated effluents.

Limnodrilus hoffmeisteri: One of the most abundant and widespread oligochaet taxa. Extremely tolerant and frequently the most abundant taxa in habitats having high organic enrichment. However, can be found in oligotrophic to grossly polluted and enriched waters.

OLIGOCHAETA: Naididae

Dero digitata: Wide spread.

Ophidonais serpentina: Widespread.

Stylaria lacustris: Widespread.

HIRUDINEA: Glossiphoniidae

Helobdella stagnalis: Lentic, littoral. Predator. Very widespread. Tolerant.

CLADOCERA

Daphnia pulicaria: Lentic, limnetic. Collector-filterers.

Daphnia galeata: Lentic, limnetic. Collector-filterers. Northeastern and Northwestern U.S.

Eurycercus: Lentic, limnetic, shallow waters. Collector-filterer. Widespread.

Simocephalus vetulus: Lentic, limnetic. Collector-filterer. Widespread but not common.

OSTRACODA

Cyclocypris: Lentic, littoral and profundal. Collector-gatherers. Widespread.

Candona: Lentic, littoral and profundal. Found on a wide variety of substrates. Collector-gatherers. Widespread and very common.

COPEPODA

Macrocyclops albidus: Widespread and common. Lentic, littoral and profundal. Soft sediments and vegetation. Collector-gatherer.

AMPHIPODA

Hyalella azteca: Widespread and very common. Lentic and slow-moving lotic waters. Generally littoral. Collector-gatherers. Tolerant.

MOLLUSCA

Menetus (M.) callioglyptus
Gyraulus (Torquis) parvus (Planorbidae): Lentic, littoral habitats. Sediment surface and hydrophytes. Scrapers and collector-gatherers. Widespread.

Physella (P.) gyrina (Planorbidae): Lentic, littoral habitats. Sediment surface and hydrophytes. Collector-gatherers. Widespread.

Sphaeriidae: Lentic, littoral and profundal. Sediments. Collector-filterers. Widespread.

ACARI: Parasites.

Lebertia (Lebertia)
Piona Exilis Group
Neumania (Neumania)

DIPTERA: Ceratopogonidae

?Culicoides: Lentic, littoral habitats. Burrowers. Predators and collector-gatherers. There are no keys to larval genera. Widespread. Highly tolerant.

DIPTERA: Chaoboridae

Chaoborus: Lentic, limnetic, profundal and littoral. Sprawlers on sediments during the day and planktonic at night. Predators. Widespread.

DIPTERA: Chironomidae

Chironomus: Lentic, littoral and profundal habitats. Burrowers (tube builders). Collector-gathers mainly. Widespread. Larvae found mostly in soft sediments. Members of this genus are generally tolerant to organic enrichments, and some are extremely tolerant (e.g. "bloodworms"). Taxa present in samples are not "bloodworms". Generally indicators of mesotrophic or eutrophic conditions. Common in the littoral zone of reservoirs.

Cladopelma: Lentic, littoral habitats. Burrowers. Collector-gatherers. Widespread. Found in sandy and muddy substrates of lakes and rivers. Common in the littoral zone of reservoirs.

Parachironomus: Lentic, littoral habitats. Sprawlers. Predators and collector-gatherers. Widespread. Larvae occur in a diverse array of lentic and lotic habitats.

Polypedilum: Lentic, on vascular hydrophytes. Climbers, clingers. Macrophyte-herbivores, collector-gatherers, predators. Widespread. Larvae occur in a diverse array of lentic and lotic habitats. Common in the littoral zone of reservoirs.

Procladius: Lentic, profundal and some littoral. Sprawlers. Predators and collector-gatherers. Widespread. Larvae of most species prefer muddy substrates of lentic and slow moving lotic waters. Abundant in the littoral zone or reservoirs.

Identifications by:

R.W. Wisseman, Western Aquatic Institute, Corvallis, OR (general identifications).
D.R. Spencer, Fowlerville, MI (Oligochaeta)
L. Ferrington, Lawrence, KS (Chironomidae)
W.B. Morton, Guelph, Ontario (Acari)
J. Cordell, University of Washington, Seattle, WA (Cladocera, Ostracoda, Copepoda).

T. Frest, Seattle, WA (Gastropoda)
G.L. Mackie, University of Guelph, Ontario (Sphaeriidae)

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Washington Dept. of Ecology Benthic Invertebrates Lake Samples
Hilsenhoff Biotic Index tolerance values and functional
feeding group designation used in data analysis.

TAXON		ORD	FAM	HBI	FFG
MISC. TAXA					
Nematoda		MIS	NMA	5	PA
Imm. Tubificidae w cap. setae		MIS	TUB	10	CG
Imm. Tubificidae w/o cap. setae		MIS	TUB	10	CG
Aulodrilus	limnobius	MIS	TUB	8	CG
Aulodrilus	piqueti	MIS	TUB	8	CG
Aulodrilus	plurizeta	MIS	TUB	8	CG
Bothrioneurum	vejdovskyanum	MIS	OLG	8	CG
Branchiura	sowerbyi	MIS	TUB	10	CG
Dero	digitata	MIS	NAI	10	CG
Limnodrilus	hoffmeisteri	MIS	TUB	10	CG
Ophiodonais	serpentina	MIS	NAI	6	CG
Stylaria	lacustris	MIS	NAI	8	CG
Helobdella	stagnalis	MIS	HIR	6	PR
Physidae		MIS	PHY	8	CG
Planorbidae		MIS	PLA	8	SC
Sphaeriidae		MIS	SPH	8	CF
Daphnia	galeata	MIS	CLA	8	CF
Daphnia	pulicaria	MIS	CLA	8	CF
Eurycercus		MIS	CLA	8	CF
Simocephalus	vetulus	MIS	CLA	8	CF
Cyclocypris		MIS	OST	8	CG
Candona		MIS	OST	8	CG
Macrocyclops	albidus	MIS	COP	8	CG
Hyallela	azteca	MIS	AMP	8	CG
Lebertia	Lebertia	MIS	ACA	6	PA
Neumania	Neumania	MIS	ACA	6	PA
Piona	Exilis Group	MIS	ACA	6	PA
MISC. DIPTERA					
?Culicoides		DIP	CER	10	PR
Chaoborus		DIP	CUL	8	CG
CHIRONOMIDAE					
Chironomus		DIP	CHI	10	CG
Cladopelma		DIP	CHI	9	CG
Parachironomus		DIP	CHI	10	PR
Polypedilum		DIP	CHI	6	OM
Procladius		DIP	CHI	9	PR

PR= Predator
PA= Parasite
CG= Collector-gatherer
CF= Collector-filterer
OM= Omnivore
PH= Piercer-herbivore
MH= Macrophyte-herbivore
SC= Scraper
SH= Shredder
UN= Unknown

Hilsenhoff Biotic Index

higher numbered square. An arthropod on a line is considered to be in the square that contains its head, or in the square closest to its head. After the highest numbered square has been sampled, return to square 1. Remove and preserve at least 100 arthropods. Remove all arthropods from the last square to be picked. Do not collect Hemiptera, or Coleoptera other than Dryopoidea. Except for adult Elmidae and fifth instar Hydroptilidae larvae, which have expanded abdomens and are usually in cases, do not collect arthropods less than 3 mm long because most cannot be identified. An illuminated 5X magnifier on a long, movable arm (Luxo®) will facilitate finding and removing arthropods from the pan.

7. Preserve all arthropods in 70% ethanol for identification to genus or species in the laboratory. Isopropyl alcohol may also be used.

8. Sort and identify all arthropods to genus, except Chironomidae, which should be placed in a separate vial. When all samples have been identified to genus, species identification should be made whenever necessary and possible. This is best accomplished by working on one genus at a time and identifying species in that genus from all samples before identifying species in another genus.

9. Chironomidae are sorted to genus by placing those that look alike together. Head color, head size and shape, markings on the head, antennal length and structure, number and location of eye spots, general shape and pigmentation of the mentum, length and color of preanal papillae and setae, length of prolegs and color of their claws, and general coloration are among the characters that can be used to separate genera. Mount the two most dissimilar larvae from each group in Hoyer's medium under separate cover slips on the same slide. If both are found to be the same genus, the remainder may be assumed to be also the same and need not be mounted. If they are different, further sorting and slide mounting is needed or all must be mounted on slides. An alternative is to clear all larvae in 10% KOH and make temporary mounts in glycerine for identification.

10. Record the number of each species on a data sheet and multiply the number by the tolerance value for that species. Sum the products and divide by the total number of arthropods in the sample to obtain the biotic index for the stream. Table 1 is a general guide to the water quality of streams. Replicate samples, or both spring and fall samples, will add to the confidence of the evaluation.

Table 1. Evaluation of water quality using biotic index values of samples collected in March, April, May, September, and early October.

Biotic Index	Water Quality	Degree of Organic Pollution
0.00–3.50	Excellent	No apparent organic pollution
3.51–4.50	Very Good	Possible slight organic pollution
4.51–5.50	Good	Some organic pollution
5.51–6.50	Fair	Fairly significant organic pollution
6.51–7.50	Fairly Poor	Significant organic pollution
7.51–8.50	Poor	Very significant organic pollution
8.51–10.00	Very Poor	Severe organic pollution

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Washington Dept. of Ecology Benthic Invertebrates Lake Samples
Steilacoom Lake Station S2-4 November 26, 1990
Petite Ponar Dredge Samples
Means have been adjusted to a square meter basis.

	REPLICATES						
TAXON	A	B	C	D	MEAN	STDEV	%
Imm. Tubificidae w cap. setae	5	7	6	5	192	27.6	14.02
Imm. Tubificidae w/o cap. set	0	1	0	1	17	16.7	1.22
Bothrioneurum vejdovskyanum	2	40	4	3	408	534.5	29.88
Branchiura sowerbyi	4	2	4	3	108	27.6	7.93
Dero digitata	0	2	1	0	25	27.6	1.83
Limnodrilus hoffmeisteri	0	1	0	0	8	14.4	0.61
TOTAL: MISC. TAXA	11	53	15	12	758	584.2	55.49
Chironomus	22	12	19	2	458	256.4	33.54
Procladius	0	1	1	16	150	221.7	10.98
TOTAL: CHIRONOMIDAE	22	13	20	18	608	111.5	44.51
GRAND TOTAL	33	66	35	30	1367	484.7	
% SAMPLE SORTED	100	100	100	100			

Washington Dept. of Ecology Benthic Invertebrates Lake Samples
Steilacoom Lake Station S2-11 November 26, 1990
Petite Ponar Dredge Samples
Means have been adjusted to a square meter basis.

		REPLICATES						
TAXON		A	B	C	D	MEAN	STDEV	%
Dero	digitata	4	2	1	2	75	36.3	1.31
Ophidonais	serpentina	426	2	1	20	3741	6042.8	65.26
Stylaria	lacustris	6	0	0	0	50	86.6	0.87
Physidae		60	0	0	0	500	865.9	8.72
Planorbidae		20	0	0	0	167	288.6	2.91
Daphnia	galeata	2	0	0	0	17	28.9	0.29
Eurycercus		2	0	0	0	17	28.9	0.29
Simocephalus	vetulus	36	0	0	0	300	519.6	5.23
Cyclocypris		4	0	0	0	33	57.7	0.58
Macrocyclops	albidus	4	1	0	0	42	54.6	0.73
Hyallela	azteca	4	0	0	0	33	57.7	0.58
Lebertia	Lebertia	2	0	0	0	17	28.9	0.29
Neumania	Neumania	0	0	1	0	8	14.4	0.15
Piona	Exilis Group	6	0	0	0	50	86.6	0.87
TOTAL: MISC. TAXA		576	5	3	22	5049	8172.4	88.08
Chironomus		6	26	18	23	608	254.2	10.61
Parachironomus		2	0	0	0	17	28.9	0.29
Polypedilum		0	0	0	1	8	14.4	0.15
Procladius		2	1	2	1	50	16.7	0.87
TOTAL: CHIRONOMIDAE		10	27	20	25	683	219.2	11.92
GRAND TOTAL		586	32	23	47	5733	7971.8	
% SAMPLE SORTED		50	100	100	100			

Washington Dept. of Ecology Benthic Invertebrates Lake Samples
Steilacoom Lake Station S2-12 November 26, 1990
Petite Ponar Dredge Samples
Means have been adjusted to a square meter basis.

	REPLICATES						
TAXON	A	B	C	D	MEAN	STDEV	%
Nematoda	0	2	0	0	17	28.9	0.40
Imm. Tubificidae w cap. seta	50	4	2	0	467	694.4	11.07
Imm. Tubificidae w/o cap. se	18	0	0	0	150	259.8	3.56
Aulodrilus piqueti	50	22	2	12	717	596.9	17.00
Aulodrilus pluriseta	2	0	0	0	17	28.9	0.40
Bothrioneurum vejdovskyanum	34	110	34	0	1483	1342.6	35.18
Branchiura sowerbyi	18	2	6	2	233	218.6	5.53
Dero digitata	4	6	0	2	100	74.5	2.37
Limnodrilus hoffmeisteri	4	0	0	0	33	57.7	0.79
Helobdella stagnalis	0	4	2	2	67	47.1	1.58
Sphaeriidae	10	6	0	2	150	128.0	3.56
Daphnia galeata	20	8	4	2	283	232.7	6.72
Daphnia pulicaria	4	4	0	0	67	66.7	1.58
TOTAL: MISC. TAXA	214	168	50	22	3783	2659.9	89.72
Chironomus	6	2	2	0	83	72.6	1.98
Procladius	14	8	14	6	350	119.0	8.30
TOTAL: CHIRONOMIDAE	20	10	16	6	433	179.5	10.28
GRAND TOTAL	234	178	66	28	4216	2767.1	
% SAMPLE SORTED	50	50	50	50			

Washington Dept. of Ecology Benthic Invertebrates Lake Samples
Black Lake Station B2-2 November 27, 1990
Petite Ponar Dredge Samples.
Means have been adjusted to a square meter basis.

		REPLICATES						
TAXON		A	B	C	D	MEAN	STDEV	%
Nematoda		0	0	2	0	17	28.9	0.99
Aulodrilus	limnobius	26	24	16	94	1333	1046.6	79.21
Aulodrilus	piqueti	2	0	0	0	17	28.9	0.99
Dero	digitata	4	0	0	0	33	57.7	1.98
Ophi~~o~~donais	serpentina	0	0	0	2	17	28.9	0.99
Daphnia	galeata	4	0	0	2	50	55.3	2.97
Candona		0	0	0	2	17	28.9	0.99
TOTAL: MISC. TAXA		36	24	18	100	1483	1089.6	88.12
?Culicoides		4	0	8	0	100	110.5	5.94
Chaoborus		4	0	0	4	67	66.7	3.96
TOTAL: MISC. DIPTERA		8	0	8	4	167	110.5	9.90
Chironomus		0	0	0	2	17	28.9	0.99
Cladopelma		2	0	0	0	17	28.9	0.99
TOTAL: CHIRONOMIDAE		2	0	0	2	33	33.3	1.98
TOTAL: ALL DIPTERA		10	0	8	6	200	124.7	11.88
GRAND TOTAL		46	24	26	106	1683	1105.8	
% SAMPLE SORTED		50	50	50	50			

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Washington Dept. of Ecology  Benthic Invertebrates  Lake Samples

Steilacoom Lake  Station S2-4  November 26, 1990
Petite Ponar Dredge Samples
Abundances have beeen adjusted to a square meter basis.

 Total abundance =    1366.     Total number of taxa=  8.
 Hilsenhoff Biotic Index =  9.293

 Functional feeding group distribution:
    Group     Taxa       Abundance  Percent
     PR        1.           150.     10.98
     PA        0.             0.       .00
     CG        7.          1216.     89.02
     CF        0.             0.       .00
     OM        0.             0.       .00
     PH        0.             0.       .00
     MH        0.             0.       .00
     SC        0.             0.       .00
     SH        0.             0.       .00
     UN        0.             0.       .00

 Contribution of 10 dominant taxa:
          Taxon                              Abundance Percent
 Chironomus                                     458.    33.54
 Bothrioneurum        vejdovskyanum             408.    29.88
 Imm. Tubificidae w cap. setae                  192.    14.02
 Procladius                                     150.    10.98
 Branchiura           sowerbyi                  108.     7.93
 Dero                 digitata                   25.     1.83
 Imm. Tubificidae w/o cap. setae                 17.     1.22
 Limnodrilus          hoffmeisteri                8.      .61
                                                  0.      .00
                                                  0.      .00
                                   Totals      1366.   100.01

 Diversity measures:
  Shannon H (loge) =  1.60
  Shannon H (log2) =  2.31
  Evenness =   .77
  Brillouin H =  1.59
  Simpson D =   .240
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Washington Dept. of Ecology  Benthic Invertebrates  Lake Samples

Steilacoom Lake   Station S2-11   November 26, 1990
Petite Ponar Dredge Samples
Abundances have been adjusted to a square meter basis.


 Total abundance =    5733.    Total number of taxa= 18.
 Hilsenhoff Biotic Index =  6.919

 Functional feeding group distribution:
    Group    Taxa       Abundance  Percent
     PR       2.             67.     1.17
     PA       3.             75.     1.31
     CG       8.           5082.    88.64
     CF       3.            334.     5.83
     OM       1.              8.      .14
     PH       0.              0.      .00
     MH       0.              0.      .00
     SC       1.            167.     2.91
     SH       0.              0.      .00
     UN       0.              0.      .00

 Contribution of 10 dominant taxa:
          Taxon                                Abundance Percent
 Ophidonais        serpentina                  3741.   65.26
 Chironomus                                      608.   10.61
 Physidae                                        500.    8.72
 Simocephalus       vetulus                      300.    5.23
 Planorbidae                                     167.    2.91
 Dero               digitata                      75.    1.31
 Stylaria           lacustris                     50.     .87
 Piona              Exilis Group                  50.     .87
 Procladius                                       50.     .87
 Macrocyclops       albidus                       42.     .73
                              Totals           5583.   97.38

 Diversity measures:
  Shannon H (loge) =  1.35
  Shannon H (log2) =  1.95
  Evenness =   .47
  Brillouin H =  1.34
  Simpson D =   .449
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Washington Dept. of Ecology  Benthic Invertebrates  Lake Samples

Steilacooom Lake    Station S2-12   November 26, 1990
Petite Ponar Dredge Samples
Abundances have been adjusted to a square meter basis.

 Total abundance =    4217.     Total number of taxa= 15.
 Hilsenhoff Biotic Index =  8.545

 Functional feeding group distribution:
    Group     Taxa       Abundance  Percent
     PR        2.          417.       9.89
     PA        1.           17.        .40
     CG        9.         3283.      77.85
     CF        3.          500.      11.86
     OM        0.            0.        .00
     PH        0.            0.        .00
     MH        0.            0.        .00
     SC        0.            0.        .00
     SH        0.            0.        .00
     UN        0.            0.        .00

 Contribution of 10 dominant taxa:
          Taxon                                  Abundance Percent
 Bothrioneurum        vejdovskyanum               1483.    35.18
 Aulodrilus           piqueti                      717.    17.00
 Imm. Tubificidae w cap. setae                     467.    11.07
 Procladius                                        350.     8.30
 Daphnia              galeata                      283.     6.72
 Branchiura           sowerbyi                     233.     5.53
 Imm. Tubificidae w/o cap. setae                   150.     3.56
 Sphaeriidae                                       150.     3.56
 Dero                 digitata                     100.     2.37
 Chironomus                                         83.     1.98
                                         Totals   4016.    95.27
 Diversity measures:
  Shannon H (loge) =  2.08
  Shannon H (log2) =  3.00
  Evenness =   .77
  Brillouin H =  2.07
  Simpson D =   .183
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Washington Dept. of Ecology  Benthic Invertebrates  Lake Samples

Black Lake   Station B2-2   November 27, 1990
Petite Ponar Dredge Samples
Abundances have been adjusted to a square meter basis.

 Total abundance =    1685.     Total number of taxa= 11.
 Hilsenhoff Biotic Index =  8.138

 Functional feeding group distribution:
    Group    Taxa       Abundance  Percent
     PR       1.          100.       5.93
     PA       1.           17.       1.01
     CG       8.         1518.      90.09
     CF       1.           50.       2.97
     OM       0.            0.        .00
     PH       0.            0.        .00
     MH       0.            0.        .00
     SC       0.            0.        .00
     SH       0.            0.        .00
     UN       0.            0.        .00

 Contribution of 10 dominant taxa:
          Taxon                              Abundance  Percent
 Aulodrilus         limnobius                  1333.     79.21
 ?Culicoides                                    100.      5.94
 Chaoborus                                       67.      3.96
 Daphnia            galeata                      50.      2.97
 Dero               digitata                     33.      1.98
 Nematoda                                        17.       .99
 Aulodrilus         piqueti                      17.       .99
 Ophidonais        serpentina                   17.      .99
 Candona                                         17.       .99
 Chironomus                                      17.       .99
                                      Totals   1668.     99.01

 Diversity measures:
  Shannon H (loge) =   .94
  Shannon H (log2) =  1.35
  Evenness =   .39
  Brillouin H =   .93
  Simpson D =   .633
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