A Sediment Triad Analysis of Lakes Sammamish, Washington, and Union

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Glossary

AVS-Acid Volatile Sulfides
BNA-Base Neutral Acid
CCME - Canadian Council of Ministers of the Environment
CSL-Washington State Cleanup Screening Level
EPA-Environment Protection Agency
HQ-Hazard Quotient
QA/QC-Quality Assurance/Quality Control
FP – Floating Percentile
FSQV-Draft Freshwater Sediment Quality Values
HPAH- High Molecular Weight Polycyclic Aromatic Hydrocarbons
KC-King County
LOE-Line of Evidence
LPAH-Low Molecular Weight Polycyclic Aromatic Hydrocarbons
MDL- Minimum Detection Limit
MSD-minimum significant difference
PAH- Polycyclic Aromatic Hydrocarbons
PCA-Principle Component Analysis
PCB- polychlorinated biphenyls
PEL- Probable Effect Level
PSEP- Washington State Puget Sound Estuarine Protocols
RTR-Ratio to Reference
SEDQUAL -Sediment Quality Information System
SRM-Standard reference method
SWAMP -Sammamish Washington Assessment and Modeling Program
SAP-Sampling and Analysis Plan
SQG-Sediment Quality Guidelines
SQS- Washington State Sediment Quality Standard
SQT-Sediment Quality Triad
TEL- Threshold Effects Levels
WOE-Weight of Evidence
WRIA-Washington Water Resources Inventory Area
WWTP-Waste Water Treatment Plant

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1.0 Introduction

1.1 Background

Sediment contamination is a pervasive environmental problem that threatens aquatic ecosystems worldwide. Once released into surface waters, many toxic and persistent contaminants become adsorbed to sediment and can become incorporated into aquatic food webs. In this manner, contaminated sediments can have toxic and bioaccumulative effects on aquatic life (USEPA, 1994, Canfield *et al.* 1996, Anderson *et al.* 1987, USEPA, 1997). The USEPA estimated that roughly 10% of the sediment in US surface waters is contaminated enough to pose potential risks to fish, as well as to the humans and wildlife who consume contaminated fish (USEPA, 1997).

The King County Department of Natural Resources (KCDNR) initiated a sediment characterization study with the objective of addressing four study questions as they pertain to Lakes Sammamish, Washington, and Union:

- What are the contaminants of concern (COC)?
- Is there a measurable response to this contamination? If so, are contaminants causing the observed response?
- Which lakes or areas of the lakes are most impacted?
- What are the potential contaminants sources?

To address these questions, a Sediment Quality Triad (SQT) study (discussed below), which is an integrated method for evaluating sediment contamination, was initiated.

2.0 Sediment Quality Triad

Recognizing that contaminated sediments can have detrimental impacts on ecosystems and pose risks to wildlife and humans has resulted in many detailed sediment quality investigations. The sediment quality triad (SQT) is a conceptual framework for collecting synoptic measurements of sediment chemistry, toxicity, and benthos, and using these data to evaluate sediment quality (Long and Chapman 1985, Chapman 1990, Chapman 1992). SQT studies generally have three components: 1) sediment chemistry that assesses contaminant concentration, 2) bioassays that evaluate the sediment's toxicity to aquatic organisms, and 3) the benthic invertebrate community composition that provides direct evidence of *in situ* biotic alterations (Long and Chapman 1985, Chapman 1990, Chapman 1992). Taken separately, each component represents an independent line-of-evidence (LOE). Evaluated simultaneously, the combined LOE represent a "weight-of-evidence" (WOE) approach. While each individual LOE provides limited information about the state of the sediment, the combined WOE approach provides a more robust assessment. This combined approach is significant since sediment toxicity can vary spatially and temporally due to variable contaminant concentrations and sediment conditions that may influence bioavailability, including pH, grain size, chemical form, redox potential (Eh), and the presence of other chemicals (Chapman 1992). This effects based approach has been used worldwide, including the Puget Sound (Long and Chapman 1985), San Francisco Bay (Chapman *et al.* 1987), and the Gulf of Mexico (Carr *et al.* 1996). Freshwater applications include the Great Lakes (Canfield *et al.* 1996), the Mississippi River (Canfield *et al.* 1998) and Lake Union (Yake *et al.* 1986).

Key assumptions in SQT studies are that the chemicals evaluated are appropriate indicators of contamination and that the bioassay endpoints and benthic metrics are appropriate indicators of biological effects and bioavailability. There are distinct advantages and disadvantages to using this approach. Key advantages of the SQT are that the analysis combines lines-of-evidence into an integrated approach, does not require *a priori* assumptions about the specific mechanisms of biota and chemical interactions,

provides empirical evidence of sediment quality, and allows ecological inferences about both physical-chemical and biological properties (Chapman 1992). Disadvantages include the lack of a set of standardized statistical criteria for this approach and the time and cost associated with the substantial amount of data required to do these analyses (Chapman 1992). Each LOE plays an important role in addressing one or more of the study questions discussed in Section 1.0.

2.1 Sediment Chemistry

As detailed above, sediments affect the environmental fate and transport of many contaminants in aquatic ecosystems, and the SQT approach uses measured chemical concentrations to gauge the overall degree of contamination. However, chemical concentrations do not necessarily provide information on bioavailability or the additive effects of complex mixtures (Anderson *et al.* 1987, Di Toro *et al.* 2000, Landrum *et al.* 2003). Sediment associated chemicals are generally less toxic than dissolved forms because they are not as bioavailable. For example, the toxicity of sediment-associated divalent metals is influenced by the amount of sediment acid volatile sulfides (AVS). If sufficient AVS is present, metals bind to sediment phase sulfides and bioavailablity is decreased (Di Toro *et al.* 1990). Thus, sediment associated divalent metals are not expected to cause adverse effects if AVS concentrations are greater than SEM levels. Additionally, the sediment organic carbon content mediates the bioavailability of hydrophobic organic chemicals. Organic chemicals form complexes with organic matter that reduce the bioavailability of potentially toxic chemicals (Anderson *et al.* 1987).

2.2 Benthic Community

Since aquatic organisms living in or near sediment can be impacted by sediment associated contaminants, the spatial and temporal distribution of benthic organisms may reflect the extent of bioavailable sediment contamination (USEPA 1994, Canfield *et al.* 1996). Additionally, benthic organisms are an important food source for ecologically and commercially important organisms such as food and sport fish (Chapman 1990). Therefore, benthic organisms are a convenient means for assessing sediment contamination because they are relatively easy to collect, spend all or most of their life

cycle in the sediments, and the overall community composition is useful for evaluating ecosystem integrity (Canfield *et al.* 1996). However, there are important constraints that should be considered when utilizing benthic organisms as indicators of contamination. Benthic assemblages can reflect biotic and abiotic (e.g., substrate composition, depth) factors other than contamination. In addition, it is very difficult to relate benthic metrics to individual contaminants. While an effect may be observed in the benthic community, it is very difficult to determine the particular chemical or group of chemicals that caused the observed impact. Lastly, due to the naturally heterogeneous distribution of many benthic organisms, large sample sizes are typically necessary to overcome natural variance (Canfield *et al.* 1996).

2.3 Sediment Toxicity

While sediment contamination presents a potential ecological risk, sediment toxicity tests evaluate the degree to which aquatic organisms respond to the potential risk (Chapman *et al.* 2002). However, it is important to note that there is no one "sentinel species" that can serve as an accurate indicator for ecosystem health and that lab-to-field extrapolations are less that perfect (Power 1997). There are also non-chemical physical factors such as sediment grain size composition that may affect bioassays and acute exposure may be insufficient for contaminants that cause sub-lethal effects (Chapman *et al.* 1997, Reynoldson *et al.* 1997).

3.0 Literature Review

3.1 Evaluating Triad Components

The task of simultaneously evaluating the results of the chemical analyses, bioassays with multiple species and endpoints, and benthic data with multiple metrics and taxa generally requires appropriate data reduction techniques. The discussion presented below provides an overview of existing methods commonly used to analyze these sometimes complex data.

While the concept of synoptic evaluation of chemical, toxicity, and benthic data has existed for over 20 years, Long and Chapman (1985) published the first paper that applied the term 'Sediment Quality Triad' to this type of data. Early conceptualizations of SQT analyses were that all data should be compared on a quantitative basis and all data should be normalized to a reference, creating a ratio to reference (RTR) for each LOE. (Chapman 1992). Once a single chemistry RTR, mean bioassay RTR, and benthic RTRs are developed, all of the values are normalized to the reference value and the results are plotted on a triaxial graph with a common origin (Chapman 1990). While normalizing data to a reference is commonly done, the reduction of data to triangular graphical plots is no longer recommended since it causes a significant loss of information, as well as disguises the spatial aspect of contaminants (Chapman 2000).

A more qualitative method of analyzing triad data are tabular decision matrixes that utilize both LOE and best professional judgment in a set of *a priori* comparisons. Table 1 provides an example of a tabular decision matrix. One of the advantages of this method is that the WOE approach and analyses are applied in a straightforward manner. A disadvantage is that it does not incorporate variance in the individual LOE components (Chapman 1992). Thus, studies using tabular decision matrices typically use more qualitative analyses such as Mantel's test, ANOVA, or Spearman correlations to provide an assessment of the relationships within and between study components (Chapman 1992, Chapman 1996).

Table 1. (modified from Chapman 1992) Tabular Decision Matrix

Triad Component			
Chemistry	**Toxicity**	**Benthic Community Alteration**	**Possible Conclusions**
+	+	+	Evidence for pollution-induced degradation
-	-	-	Evidence that there is no pollution-induced degradation
+	-	-	Chemicals are not bioavailable
-	+	-	Unmeasured chemicals or conditions exist with the potential to cause degradation
-	-	+	Alteration is not due to toxic chemicals, or benthos is more sensitive than bioassays and SQGs are not appropriate for community alterations
+	+	-	Toxic chemicals are bioavailable in the laboratory but not *in situ or* benthos have adapted to the toxicants
-	+	+	Unmeasured toxic chemicals are causing degradation or SQGs are insensitive and not an appropriate indicator of biological impairment. Also, many measured toxicants do not have SQGs.
+	-	+	Chemicals are not bioavailable, alteration is not due to toxic chemicals, or benthos are more sensitive than bioassays.

+ = Measured difference between test and control or reference conditions
- = No measured difference between test and control or reference conditions

Researchers have also used quadrant frequency analysis (or "importance/performance" plots) to identify the benthic indices and bioassay endpoints most sensitive to elevated concentrations. To demonstrate this approach, example data are used in the figures and tables described below. In quadrant analysis, a scatter plot of the sediment quality guideline (SQG-discussed in Section 5.7) quotient of chemistry vs. a bioassay endpoint is created (See Figure 1 for plot and Table 2 for interpretation). After drawing a line on the SQG axis above which no non-toxic examples exist (39 in Figure 1) and a line on the bioassay axis above which no non-toxic samples exist (33 in Figure 1), four quadrants are created. Samples in quadrants I and II can be identified as potential Type II and Type I statistical errors, respectively (Canfield *et al.* 1996). To evaluate the benthic data, a benthic metric is plotted against the same SQG quotients (SQG-Q) and the quotient value identified in the previous plot is redrawn. SQG-Qs are calculated by dividing each

chemical point concentration by its SQV and then taking the average of all quotients at a station. These average values can also be broken down by chemical group. Lastly, a line is drawn that corresponds to the benthic metric that put the smallest number of points in quadrants I and II. Benthic indices have been compared to laboratory toxicity results in a similar manner (*Ingersoll et al. 1996*).

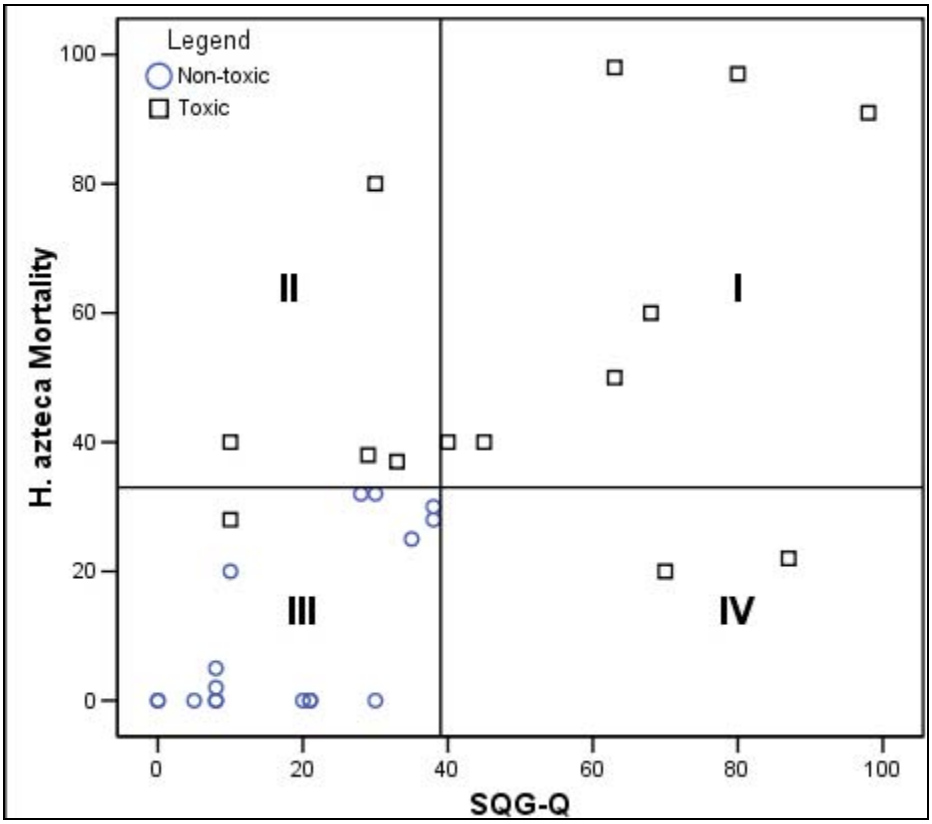

Figure 1. Quadrant Analysis with the *H. azteca* Mortality and SQG-Q.

Table 1. Quadrant Analysis

Quadrant	**Chemical Concentration**	**Benthic Metrics**	**Conclusion**
I	Low	Impacted	False Positive
II	Elevated	Not Impacted	False Negative
III	Low	Not Impacted	Valid Data
IV	Elevated	Impacted	Valid Data

More recent SQT studies have tended towards using multivariate approaches to analyze the data (Green *et al.* 1993, Chapman et al, 1997, Chapman 2000). These multivariate approaches frequently begin with analyses of individual SQT components (ANOVA,

etc.) (Chapman 1992). Multivariate approaches such as principle component analysis (PCA) can then be used to reduce the dimensionality of each component or examine the relationship between components (Zar 1984).

3.2 Bioassay Data

A significant challenge in dealing with bioassay data is the question of whether to designate bioassay data from a particular station a "hit" or "no-hit". This issue is further confounded by the fact that there are often multiple samples from the same station. Bioassays using the same sample could have been conducted on several test species, some with multiple endpoints. In addition, since both control and reference data are available for each endpoint, it is not obvious which is appropriate for statistical comparison. While there are no standardized approaches to evaluate the toxic nature of a station, some are more commonly utilized than others. Chapman *et al.* (1997) suggest for a station to be classified as 'impaired', it should have bioassays that are significantly different from the control (t-test), exceed the minimum significant difference (MSD), and it should be outside the reference envelope (described below). Alternate methods of evaluating whether bioassay data indicates a sample is toxic include assessing between station differences in the mean response using ANOVA and *a priori* contrasts (Chapman 1996). For tests which have been affected by factors other than contamination (i.e. grain size) comparisons using those factors as a covariate in ANCOVA may be appropriate (Chapman 1996). To further enhance the ecological relevance of bioassays, the toxicity tests are often related to one or more benthic metrics (Chapman 2000).

The reference envelope approach is becoming an increasingly popular method for interpreting the ecological relevance of bioassays (Chapman 2000). This approach enables the statistical determination of whether the conditions at any given test site are distinct from the reference population. Using bioassay data from reference stations, where localized pollution is presumably absent, tolerance limits from the mean bioassay response can be calculated. In this case, the tolerance limits are the upper or lower confidence interval bound around some percentile of the underlying data distribution. Figure 2 illustrates this concept. Samples with toxicity values more extreme than the

tolerance limit are considered toxic relative to the reference sediment conditions. Since the example in Figure 2 pertains to survival, a value more 'extreme' than the reference envelope would be a value less than the reference envelope. While the t-test to control comparison uses lab replicates to characterize variance, the reference envelope approach incorporates variance from both laboratory replicates and natural within-lake variability. Reference envelopes can be calculated for both parametric and non-parametric variables (Smith 2002).

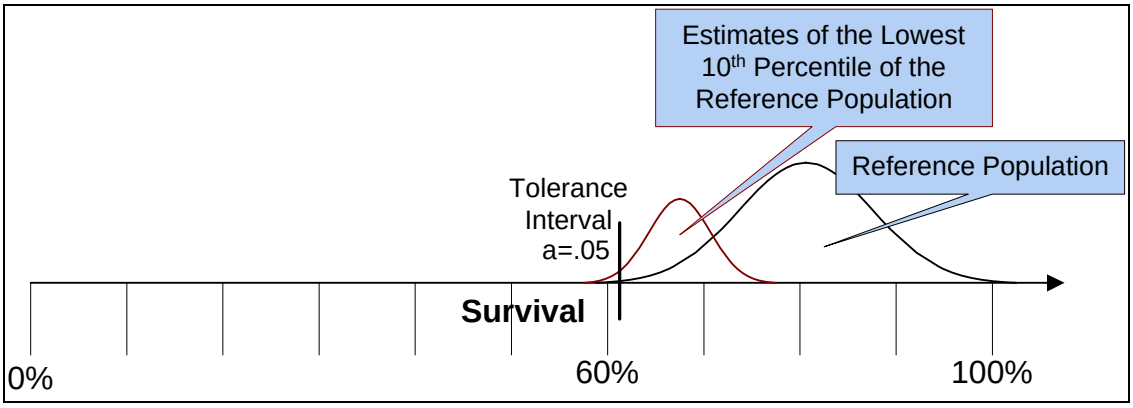

Figure 2. Illustration of reference envelope approach for calculating tolerance interval. There is a 95% probablity that survival values less than the tolerance interval are as low or lower than the 10th percentile of the distribution of reference site toxicity results (Hunt et al. 1989).

3.3 Benthic Data

Benthic data are often summarized through the use of benthic metrics, such as taxa richness, total abundance, numerical dominance, mean abundance, and the number of pollution intolerant species (Canfield *et al.* 1996, Chapman 1996). Due to the abundance of available data, PCA and classification (clustering) can be used to reduce the number of variables (Chapman 1996, Chapman 1997, Chapman 2000). In cluster analysis, the data are bootstrapped and then statistically tested to determine whether samples are significantly different from each other (Chapman 1992).

4.0 Study Area

4.1 Study Area Description

Lakes Sammamish, Union, and Washington are located in the Greater Lake Washington Water Resources Inventory Area 8 (WRIA 8), which drains over 1554 km^2. While the toxicity, chemistry, and benthic community structure of sediment adjacent to industrial sites in Lakes Washington and Union have previously been assessed (Yake *et al.* 1986, Norton, 1991, Norton, 1992, Cubbage 1992, Bennett and Cubbage 1992) no large-scale whole–lake sediment characterization has been conducted until the present study.

Lakes Sammamish, Washington, and Union were carved out by glaciers approximately 15,000 years ago in the late Pleistocene during the Vashon glaciation (Leisch 1963 from Perkins 1995). Figure 3 shows all watersheds, lakes, major tributaries and two contaminant sources (discussed below) in the study area. Issaquah Creek is the primary tributary to Lake Sammamish, contributing about 70% of the surface water (Moon 1973 from KC 1999). Lake Sammamish drains to the North into the Sammammish River which drains into the north end of Lake Washington. The Cedar River, which historically drained into the Black River and in turn, into the Duwamish River, now drains into south Lake Washington (Perkins 1995, KC 1999, KC 2000, KC 2001). Lake Washington drains through the Montlake Cut into Lake Union, which drains into Hiram Chittenden Locks and finally, to Puget Sound (KC 2001). Both the Montlake Cut and the Hiram Chittenden Locks were created in the early 1900s to allow for ship passage between Puget Sound and Lake Washington (Edmondson 1994). On the next figure you have the Samm watershed labeled Samm River watershed – should be the Lake Sammamish Watershed

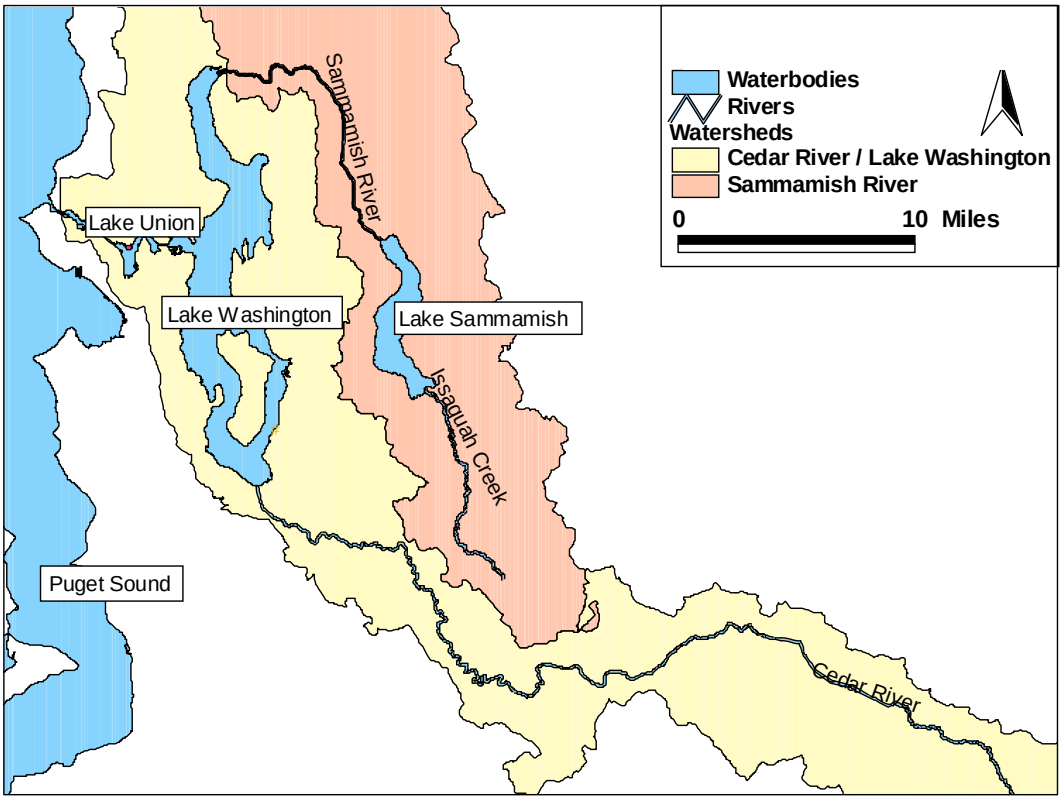

Figure 3. Map of Study Area

The sedimentation rate in Lake Washington was estimated using ^{210}Pb profiles, which suggests historic sedimentation rates were roughly 5 mm/yr previous to 1968 and 2.5 mm/yr after (Wakeham *et al.* 2004). Since the grab samples in this study were taken to a depth of 10 cm, the sediments in this study represent the time period from the mid-1960s to the time of sampling (1999-2001). Table 3 summarizes the morphometric characteristics of the three lakes. Table 4 provides a summary of land use/land cover characteristics of the Cedar-Washington and Lake Sammamish watersheds, generated from a 2001 Landsat image. Note that Lake Union is part of the Cedar-Washington watershed. Above the Landsburg Dam, the Upper Cedar River sub-watershed covered is protected as Seattle's water supply. Below the dam, the watershed is covered primarily by forest and with urban development. The Lake Sammamish watershed is also covered with mixed forest and urban development. Rapid population growth is currently occurring on the Sammamish Plateau east of Lake Sammamish, and throughout the northern areas of the Sammamish watershed (KC 2000).

Table 2. Lake MorphometricCharacteristics (Taken from: http://dnr.metrokc.gov/wlr/waterres/lakes)

Lake	Drainage Area (km^2)	Retention Time (years)	Length (km)	Area (km^2)	Mean Depth (m)	Maximum Depth (m)	Volume (m^3)
Sammamish	254.9	2.3	13	19.8	17.7	32	$3.5x10^8$
Washington	1,274	1.8	21	87.6	32.9	65.2	$2.9x10^9$
Union	1,554	0.02	2	2.35	10	15	$2.5x10^7$

Table 3. Watershed Land Use Characteristics (Note that Lake Union is part of the Cedar River/Lake Washington Watershed)

Land Cover	Cedar River / Lake Washington	Lake Sammamish
Urban/High Density	14.3%	10.7%
Mixed Urban/Low Density	19.9%	30.0%
Water	10.7%	3.7%
Bare Earth	1.8%	0.7%
Conifer Forest	25.0%	9.7%
Deciduous Forest	0.5%	1.3%
Mixed Forest	19.4%	32.5%
Recent Regenerated Forest	0.7%	0.1%
Recent Clearcuts	0.0%	0.1%
Herbaceous Vegetation	4.7%	7.7%
Shrub/Scrub Vegetation	3.0%	3.7%

Both Lakes Sammamish and Washington are used for recreational purposes, including swimming, waterskiing, and fishing (Metro and KC 1995, Parametrix 2003). Lake Union is a heavily urbanized catchment; lake use is dominated by marine-oriented commerce and industrial facilities intermixed with marinas, houseboats, offices, restaurants, and residences (KC 2001).

4.2 Contaminant-Related Studies of the Lakes

Until the late 1960s untreated sewage was released directly into Lake Washington and treated effluent was released into Lake Sammamish, resulting in eutrophication and associated low water quality and frequent late summer cyanobacteria blooms (Frodge, King County, pers. comm.). In the late 1960s, the wastewater treatment plant (WWTP) effluent was diverted from the two lakes (Perkins 1995, Edmondson 1994). Following the WWTP effluent diversion, Lake Washington's water quality improved dramatically,

and Lake Sammamish also recovered to a lesser degree. The differences in the improvement of the two lakes are attributed to the varying nutrient loads and differences in basin morphometry (Edmondson 1994, Metro 2002).

Numerous studies have investigated the quality of the water, sediment and biota in Lakes Sammamish, Washington, and Union. A brief review of the available studies is provided below. Appendix A provides greater detail about the studies discussed below and lists additional documents that discuss Lakes Washington, Sammamish, or Union. Heavy metals in Lake Washington sediments were studied intensively in the early to mid 1970s due to concerns over environmental degradation (Crecelius 1975). These studies revealed elevated sediment concentrations of lead, antimony, mercury, arsenic, and copper possibly associated with atmospheric deposition of these metals associated with historic release from the ASARCO copper smelter located near Tacoma, WA (Crecelius and Piper 1973). Another historical source of sediment contamination to Lake Washington is located in the southeast corner of the lake (Figure 1), where the wood processing plants at the J.H. Baxter property and Quendall Terminals were located. While in operation, these facilities were responsible for contaminating the nearby soil, groundwater, surface water, and sediments with polycyclic aromatic hydrocarbons (PAHs) and the volatile organic compounds (VOCs) benzene, toluene, ethylbenzene, and xylene (BTEX) (Ecology 2002). Several studies (Norton 1991, Norton 1992, Bennett *et al.* 1992) have confirmed elevated PAH concentrations, reduced benthic macroinvertebrate diversity, and sediment toxicity at the Quendall and Baxter sites.

Lake Union has experienced a reduction in both sediment and water quality due to commercial and industrial use, as well as from urban runoff (Cubbage 1992). Sediment chemical concentrations detected in the Salmon Bay area of Lake Union are some of the the highest found in Washington State (GLWTC 2001). Gas Works Park (GWP), a 20-acre park on the north shore of Lake Union, is located on the site of a former coal gasification plant that operated from 1906 to 1956 (Figure 1). Yake *et al.* (1986) conducted a SQT analysis adjacent to GWP and found sediment toxicity and elevated PAH concentrations. This study also revealed one site with elevated polychlorinated

biphenyls (PCB) concentrations and multiple sites with elevated cyanide levels (Yake *et al.* 1986). Subsequent sediment sampling confirmed the observed sediment toxicity and elevated sediment PAH concentrations near GWP, as well as elevated PCBs near the Seattle City Light Steam Plant, and elevated metals throughout the lake (Cubbage 1992). These findings led to Lake Union being listed on the Washington State 303(d) list for exceeding sediment bioassay standards in 1996, 1998, and 2002/2004 (Ecology 1996, Ecology 1998, Ecology 2004). Work by Crecelius *et al*. (1989) also found high levels of dieldrin in edible fish tissue. As a result, Lake Union was also on the 1996 and 1998 303(d) lists for dieldrin. Recent sampling by the Washington State Department of Ecology (Ecology) has shown that sediments adjacent to GWP have probably adversely affected the benthic community and are toxic to *C. tentans* (Jack 2003).

5.0 Methods

5.1 Sediment Samples

Sixty-one stations in Lakes Sammamish, Washington, and Union were sampled in the summers of 1999-2001. Figure 4 shows all sampling sites in the three lakes. Sampling followed the protocol established in the sampling and analysis plans (SAP) developed for this project where additional details about the sampling methods can be found (KC 1999, KC 2000, KC 2001).

Sediment samples were collected from 17 stations in Lake Sammamish between August 9th, 1999 and September 16th, 1999. Two of these stations were located in mid lake deep areas and two shallow stations were located in near shore areas removed from stream inputs. Thirteen samples were collected from shallow areas where sediment quality was likely to be influenced by creeks, storm drains, or emergency bypass outfalls associated with the wastewater conveyance system (KC 1999).

Sediment samples were collected from 29 stations in Lake Washington between August 7th, 2000 and September 13th, 2000. Three general categories of sampling location were identified. Five stations were located in the middle and deeper part of the lake, one station was located near shore, away from the influence of any tributaries, and the rest of the stations were located near shore in areas potentially influenced by creeks, storm drains, emergency bypass outfalls, or combined sewer overflows from the wastewater conveyance system (KC 2000).

Sediment samples were collected from 15 stations in Lake Union between August 7th, 2000 and September 13th, 2000. Because there was quite a bit of existing sediment chemistry data for Lake Union, a somewhat different sampling scheme was identified. Existing Lake Union sediment chemistry data were evaluated to find existing data that had been collected in the last 11 years that had received a QA 1 level review. Once the usable existing data evaluation was conducted, a GIS map of existing stations locations was created. A grid was then placed over the existing data map and new sampling locations were identified in grid cells where few or no data existed. Stations locations

were also selected to provide this study with broad spatial coverage within the greater Lake Union area (Union Bay, Portage Bay, Ship Canal, and Salmon Bay) (KC 2001)

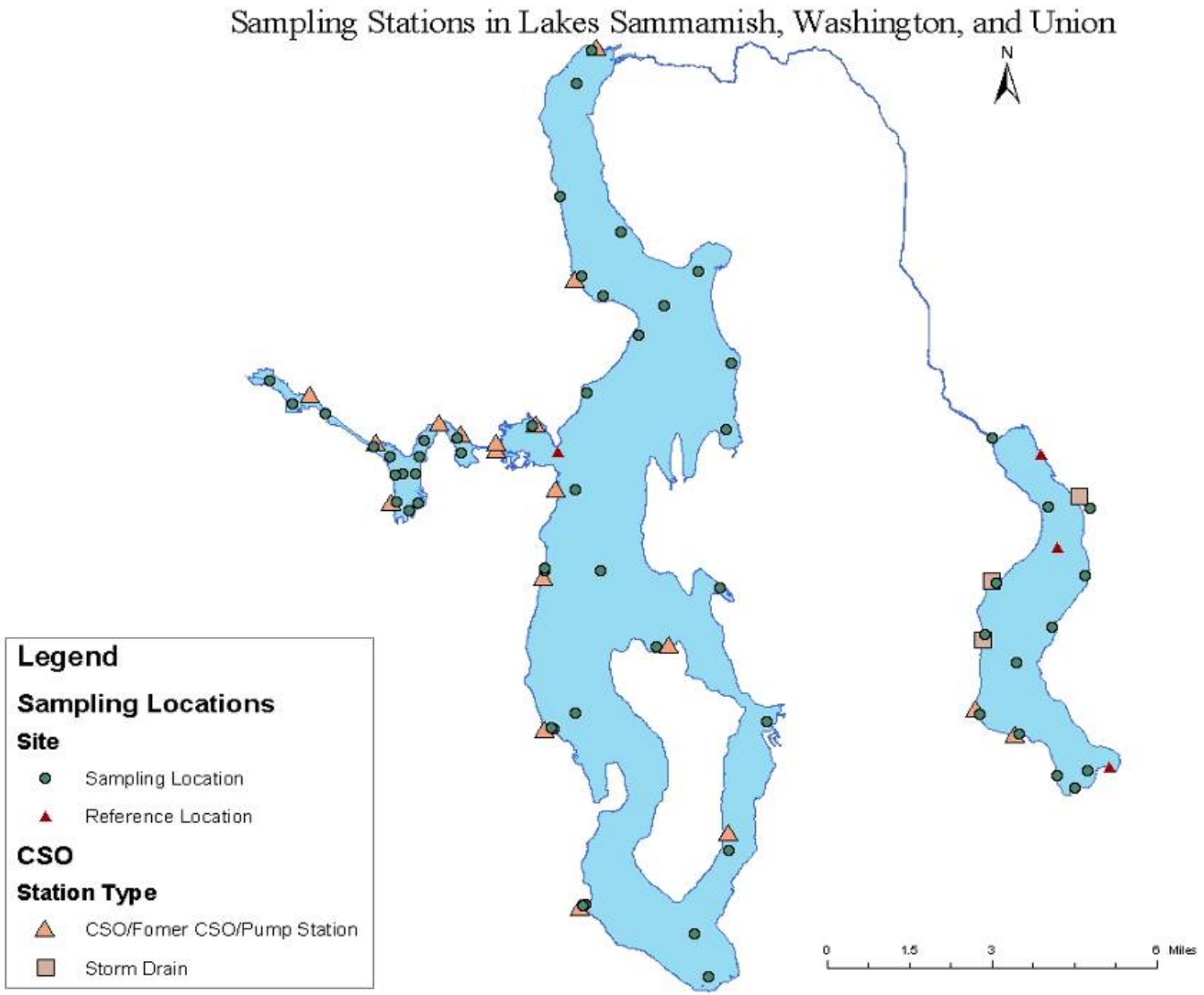

Figure 4. Sampling Locations

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5.1.1 Reference Sites

Reference sites were selected from areas with physical characteristics similar to the test sites and that theoretically represent the least impacted sites within the study area (Burton *et al.* 2002b). To accomplish this, the reference sediments for all sites were collected from either sediment in the least impacted lake (i.e. Lake Sammamish) or from locations in Lake Washington known to be relatively uncontaminated. For the purposes of the bioassay testing, the reference sediment for all three lakes was collected from both fine grained and coarse grained locations. Thus, the sediment samples from each lake were divided into two groups, based on their composition of percent fines. When the bioassays were conducted, the samples with percent fines closest to the coarse reference station were run in the same batch with the 'coarse' reference station. The same is true for the fine grained reference stations.

5.2 Sediment Collection

Samples were collected from the top 10 centimeters (cm) of sediment, enabling the characterization of the biologically active zone (King County 1999, King County 2000, King County 2001). Surface sediment grabs for chemical analysis and toxicity testing. were collected with a 0.1 m^2 stainless steel modified Van Veen grab sampler. The sample aliquot was collected with a stainless steel spoon from the top 10 cm of sediment in the sampler and placed in a stainless steel bowl. A sufficient number of sediment grabs were collected to provide approximately eight liters of sediment. The sample was homogenized and transferred to pre-labeled, laboratory-supplied containers. Benthic macroinvertebrate samples were obtained using a small Van Veen grab sampler (0.025 m^2) in Lake Sammamish and a Petite Ponar sampler (0.0231 m^2) in Lakes Washington and Union. Initially there was a concern that the small Van Veen grab sampler did not provide an adequate volume of sample material, as such, two grabs were used for each sample. The benthic samples were preserved using a 10% buffered formalin solution with Rose Bengal (KC 1999, KC 2000, KC 2001). After approximately two weeks all benthic samples were transferred to 70% ethyl alcohol.

5.3 Chemical Testing

Sediments collected from the three lakes were analyzed for chemical constituents including base/neutral/acid extractable organic compounds (BNAs), pesticides and herbicides,PCBs, metals, tributyltin and other butyltin metabolites, total petroleum hydrocarbons, ammonia, percent solids, total organic carbon (TOC), total phosphorus and other sediment conventional parameters. More detailed information about the chemical analyses and specific methods can be found in (King County 1999), (King County 2000), and (King County 2001). The analysis methods and detection limits for all analyzed chemicals are listed in Appendix B.

5.4 Biological Testing

5.4.1 Amphipod Bioassay

Hyalella azteca is an amphipod common to freshwater systems. It is frequently used as an indicator of the health of aquatic systems and is widely accepted as an ideal organism for evaluating sediment toxicity (Burton *et al.* 1996, Canfield *et al.* 1996, Chapman *et al.* 2001). Amphipod survival has been correlated with amphipod abundance and species richness (Swartz et al. 1994). In this study, *H. azteca* was used to test freshwater sediment for acute toxicity in a 10-day static renewal test with a mortality endpoint. Testing protocol followed EPA Method 600/R-94/024, 1994, in addition to E 1706-95b ASTM (1997) (King County, 1999, King County, 2000, King County, 2001).

5.4.2 Chironomus tentans

The freshwater macroinvertebrate *Chironomus tentans* has been widely used for the acute assessment of sediment contamination. This is due in part to its ubiquitous occurrence and importance in many aquatic environments and the fact that its larval development occurs in sediment. Results from acute exposures with *C. tentans* have been shown to effectively extrapolate to benthic community structure and other population-level effects determined under field conditions (Benoit *et al.* 1997, Canfield *et al.* 1996).

In this study, *C. tentans* was used to test freshwater sediment for acute toxicity in a 10-day static renewal test, with mortality and growth as endpoints. Testing protocol followed the USEPA Method 600/R-94/024 (1994) in addition to E 1706-95b ASTM (1997), ((King County 1999, King County 2000, King County 2001). Replicates were excluded from the mortality tests if the number or organisms increased and from the growth tests if the organism died. This resulted in 2 replicates being excluded from one station (M621).

5.4.3 Microtox® Bioassay

Microtox® is a rapid method of evaluating toxicity in an aqueous medium using the bioluminescent bacteria *Vibrio fishceri*. Assuming that light emitted by Microtox® is an indication of the overall biological condition of the bacteria, this test measures changes in the light output of the sample, as monitored by a photometer. Light emitted by the bacteria exposed to test sediment is compared to light emitted by bacteria exposed to control sediment, with a significant difference in luminescence between the test and control sediment indicating toxicity (King County 1999, King County 2000, KC 2001). This study used the Washington State Puget Sound Estuarine Protocols (PSEP 1995) for Microtox®, with both an organic and an aqueous extraction protocol to assess sediment toxicity. However, the extraction method used here was a modification of the saline extract procedure which used laboratory grade freshwater with similar characteristics as the site water (King County 1999, King County 2000, King County 2001).

Microtox® is an extremely sensitive bioassay, and some authors have suggested this high sensitivity makes Microtox® less effective at distinguishing between reference and control sites (Bombardier and Blaise, 2000). Further, there is broad agreement that the Microtox® bioassay is particularly sensitive to organic and conventional contaminants, but is not very sensitive to heavy metals (Pastorok and Becker, 1989, Lappalainen *et al.* 2000). Due to this over sensitivity, Microtox® was only used in this study to establish the "hit/"no-hit" status of the stations for the purposes of comparison to Ecology's Floating Percentile guidelines (discussed below).

5.5 Taxonomic evaluation

Taxonomic identification of benthic samples was completed by EcoAnalysts, Inc in Moscow, Idaho. Lab personnel used low power scanning or dissecting microscopes to sort the samples into major groups (e.g., oligochaetes, diptera, etc.). Once the samples were sorted, taxonomic analyses were conducted with all organisms identified to the lowest practical level (King County 1999, King County 2000, King County 2001). The benthic taxa were classified and several benthic metrics, including the Shannon-Wiener Diversity Index, Taxa Richness, and the Hilsenhoff Biotic Index were calculated**.**

5.6 Data Organization and Acceptability

After sampling, all appropriate chain-of-custody procedures, as well as sample preservation and storage requirements were followed. Concurrent with the chemical analyses, laboratory blank, replicate, matrix spike, standard reference method (SRM), and surrogate Quality Assurance/Quality Control (QA/QC) analyses were conducted. The majority of the chemistry data were within acceptable QA/QC limits. Any chemical concentrations corresponding to samples with low or high recovery were annotated with the appropriate data qualifier. The potential importance of high or low recovery is discussed in the uncertainty section below. A limited number of data points outside the acceptable QA/QC range were excluded from the majority of these analyses. The toxicity data were all within acceptable QA/QC ranges as were the benthic invertebrate analyses conducted by EcoAnalysts. The synoptic chemistry, bioassay, and benthic community composition data were stored in a relational Microsoft Access database. Due to the large size of the dataset, the data are not reprinted in this report. However, the Access database can be obtained from the author.

5.7 Using Sediment Quality Guidelines to Predict Toxicity

As discussed above, aquatic organisms are can be directly and indirectly exposed to chemical contaminants through interactions with sediments. SQGs enable evaluation of the toxicological significance of sediment associated chemicals by providing a benchmark from which to gauge the potential effects that exposure to sediment will have on aquatic organisms. However, many researchers now agree that SQGs should be

utilized in conjunction with other methods (Long and MacDonald, 1998, Chapman *et. al*. 1999, O'Connor *et. al*. 2000).

While several national, federal and nongovernmental organizations have developed SQGs, neither the Environmental Protection Agency (EPA) nor Ecology has promulgated formal numerical freshwater sediment standards. However, Ecology has provided an evaluation of existing SQGs for use in Washington State in terms of their reliability (correct predictions/total stations- the repeatability of each measurement), sensitivity (correctly predicted hits /total number of hits), and efficiency (both correctly predicted hits/total predicted hits and no-hits correctly predicted/total number of no-hits) (Ecology, 2002). Ecology (2002) concluded that none of the existing SQGs have ideal reliability, and proposed using a new technique for generating SQGs, using what they refer to as the floating percentile (FP) method (discussed below). While the FP method is an innovative way of calculating SQGs, it has yet to be adopted by other agencies, there is no peer reviewed literature that formally presents it, and its predictive ability has not yet been tested on a large dataset different than the one from which it was generated.

Thus, in addition to using the FP, a more widely used set of SQGs was selected. The probable effect levels (PEL) (Smith et al. 1996) is an older, more widely used SQG set. Since PELs represent a good balance between sensitivity and efficiency, PELs and thiere companion and more conservative threshold effect levels (TELs) were selected (discussed below) (Smith *et al.* 1996, Ecology 2002) for use in this study. Further, the TEL's and PEL's include SQGs for organochlorine pesticides (DDT, dieldrin, etc.), which are not included among the FP chemicals. Thus, another reason for selecting the TEL/PELs SQGs was that they enable evaluation of a group of chemicals commonly associated with sediment toxicity (Hoke *et al.* 1994, Swartz *et al.* 1994). The following sections provide more details on these guidelines.

5.4.1 Threshold Effect Levels and Probable Effect Levels

The Canadian Council of Ministers of the Environment (CCME) developed freshwater threshold effect levels (TEL) and probable effect levels (PEL) from a large database

containing freshwater sediment chemistry and toxicity data from North America. The TEL is intended to represent the level below which adverse biological effects rarely occur, while the PEL is intended to correspond to the level above which adverse biological effects frequently occur (CCME 1995, Smith *et al.* 1996). The analysis by Ecology (2002) showed the TELs to be very efficient (low sensitivity) and have high rates of false positives, while the less conservative PELs are more balanced in terms of sensitivity and efficiency.

5.4.2 Floating Percentile

The floating percentile (FP) guidelines were developed by Ecology from a large freshwater dataset from Oregon and Washington (Ecology, 2003). A key goal in the development of these guidelines was to reduce false positives and false negatives by allowing chemical guidelines to represent varying percentiles of biological effects. There are three biological significance levels associated with these guidelines: statistical significance (stat), sediment quality standards (SQS), and cleanup screening level (CSL). Evaluating data under these guidelines involves designating each station as a "hit" or "no-hit" by comparing the bioassay data for each endpoint to its control and applying the difference between test and control shown in Table 5. It is important to note that the terms (CSL, SQS) are borrowed from regional sediment management and related only to conceptual levels of biological effects and do not imply formally promulgated sediment standards (Ecology, 1995). The stat, SQS, and CSL classifications correspond to "a no adverse effects level, a level above which minor adverse effects may occur, and a level above which more significant adverse effects may occur", respectively (Ecology 2002). More detail on the FP guideline derivation can be found in Ecology 2002 and Ecology 2003. The actual FP guideline values were obtained via personal communication with Brett Betts (Ecology) on November 25, 2003.

Table 4. Endpoints for Stat, SQS, and CSL significance levels (Summarized from Ecology, 2003.)

Test	Endpoint	Stat (α=.05)	SQS	CSL
H. azteca	10-day mortality	Signif. compared to C	T – C > 10%	T – C > 25%
C. tentans	10-day mortality	Signif. compared to C	T – C> 10%	T – C > 25%
C. tentans	10-day growth	Signif. Compared to C	T/C < 0.8	T/C < 0.7
Microtox®	Decrease in luminescence	Signif. Compared to C	T/C < 0.85	T/C < 0.75

C= Control, T = Test Sample

It is important to note that the synoptic chemistry and bioassay data from Lakes Sammamish and Washington, but not Lake Union, are part of the freshwater sediment dataset included in the SEDQUAL dataset from which the FP guidelines were derived. The data from Lakes Sammamish and Washington represent roughly 17% of the toxicity data points, and roughly 14% of the chemistry data points in the database. Because the Lake Union data were not included in the database used to derive the FP guidelines, it was used to evaluate the predictive ability of the FP guidelines. A preliminary evaluation of the FP guidelines using the Lake Union data showed the reliability of the Stat, SQS, and CSL levels to be 81%, 70%, and 50%, respectively. Due to the fact that the Stat and SQS guidelines levels have both fairly similar narrative descriptions and reliability values, only the Stat and CSL levels were used for further evaluation of the data.

6.0 Results

6.1 Sediment Chemistry

Eighty two sediment samples collected from 62 stations were analyzed for more than 150 contaminants, including metals, PAHs, pesticides, herbicides, phthalates, PCBs, *etc*. The full list of contaminants and associated analytical results are listed in Appendix C, Table 1. To streamline the data analyses, a subset of this larger contaminant list was selected which only included chemicals that were detected at least five times, were already of concern in the study lakes, or were included in the FP or TEL/PEL SQGs. This subset resulted in 59 chemicals, which are identified in Appendix C, Table 1. For most of the analyses, a third subset of chemicals was created, consisting data from all lakes, but only those chemicals with a minimum of 30 detects. The goal of this analysis was to both reduce data dimensionality and to identify contaminants that exceeded SQGs.

Since no numerical value was assigned to chemicals not detected above their detection limit (they were either not present in a sample or were present in quantities smaller than can be detected by the analytical techniques), all non-detect data were populated with ½ the method detection limit (MDL). The sums for Total PCBs, Total PAHs, Total DDTs, and Total Benzofluoranthene were calculated as the sum of the constituent values. Appendix C, Table 2 shows the chemicals included in each sum. All metal concentrations were reported as total, and the simultaneously extracted metals (SEM), were used only in the AVS analyses. The data points that were rejected based on QA/QC were excluded from the analyses, excepting the PCA and bootstrap analyses, where missing values would have rendered a sample unusable. The rejected chemical concentration data consisted of 60 data points from the 2001 sampling, primarily for Benzo(b)fluoranthene and Benzo(k)fluoranthene. For many of the analyses, the chemical data were ln- normalized to transform the data to an approximately normal distribution.

6.2.1 Comparison to SQGs

As discussed above, all chemical concentrations were compared to numerical SQGs. Appendix C, Table 3 lists each chemical and corresponding SQG for the two guidelines discussed above. To summarize these data, chemicals were divided into eight groups

based on chemical group. Appendix C, Table 3 presents a list of these chemical groups. The combined influence of all contaminants in a chemical group was calculated by dividing the measured concentration of each contaminant in a group by its associated guideline and taking the average of all quotient values. This value is termed a hazard quotient (HQ). Thus, a value >1 indicates that the particular group, on average, exceeded the guideline for that station. Note that since if a station was sampled multiple times, the HQ values represent the average of all sampling events. A 'station average' HQ, that represents the average HQ across all chemicals for a station is also shown. Chemical concentrations were excluded if they were below the SQG and/or were not detected above analytical detection limits. The results of this analysis are shown in Appendix C, Table 4. Table 6 below summarizes the results shown in Appendix C, Table 4 by showing the percentage of stations that exceeded the SQG. PCBs were found at elevated concentrations throughout the three lakes. Additionally, Lake Union sediments had elevated concentrations of TBT, metals, and PAHs. Table 6 shows that, generally, Lake Union had higher chemical concentrations than Lake Washington, which generally had higher chemical concentrations than Lake Sammamish.

While this comparison to SQGs provided important information about sediment chemical concentrations, it still leaves the chemicals in the form of up to 8 parameters (each chemical group) and provides little information about how individual chemicals in each chemical group may covary. To further investigate patterns within and between chemicals, Principal Component Analysis (PCA) was used.

Table 5. Percentage of stations in each lake with SQG-Qs >1

Chemical Group	SQG	Lake Sammamish	Lake Washington	Lake Union
Dibenzofuran	FP-Stat	0%	0%	13%
TBT	FP-Stat	0%	10%	73%
Organochlorine Pesticides	TEL	0%	0%	33%
DDT	TEL	0%	34%	53%
Phthalates	FP-Stat	31%	34%	73%
PAHs	FP-Stat	0%	0%	20%
	TEL	25%	17%	73%
Metals	FP-Stat	0%	28%	80%
	TEL	25%	76%	93%
PCBs	FP-Stat	94%	97%	80%
	TEL	100%	100%	93%

6.2.1 Principal Component Analysis (PCA)

PCA is a multivariate statistical technique for reducing the number of variables in a data matrix. In the present case, PCA was used to reduce the number of chemicals parameters from 30 to 4. While there were 82 samples analyzed for the full suite of contaminants, only chemicals with a minimum of 30 detections were used in this analysis. This did not affect the number of samples in the analysis, only the limited the suite of chemicals evaluated for each sample. This enabled chemicals to load to components based on the correlation and magnitude of detected values rather than patterns in non-detected chemicals. In order to reduce the influence of spurious correlations between chemicals that tended to have similar trends in the magnitude of detection limits, all non-detect values for each chemical were assigned one half of the highest MDL concentration for that chemical. Data originally qualified as rejected were also populated with this value since PCA cannot be run on data matrices with missing values. Finally, all data were ln-normalized to transform the data to an approximately normal population.

To simplify the factors the Varimax rotation with Kaiser Normalization was used (Kaiser, 1958). The Varimax rotation criteria uses orthogonal rotation to minimize variation in the PCs and was selected based on its widespread acceptance as the optimal PCA rotation. This analysis yielded four factors with Eigenvalues >1 which explained 92% of

the total variation in the overall chemical dataset. Table 7 shows the factors and the correlation between the factors and each PC. The shading indicates which chemicals were correlated with the PC. For example, Fluoranthene had a coefficient of correlation of 0.98 with PC1, indicating that 98% of the variance in Fluoranthene's concentration can be accounted for by PC1. The first PC was correlated with 11 PAHs. The second PC was primarily correlated with metals, Total PCBs, Bis(2-Ethylhexyl)Phthalate, and Aroclor 1254. The third PC was correlated with Arsenic, Total DDT, 4, 4 DDT, and 4, 4 DDE. The fourth PC was correlated with the Butyl Tins; Mono-n-Butyltin, Tri-n-Butyltin, and Di-n-Butyltin. Mercury was not correlated with any of the PCs. For the sake of simplicity, PC1 is henceforth termed the "PAH PC", "PC 2" the "Metals PC", PC3 the "Pesticide PC", and PC 4 the "Butyl Tin PC". In this analysis, these PCs were extremely useful because they enable comparison of the chemistry data to the bioassay results and benthos data using four parameters instead of 30. The high correlations within each PC may also signify a similar origin and/or fate and transport for some of the chemicals.

Table 6. Principle Component Correlations. Shading indicates which chemicals correlate with each PC.

	Chemical Group	PC1	PC2	PC3	PC4
Anthracene	PAHs	0.98	0.13	0.08	0.03
Benzo(a)anthracene	PAHs	0.99	0.06	0.11	0.04
Benzo(a)pyrene	PAHs	0.99	0.04	0.14	0.04
Benzo(a)fluoranthene	PAHs	0.98	0.06	0.14	0.06
Benzo(g,h,i)perylene	PAHs	0.96	0.09	0.20	0.05
Benzo(k)fluoranthene	PAHs	0.97	0.07	0.10	0.08
Chrysene	PAHs	0.99	0.08	0.12	0.04
Fluoranthene	PAHs	0.98	0.09	0.11	0.04
Indeno(1,2,3-Cd)Pyrene	PAHs	0.97	0.11	0.18	0.05
Phenanthrene	PAHs	0.95	0.22	0.02	0.04
Pyrene	PAHs	0.99	0.08	0.14	0.00
Total PAHs (Molar Sum)	PAHs	0.97	0.14	0.13	0.04
Aroclor 1254	PCBs	0.15	0.88	0.36	0.19
Bis(2-Ethylhexyl)Phthalate	Phthalate	0.05	0.69	-0.06	0.36
Cadmium	Metals	0.16	0.78	0.53	0.19
Chromium	Metals	0.09	0.66	0.58	0.15
Copper	Metals	0.11	0.95	0.16	0.09
Lead	Metals	0.08	0.96	0.22	0.09
Silver	Metals	0.08	0.94	0.26	0.02
Total PCBs	PCBs	0.08	0.84	0.39	0.02
Zinc	Metals	0.18	0.86	0.38	0.29
Arsenic	Metals	0.19	0.37	0.68	0.41
Nickel	Metals	0.19	0.35	0.68	0.17
Total DDT	Pesticicdes	0.17	0.36	0.82	0.20
4,4 DDT	Pesticicdes	0.12	0.38	0.82	0.18
4,4 DDE	Pesticicdes	0.42	0.30	0.73	0.17
Di-n-Butyltin	Butyl Tin	0.04	0.25	0.27	0.92
Mono-n-Butyltin	Butyl Tin	0.01	0.13	0.28	0.92
Tri-n-Butyltin	Butyl Tin	0.10	0.50	0.28	0.79
Mercury	Metals	0.39	0.55	0.51	0.37

6.2.2 Grain Size Analysis

Due to the increased surface area and increased surface charge associated with finer particles, hydrophobic contaminants are typically associated with finer sediment particles

(Horowitz 1991). To evaluate the extent to which grain size influences sediment contamination, the relationship between % clay, % silt, and % fines (silt + clay) and ln-normalized contaminant concentrations was evaluated for Total PAHs, (which was positively correlated with observed adverse effects (see section 6.4.1)) as well as Zn and Cu, which were the metals predicted to be most biologically available (see section 6.4.2). The strongest relationship was found between % clay and Cu, Zn, and total PAHs, which had r^2 values of 0.25, 0.30, and 0.14, respectively. Figure 5 below illustrates the relationship between Zn (the highest r^2) and percent fines. While the relationship is not strong, it does confirm that sediment grain size plays some role in influencing contaminant concentrations.

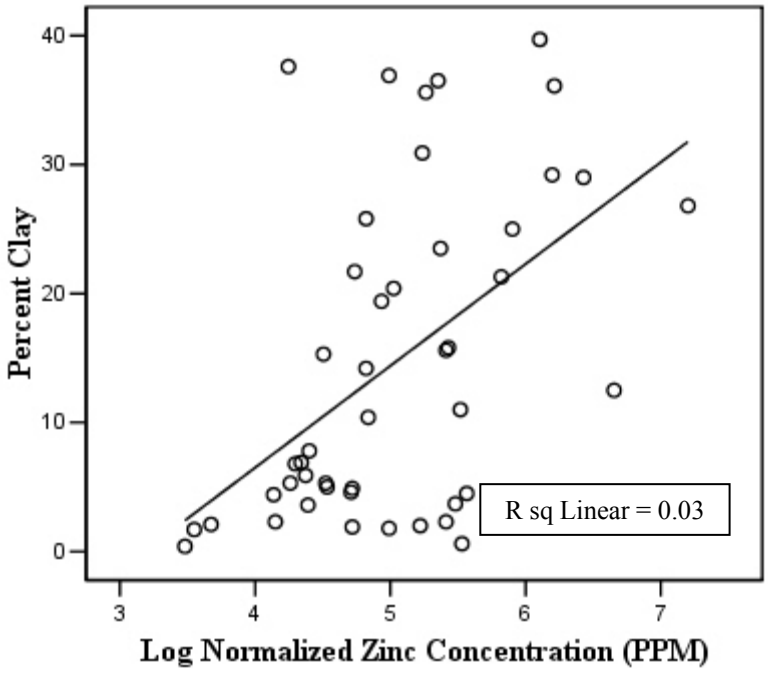

Figure 5. Zn Concentration vs. Percent Clay R=0.3

6.2.3 Temporal Perspective: DDT and its Derivatives

The existence of an evaluation of Lake Washington sediment contaminant concentrations in early 1981 enables the comparison of historical and current levels of DDT-related sediment contamination. When evaluating DDT contamination, an important consideration is that high ratios of DDT to its degradation by products (DDD and DDE) generally indicate that contamination is more recent (Davies *et al.* 1975).

The 1981 Toxicant Pretreatment Planning Study (TPPS) conducted by Metro (the precursor to King County) evaluated sediment samples from the top 2 cm, and many of the stations were focused around CSOs and storm drains (TPPS 1984). DDT was detected at more stations, and in higher concentrations than in the present study. Additionally, the ratios of DDT to its degradation byproducts (DDD and DDE) were also higher (Wilson, pers. comm.). In Lake Washington data from the present study, DDT or its degradation byproducts were found at 16 stations and DDT itself was only detected at 7 stations. At these 7 stations, the ratio of DDT to total DDT (DDT + DDD + DDE) ranged from 10 to 30 percent. This information suggests that DDT contamination is historical, is being degraded, and is being buried. It also appears that no new sources of DDT contamination have been identified (Wilson, pers. comm.).

6.3 Bioassay Data

Sixty-three grab samples collected from the three lakes were analyzed for sediment toxicity. The results from all four endpoints (Microtox® luminescence, *H. Azteca* growth, and *C. tentans* growth and survival) were evaluated to determine if they were statistically different than the control, at $\alpha = 0.05$. The statistics were run on raw toxicity data (with individual replicates), as opposed to the summary toxicity data (average of replicates). The term 'test' refers to a bioassay conducted on any sample that is not a control and the term 'test results' refers to the numerical result of a bioassay test run on a non-control sample (e.g., 80% survival at station 0612). Control normalization (dividing the bioassay test result by the control result) is a common technique to reduce the influence of test conditions on individual test results. However, since the control normalized bioassays generally had weaker correlations with the chemistry data, the bioassay data were only control normalized to facilitate comparisons between endpoints or between this study and other freshwater toxicity datasets.

Statistical significance of each test sample was assessed using one-way analysis of variance (ANOVA), and *post hoc* Dunnett's test with a significance level of $\alpha = 0.05$, to account for the fact that multiple test samples were run with 1 control. To fit the

assumption of normality for the ANOVA test, ln-transformations were applied to all bioassay data. The normality of the data was visually analyzed using histograms and P-P plots (used to determine whether the distribution of a variable matches a normal distribution), and the equality of variances was evaluated using Levene's test (Zar, 1984). The *C. tentans* growth data was ln-normalized and mortality results were ln +1 normalized because there were samples where survival was zero. Because the *H. Azteca* mortality data fit a gamma-distribution, initially they were not transformed and a Mann-Whitney test with a Bonferroni correction was used. Ultimately, this yielded results identical to the ANOVA tests run on the ln-normalized *H. Azteca* data. The results of the statistical analysis of the bioassay data are shown in Appendix D, Table 1. Table 8 summarizes the number of samples statistically different from the control for each species and the endpoint in each of the three lakes. If a station was sampled multiple times, and at least one of the samples was significantly different from the control, that station was considered statistically different from the control.

Table 7. Number of Stations Significantly Different from Control (α = 0.05).

Lake	Total Number of Samples	*C. tentans* Growth	*C. tentans* Mortality	*H. azteca* Mortality	Microtox® Luminescence
Sammamish	19	0	1	4*	9
Washington	29	2	4	3	12
Union	15	5	3	4	9

*1 of these hits was at a Lake Sammamish reference station.

Section 1.01

Following this step, the Microtox® data were excluded from further analyses. This exclusion occurred because many scientists involved in this project considered the Microtox® results suspect due to their high very sensitivity. Additionally, there are many questions about the relevance of using saltwater luminescent bacteria to evaluate freshwater ecosystems. Furthermore, in this study, the Microtox® data was poorly correlated with the other bioassay data.

6.3.1 Reference Envelope

The reference envelope statistical method uses bioassay test results from reference sites to develop relative standards against which to compare results from test sites (Smith 2002). The reference envelope establishes a numerical value representing the lower boundary of optimal conditions. Values below this boundary can be considered to be sampled from distinct populations with values lower than the reference envelope, and are deemed "impaired" as compared to the reference population.

Variance partitioning, which evaluates how much of the variance in a dataset is attributable to error, was used to determine the appropriate method for calculating the reference envelope. Since the error was primarily error variance (the data points were correlated by date or location), the 'naïve variance' approach, which gives equal weight to all of observed data, is not appropriate. Since the data points were correlated, the observed variability in the data may misrepresent the actual amount of information the data contain. Thus, the reference envelope was calculated following the bootstrapping method discussed in Smith (2002) using software provided by the author. Due to the computational complexity of the calculations, details are omitted here and can be found in Smith and Reige (1998) and Smith (2002). Note the reference envelope was calculated

for all endpoints. However, since the *H. azteca* data were previously determined to be taken from a gamma distribution and the ln-normalization did not entirely parameterize these data, these values should be viewed with more caution.

The data used for the reference envelope calculation were selected three ways; 1) stations originally designated as reference sites, 2) all data from Lake Sammamish and 3) two relatively uncontaminated stations from Lake Washington (0862/0801A). The second group was selected based on its relatively uncontaminated condition and results of the statistical comparison of bioassay results to the controls. This resulted in 24 samples, or 19 unique stations. These samples were then broken down into two groups based on whether the bioassay tests were conducted with a fine-grained or coarse-grained sediment reference station group (see Section 4.1.1). The summary bioassay data were ln-normalized and the parametric bootstrap with α=0.05 and the 10th percentile of the reference and *H. azteca* and Table 10 shows the number of stations less than the reference envelope in each lake. Since a few stations were sampled multiple times, a station with one value less than the reference envelope for a given endpoint was considered to be distinct from the reference population (impaired).

These results show that stations with fine-grained sediment most frequently had bioassay results below the reference envelope value. In addition, Lake Union has more statistically significant bioassays than Lakes Washington and Sammamish.

Table 8. Reference Envelope Values for *C. tentans* and *H. azteca*

Species	Endpoint	Ref Envelope-Coarse	Ref Envelope-Fine
C. tentans	Mortality	55% Survival (-0.607)	54% Survival (-0.618)
C. tentans	Growth	2.09 MG DW (0.737)	1.80 MG DW (0.588)
H. azteca	Mortality	85% (-0.161)	76% (0.274)

Table 9. Percent of Stations Less than Reference Envelope

Lake	Number of Stations	Endpoint	Coarse (# less than RE/Total)	Fine (# less than RE/Total)
Sammamish	17	*C. tentans* mortality	0	0
		C. tentans growth	0	0
		H. azteca mortality	0	0
Washington	29	*C. tentans* mortality	11*	10
		C. tentans growth	11*	5**
		H. azteca mortality	11*	5**
Union	15	*C. tentans* mortality	0	0
		C. tentans growth	0	25***
		H. azteca mortality	0	17***

*Same Station: 4903B (Henderson CSO mouth)
**Same Station: 0864A (Sayer CSO)
***0569 (Southwest Lake Union, Dexter Ave) represented one of the three C. *tentans* growth stations and one of the two *H. azteca* mortality
RE – reference envelope

6.4 Toxicity

To frame the sediment toxicity observed in this study within a larger context, toxicity data were acquired from other freshwater studies. Figures 6, 7, and 8 compare the toxicity observed in this study to that observed in four other temperate North American lakes. Generally, similar test durations, test methods, and species life stages were used in the comparison lakes. Exceptions to this were that the Lake Waukegan tests were run using juvenile (4th instar) *C. tentans*, and the Lake Roosevelt growth data represent the results of a 20-day exposure instead of a 10-day exposure. The Lake Champlain bioassays were run on samples from stations previously found to be toxic. For both of the *C. tentans* endpoints, Lake Union showed more significant biological effects than the other lakes, while Lake Sammamish and Lake Washington exhibited toxicity more

similar to the comparison lakes. For *H. azteca*, the three study lakes were less impacted than the lakes used for comparison.

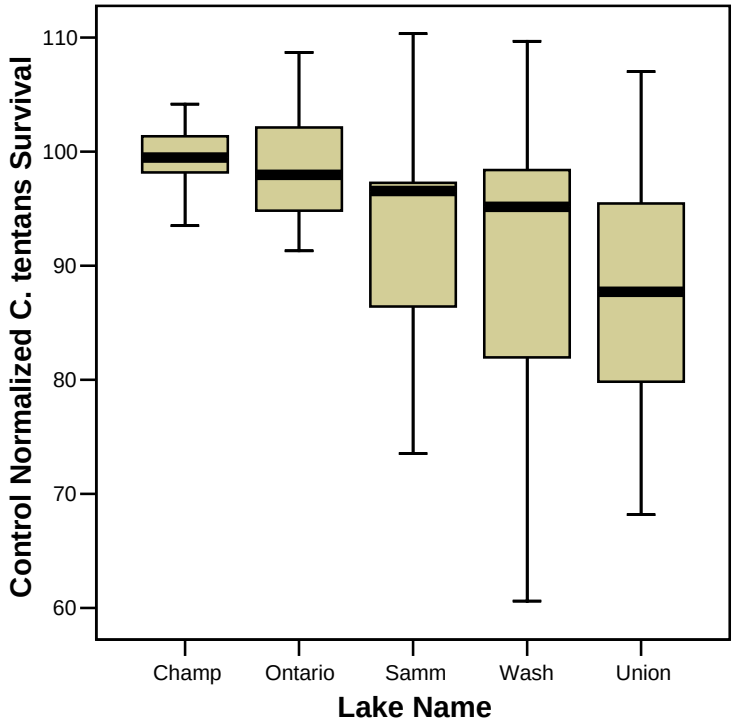

Figure 6. Control Normalized *C. tentans* Survival vs. Lake. The ends of the box represent the upper and lower quartiles. The median is marked by the bold vertical line inside the box the whiskers are the two lines outside the box that extend to the highest and lowest observations.

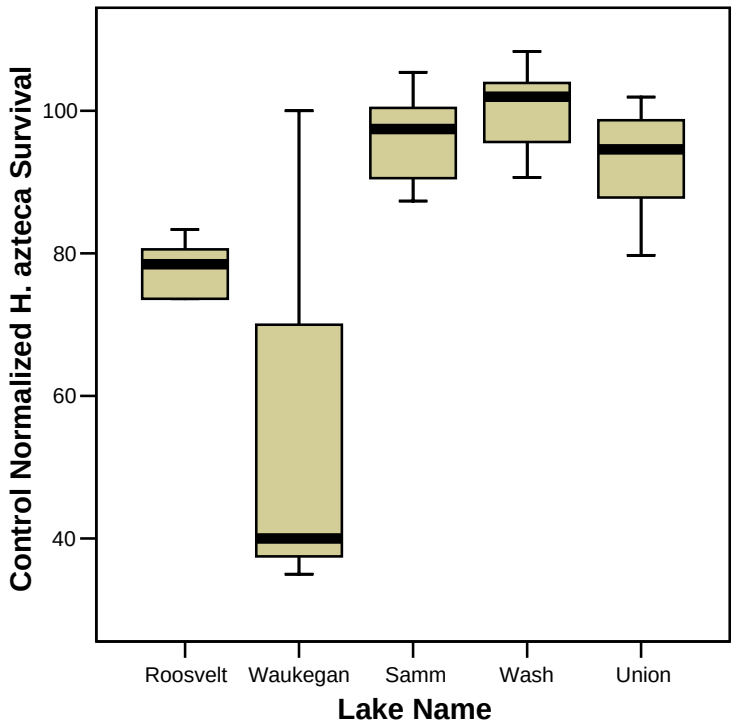

Figure 7. Control Normalized *H. azteca* Survival vs. Lake. The ends of the box represent the 1st and 3rd quartiles. The bold vertical line inside the box indicates the median and the whiskers extend to the highest and lowest observations.

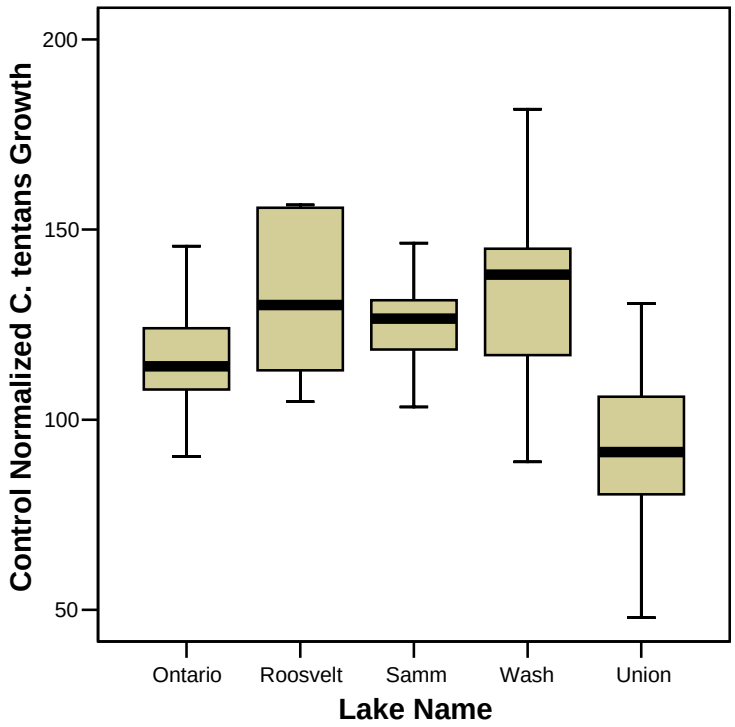

Figure 8. Control Normalized *C. tentans* Growth vs. Lake. The ends of the box represent the 1st and 3rd quartiles. The bold vertical line inside the box indicates the median and the whiskers extend to the highest and lowest observations.

6.4 Chemistry to Toxicity Comparison

Several analysis techniques were used to investigate the relationship between the chemical contaminant concentrations and bioassay results. A bootstrap procedure was used to validate correlations between particular contaminants and bioassay results. AVS/SEM normalization was used in an attempt to predict toxicity from metals, and correlation analysis was used to evaluate the relationship between the chemical PCs and the bioassay results.

6.4.1 Bootstrap Techniques

To investigate the relationship between the individual contaminant concentrations and bioassay results, the Pearson correlation coefficient between each of the three bioassay endpoints and each of the 30 chemicals evaluated in the PCA were calculated. Since some of these chemicals had weak to moderate correlations with the bioassay data, a bootstrap procedure was developed to validate the correlations. Briefly, bootstrapping is

a method of testing reliability of the dataset by randomly re-sampling the data. To complete the bootstrapping, all chemistry and bioassay data were *ln*-normalized, except Ni and Cr concentrations, which were already normal. As with the PCA, both non-detected values and data points that were qualified as rejected were populated with ½ the highest MDL concentration for that chemical. Since most chemicals had high detection frequencies, this did not dramatically alter the frequency distribution. In addition to *ln*-normalizing the data, each data point was standardized by subtracting the respective mean, and dividing by the standard deviation. To evaluate the correlation between sediment chemical concentrations and the bioassay results, the Pearson correlation coefficient (r), rather than the r^2 value was calculated since the sign of the correlation was important (i.e. high chemical concentration should give low expected survival). Scatter plots were used to confirm that a roughly linear relationship existed between variables. If several parameters (e.g. chemical concentrations) each have a weak correlation with another parameters (e.g. bioassay survival), taking the average of all chemicals and recalculating the aggregated r-value generally improves the strength of the correlation. Thus, the r and r^2 of the average of the chemical with the 5 highest r-values was then calculated with the intent of improving the correlation. This increased the strength of the correlation between the bioassay data and chemical concentrations.

The potential disadvantage of this method is that it may simply be aggregating false positives, causing the resulting correlations to be spurious. To test for this, a macro was written in Excel to randomize values from the chemical dataset and determine the correlation (r and r^2) of the relationship between the chemical averages with the strongest correlation with toxicity data. To randomize the data, one data point at a time was selected from each chemical until *n* values were selected; where *n* was the number of samples (basically sampling with replacement). As with the non-randomized data, the chemicals with the 5 highest r-values were selected, and the average of the correlation between the averages of those chemicals and the toxicity was calculated and written out to a table. This procedure constituted a 'trial' and was repeated 1000 times, saving the r value each time. This made it possible to evaluate the overlap between the 'randomized' r-values and the 'true' r-value. A t-test was then used to calculate the likelihood of the

'true' r-value being drawn from the 'randomized' population. Figure 11 shows an example of the cumulative distribution function (CDF) of the r-values for *C. tentans*. Table 11 shows the statistics and chemicals with the 10-highest r-values. To further illustrate this concept, Table 11 shows that the Pearson correlation (r) between *C. tentans* growth and the average of the 5 chemicals with the strongest correlation with *C. tentans* growth is -0.68. As seen in Figure 9, only one of the randomized (bootstrapped) values are less than -0.4, and none are as extreme as -0.68. Thus, for this and other endpoints, at α=0.05, the 'true' r is distinct from the randomized values and the correlations calculated were not due entirely to false positives. Chemicals with the five highest r^2 values common to all three endpoints were Anthracene and Benzo(k)fluoranthene. While not in the top five chemicals for each endpoint, Cu, Zn, and Pb were the metals with the highest r^2 associated with the toxicity data. Thus, these chemicals were all weakly to moderately correlated with bioassay results.

While the majority of the chemistry data are *ln*-normally distributed, a moderately strong correlation was found between a few non *ln*-normalized chemicals and the *ln*-normalized bioassay data. In particular, Bis(2-Ethylhexyl)Phthalate (also known as BEHP), had an r^2 of 0.577 and 0.545 with the ln-normalized *C. tentans* growth and *H. azteca* mortality data, respectively. The *C. tentans* survival data was not correlated with BEHP. Scatter plots of the *ln*-normalized *C. tentans* growth and *H. azteca* mortality data vs. BEHP concentration are shown in Figure 10 and 12, respectively.

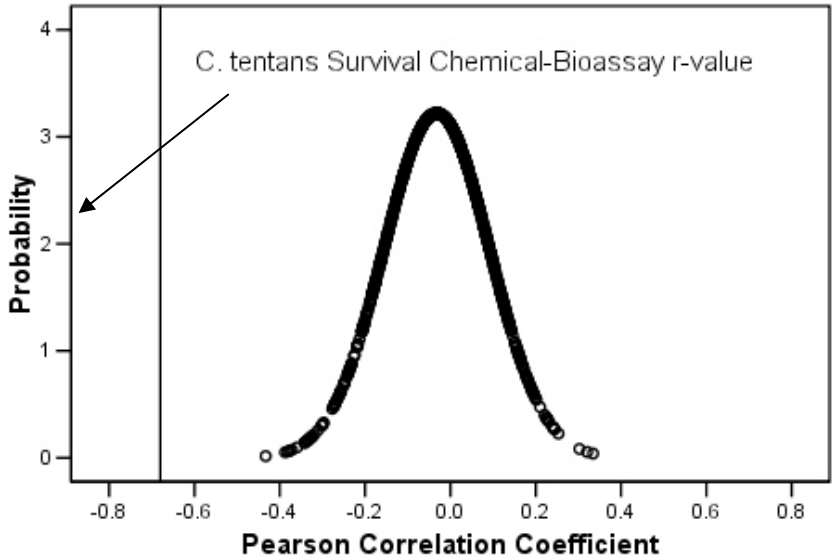

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Figure 9. Probability Distribution Function (PDF) of Pearson r-Value for *C. tentans* Bootstrap

Table 10. Bootstrap Results. The higher the r^2 represent the correlation between the aggregated chemicals and bioassay results.

Endpoint	**r^2 of 30 Chemicals**	**r^2 (r) of top 5 Chemicals**	**# of Values More Than 5 Chemical Average**	**Signif.**	**Chemicals with 5 Highest r^2 values (r^2)**
C. tentans Survival	0.123	0.16 (-0.47)	0.02	p<0.001	DBT (0.15), Pyrene(0.14), Phenanthrene(0.14), Chrysene(0.14), Benzo(a)Anthracene(0.14),
C. tentans Growth	0.313	0.47 (-0.68)	0.0	p<0.001	Aroclor 1254(0.35), Anthracene(0.35), Bis(2-Ethylhexyl)Phthalate(0.35), Benzo(k)fluoranthene (0.32), Total PCBs(0.32)
H. azteca Survival	0.18	0.30 (0.56)	0.0	p<0.001	Bis(2-Ethylhexyl)Phthalate(0.32), Cu (0.23), Phenanthrene(0.22), Anthracene(0.19), Benzo(k)fluoranthene(0.18), Benzo(a)anthracene(0.18)

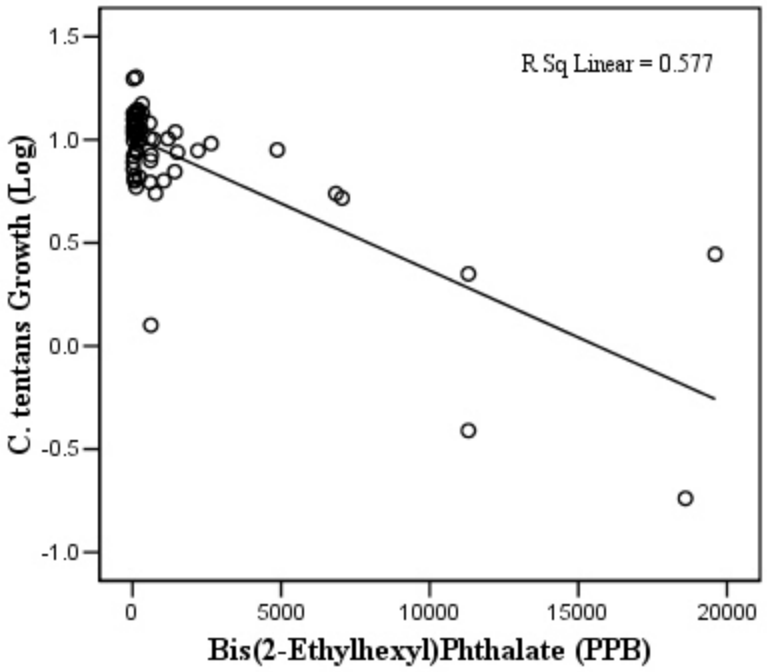

Figure 10. Bis(2-Ethylhexyl)Phthalate Concentration vs. *Ln* Normalized *H. azteca* Growth.

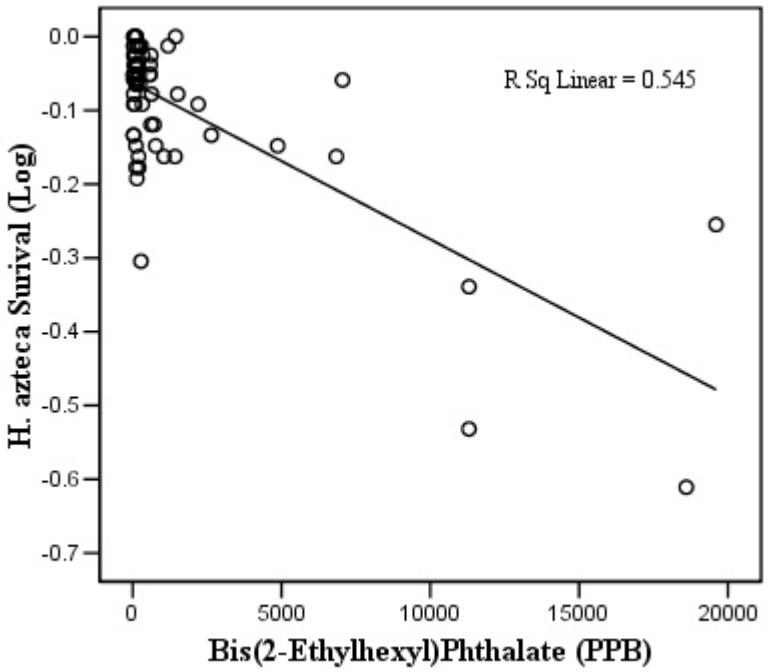

Figure 11. Bis(2-Ethylhexyl)Phthalate Concentration vs. *Ln* Normalized *H. azteca* Growth

6.4.2 AVS/SEM Normalization

As previously discussed, the bioavailability of sediment-associated divalent metals is related to the concentration ofAVS (Section 2.1). The molar concentration of SEM metals (Cd, Cu, Pb, Ni, Zn) and AVS was calculated for each sample to determine the AVS to SEM ratio. Using Pearson's correlation, the relationship between the *ln*-normalized SEM/AVS and the *ln*-normalized bioassay data was evaluated for each endpoint and found to be non-significant. Therefore, the presence of available SEM wasn't predictive of toxicity for these three lakes. Table 12 shows that Zn, Cu, and Pb make up the greatest concentration of the SEM. According to the AVS/SEM ratio, Zn, Cu, and Pb are the most related to the observed toxicity and represent the greatest percentage the metals of the SEM concentration. Compared to Zn, Cu, and Pb, there is less bioavailable Cd and Ni in these lakes.

Table 11. Average Percentage of Total SEM Represented by Individual Metals

	SEM Metal				
	Cadmium	Copper	Lead	Nickel	Zinc
Lake Sammamish	0.8	17.7	9.4	11.7	60.4
Lake Washington	0.2	8.5	14.0	9.0	68.2
Lake Union	0.1	23.9	14.5	8.4	53.1
All Lakes	**0.4**	**15.2**	**12.8**	**9.6**	**62.1**

6.4.3 PCs and Toxicity Data

To evaluate the relationship between the PCs and the bioassay data, the significance of the relationship between the *ln* +1-normalized PCs and the *ln*-normalized toxicity data was tested. All three bioassay endpoints had a significant relationship with the metals PC and the *C. tentans* growth and *H. azteca* endpoints had significant relationships with the PAH PC. The significance and the Pearson coefficient for these relationships are shown in Table 14. Since *C. tentans* growth had the strongest relationships, Figures 12 and 13 show the relationship between *C. tentans* growth and PC1 and PC2, respectively.

Table 12. Statistical Correlation between Principle Components and Bioassay Data

Predictor	Statistic	*C. tentans* Growth	*C. tentans* Mortality	H. *azteca* Mortality
PC1 (Metals)	Pearson Correlation	-0.434	-0.282	-0.250
	Sig (2-tailed)	0.00**	0.027*	0.050*
PC2 (PAHs)	Pearson Correlation	-0.558	-0.118	-0.645
	Sig (2-tailed)	0.00**	.361	0.00**
PC3 (Pesticides)	Pearson Correlation	0.244	0.087	0.338
	Sig (2-tailed)	0.056	0.502	0.007
PC4 (Butyl Tins)	Pearson Correlation	-0.28	-0.151	-0.333
	Sig (2-tailed)	0.052	0.241	0.008

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is marginally significant at the .05 level (2-tailed)

6.5 Benthic Invertebrate Assessment

Benthic invertebrates sampled from the three lakes exhibited a wide range of abundance values ranging from 10/m^2 to 125/m^2.In the three lakes, Oligochaeta were the dominant taxa in 50.9 percent of the samples, Chironomidae dominated in 47.3 percent of the samples, and Trichoptera dominated 2.0 percent of the samples. Generally, both the abundance of dominant taxa and abundance of chronomids increased as chemical concentration increased. The percentage of tolerant species such as oligochaetes also increased with increasing chemical concentrations.

6.5.1 Benthic Metrics

In addition to taxa abundance, several benthic metrics were calculated including species richness, diversity, and dominance metrics. Most taxa richness measures including species richness, Chironomidae richness, filterer richness, gatherer richness, Margalef's richness, and scraper richness were highly inter-correlated. In addition, several diversity metrics such s Pielou's J, Shannon-Weaver, and Simpson's Heterogeneity were also highly correlated. The metric most highly correlated with the others was chosen as the representative metric from each group; species richness was chosen from the first group and Shannon-Weaver diversity was chosen from the second. Species richness measures the number of distinct taxa, while the Shannon-Weaver index evaluates both the number of species and the proportional representation of taxa within the benthic community

(Shannon and Weaver 1949). In addition, both the Hilsenhoff Biotic Index (HBI) results were included in this evaluation (Hilsenhoff, 1988).

6.5.2 Comparison of Benthic Metrics to Chemistry

Based on the assessment of the benthic metrics discussed above, regression models were created for species richness, Shannon-Weaver diversity, and the HBI index. The predictor variables were physical (depth and percent fines) and chemical (the four PCs in additional to extractable phosphorus and ammonia nitrogen). Extractable phosphorus, rather than total phosphorus, was used because it generally had a better correlation with the response variables. All four response variables, and all predictor variables with the exception of percent fines were *ln* normalized to transform them to an approximately normal distribution. Summary benthic data (indices based on the average of five replicates at each station) were used to develop the models, rather than the raw, individual replicate data. Since the benthic data had no specific sample numbers, the average PC regression score for each station was used when correlating between the benthic and chemistry data. Before correlating the benthic indices to the physical and chemical data, the correlation between the physical and chemical predictor variables was tested. Depth had a significant positive correlation with both percent fines and the pesticide PC, with r^2 values of 0.14 and 0.27, respectively. Extractable phosphorus and ammonia nitrogen also had a significant relationship with each other (r^2 =0.68) and with depth and grain size (r^2 between 0.16 and 0.34). Depth was an important factor in the regression model, potentially due to the fact that depth was correlated with grain size (higher percent fines at deeper stations).

This relationship between the predictor variables had the potential to cause multicollinearity problems, so particularly close attention was paid to the variance inflation factor (VIF) while building the regression models. Table 15 shows the Pearson's correlation coefficient between each of the indices. Again, high PC regression scores generally signify elevated chemical concentrations. Mallow's Cp, enables

comparison between regression models with different number of terms was used to determine which terms were significant in the regression model (Mallows 1973). All combinations of predictors were used to find the 'best' model. Table 15 highlights the terms that were significant in the models, and Table 16 shows the regression equations and associated r^2 values. Appendix E, Tables 1-4 shows the ANOVA tables associated with the final models. Note that all four models contained terms that were not significant when individually compared to the indices, but were significant in the linear regression model when combined with other terms. For example, the species richness model includes PC4 as a significant term. However, when PC4 was not a significant term when correlated with species richness alone. Outlier analyses were conducted on all models and their residuals, distances (Cook's and leverage) and influence statistics (DfFit) did not suggest that any observation points could be classified as outliers.

Table 13. Benthic Metric Statistics

		Metric		
Predictor	**Statistic**	**Shannon -Weaver**	**Species Richness**	**HBI**
Ammonia Nitrogen	Pearson Correlation	-0.447	-0.487	0.349
	Sig. (2-tailed)	0.000*	0.000*	0.0007*
Extractable Phosphorus	Pearson Correlation	-0.446	-0.434	0.383
	Sig. (2-tailed)	0.000*	0.001*	0.003*
Depth	Pearson Correlation	-0.32	-0.57	.17
	Sig. (2-tailed)	0.016	0.000**	.20
Percent Fines	Pearson Correlation	-0.22	-0.45	.12
	Sig. (2-tailed)	0.098	0.000**	.38
PC1	Pearson Correlation	-0.165	-0.071	.199
(PAHs)	Sig. (2-tailed)	0.216	.595	.135
PC2	Pearson Correlation	-0.229	-0.066	.238
(Metals)	Sig. (2-tailed)	0.083	0.625	.07
PC3	Pearson Correlation	-0.247	-0.477	.182
(Pesticides)	Sig. (2-tailed)	0.062	0.000*	.171
PC4	Pearson Correlation	-0.159	-0.171	.279
(Butyl Tins)	Sig. (2-tailed)	0.223	0.200	.034**

* Correlation is significant at the 0.01 level (2-tailed).
**Correlation is significant at the 0.05 level (2-tailed).
*** Correlation is marginally significant at the 0.05 level (2-tailed).
=Variable is Significant in the Regression Model (α=.05)

Table 14. Regression Model Equations and Statistics

Response Variable	Regression Equation	Signif.	Adjusted R^2	Standard Error of the Estimate
Shannon-Weaver	= 2.3-.25*ln(Depth)-0. 36*ln(PC2+1) -0.315*ln(PC1+1)	0.004	0.197	0.442
Species Richness	= 3.8-0.36*ln(Depth)-0. 21*ln(PC4+1) -.0056*PercentFines	0.000	0.457	0.412
HBI	= 2.0-0.035*ln(Depth)-0. 0475*ln(PC4+1)	0.034	0.103	0.113
Chironomid Richness	= 3.030-.416*Depth-.403*PC4-.009*PercentFines	0.000	0.303	0.77

To further analyze these data, the samples were aggregated into groups based on station depth and sediment grain size, to be statistically compared against the reference station. All samples were classified as either "coarse-grained" or "fine-grained" depending on the sample group the data initially fell into (see Section 4.1.1) and by depth, broken up into 'shallow' and 'deep' categories (0-20 and 20-60 m, respectively). These depths were chosen primarily because the deepest reference station was at 21 m, but consulting a histogram of depth for all data confirmed these were acceptable groups. Since there were no coarse, deep stations, 3 groups resulted (coarse grained-shallow, fine grained-shallow and-fine grained deep). The reference data was comprised of 2 coarse-shallow stations (0600REFNE and 0544), 1 fine grained-shallow (0600REFSE) and 1-fine grained deep (0611A). All reference data, except that for the 1 coarse grained-shallow site, were from Lake Sammamish. Using the replicate (rather than the average of the 5 replicates) benthic metric data, each station was compared to the reference group using ANOVA with a *post hoc* Dunnett's comparison (described in section 6.3 in more detail) at α=0.05. The five replicate benthic samples from each of the two coarse grained shallow stations (0600REFNE and 0544) were treated as the same station; all ten replicates were combined Appendix E, Table 5 shows the results of this statistical analysis. Table 17 below summarizes this and Appendix E, Table 5 shows the number of stations in each lake with benthic metric values significantly different from the reference stations. Generally, if a station had an index significantly different from the reference for one station, it tended to be significant for the others. This was particularly true for Lake Union, where eight of the 12 stations were significant different from the reference for all

three indices, and the rest were significant for at least two. Ultimately, Lake Union had the most statically impaired benthos, followed by Lakes Washington and Sammamish.

Table 15. Number of Stations Significantly Different from Control.

Lake	Number of Stations (Excluding Reference)	Shannon-Weaver	Species Richness	HBI
Sammamish	13	3	3	3
Washington	29	3	2	2
Union	15	12	11	9

6.5.3 Benthic Reference Envelope

Similar to the reference envelope development procedure described in Section 6.3.1, a reference envelope was developed only for species richness, since it had the strongest correlation with chemical contaminants. Like the toxicity reference envelope, all of the Lake Sammamish and other reference stations were aggregated, and broken into the same coarse-shallow, fine-shallow and-fine grained deep stations described in Section 6.3.1 above. Since there was only 1 deep-fine grained reference station in the reference group, a reference envelope could not be calculated for this group. Table 16 shows the species richness value that is the result of the reference envelope calculations.

Table 16. Reference Envelope Values for *C. tentans* and *H. azteca*

	Reference Envelope		
Metric	**Coarse-Shallow**	**Fine-Shallow**	**Fine-Deep**
Species Richness	2.36	5.93	NA

6.5.4 Benthic Metrics to Toxicity

The relationships between the *ln*-normalized benthic metricsand the *ln*-normalized toxicity endpoints were evaluated using Pearson's correlation coefficient. Table 18 shows the results of this analysis. Ultimately, both the HBI and Shannon-Weaver indices had significant relationships with *C. tentans* growth and *H. azteca* mortality. This shows that the *C. tentans* growth and *H. azteca* mortality bioassays had a week to moderate correlation with two of the benthic indices. Surprisingly, species richness (which had a

moderate correlation with contaminant concentrations) was not correlated with the bioassay response. The strongest correlation (negative) between bioassay response and benthic metric was *H. azteca* mortality and HBI, respectively. This is not surprising, given *H. azteca*'s sensitivity to organic pollution (Burton *et al.* 1992)

Table 17. Correlation Analysis between Bioassay Results and Benthic Metrics.

Predictor	**Statistic**	**HBI**	**Shannon-Weaver**	**Species Richness**
C. tentans Growth	Pearson Correlation	-0.34	0.28	0.16
	Sig. (2-tailed)	0.009**	0.035*	0.240
C. tentans Mortality	Pearson Correlation	-0.08	0.05	0.04
	Sig. (2-tailed)	0.560	0.708	0.787
H. *azteca* Mortality	Pearson Correlation	-0.46	0.29	0.12
	Sig. (2-tailed)	0.000**	0.028*	0.370

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

6.5.5 Benthic Biomass

To evaluate the benthic abundance data, a literature search was conducted to find the average dry-weight biomass for each observed taxa. These values are shown in Table 19. For each station, biomass of each individual taxa type was multiplied by the abundance of each taxa and the total biomass for each station was then calculated. Using the same reference stations as those discussed in Section 6.5.1, each station was compared to the appropriate reference using ANOVA with a 1-tailed *post hoc* Dunnett's comparison (described in section 6.3 in more detail) at α=.05. The results of the statistical analyses are shown in Appendix E, Table 5. Benthic biomass for five Lake Washington stations from the fine grained-deep reference group were statistically significant from the reference. However, these stations were located at depths between 35 and 65 m, while the reference station was located at 21m. Therefore, reduced biomass in these stations is probably attributable to the fact that they are being compared to a reference station that is considerably shallower, and thus not a robust comparison. However, this is the only reference station available for the fine grained-deep reference group.

Table 18. Benthic Biomass

Taxon	DW (grams/individual)	Citation
Annelids	1.11E-04	Svensson *et al.* 2001
Acari	7.11E-05	Diggins *et al.* 1979
Bivalvia	4.43E-03	Diggins *et al.* 1979
Coleoptera	1.83E-03	Diggins *et al.* 1979
Crustacea	2.10E-04	Jorgensen *et al.* 1991
Chironomidae	5.69E-04	Jorgensen *et al.* 1991
Diptera	7.50E-05	Diggins *et al.* 1979
Ephemoroptera-Caenis sp.	2.05E-04	Jorgensen *et al.* 1991
Ephemoroptera-Hexageneia.	2.05E-04	Diggins *et al.* 1979
Gastropoda	3.98E-03	Diggins *et al.* 1979
Odonata	4.79E-04	Diggins *et al.* 1979
Tricoptera	1.15E-04	Diggins *et al.* 1979

6.6 Representing Multiple Lines of Evidence

A quantitative decision matrix was developed to summarize the multiple lines of evidence in a succinct way. To create the decision matrix, the results from the bioassay, chemical, and benthic analyses for each station were aggregated into a quantitative decision matrix. Appendix F, Table 1 shows the chemical PCs, benthic metric values, and bioassay results before these and other data were synthesized into the decision matrix. Each LOE was given a potential of 10 points, 10 points representing the maximum impairment. Since there are 30 LOE, each station has the potential to have 30 points. Stations with less than 10 points were graded as 'A' or minimally impaired, between 10 and 20 were graded 'B', or moderately impaired, and more than 20 were graded as 'C', or severely impaired. The methods used to synthesize each LOE are described below. While this approach simplifies the data to some extent, its advantages include the summarization and ultimate visual representation of large amounts of data. This summarization and step enabled further summarization in the form of a station-grading system.

Ultimately, 42 stations were graded as ‘A’, 13 were graded ‘B’, and 3 were graded ‘C’. All ‘C’ classified stations were located in Lake Union. This grading system is clearly subjective and its primary intent was to establish groups of stations for the purpose of making recommendations for future monitoring. The results of the decision matrix calculations are shown in Appendix F, Table 2. Figure 12 shows the results of these calculations for each station.

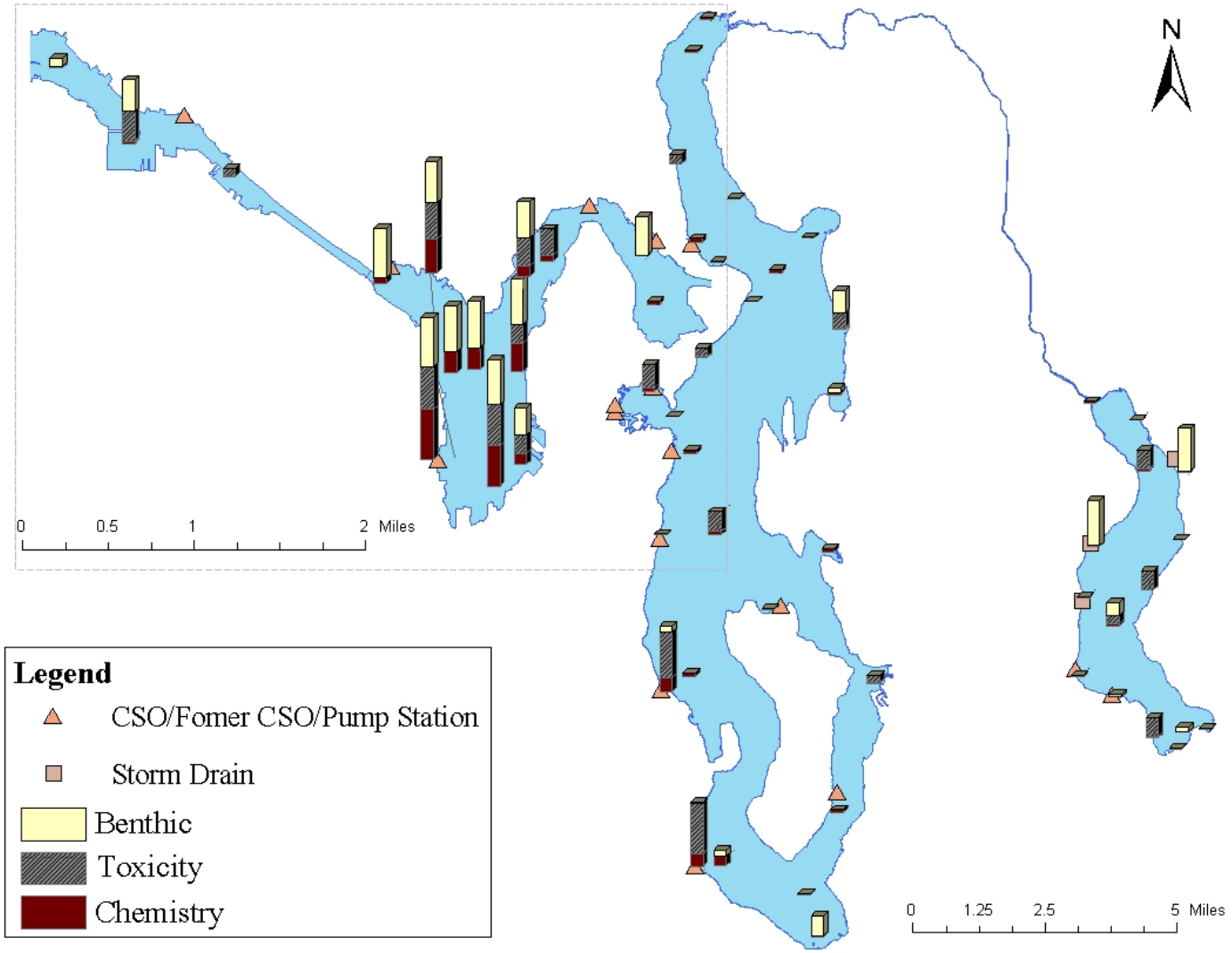

Figure 12. Summary Map of All Triad Data Using Decision Matrix

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6.6.1 Decision Matrix-Chemical Contaminant Data

For chemistry, a station-average HQ was calculated from the stat level (you had previously been using the term "stat" should be consistent) of the floating percentile SQGs, the most conservative level of the floating percentile guideline set (See Appendix C, Table 4). If replicates were analyzed for a particular station, the average of the HQs was used. The final HQ values for all stations were normalized to 10. While this approach includes some invalid assumptions, such as that all chemicals contribute equally to toxicity, it does enable the amassing of all of the chemistry data to one value representing an overall general index of contamination.

6.6.2 Decision Matrix-Bioassay Data

Both the bioassay statistical analyses and the reference envelope calculations were used to assess sediment toxicity. For comparison to control, since some endpoints were more responsive to chemical contamination than others, the control normalized data points were weighted using the 5-chemical r^2 values obtained from the bootstrap analysis (Section 6.4.1). Thus, the endpoint values for *C. tentans* growth, *H. azteca* survival, and *C. tentans* survival had weighted scores of 0.47, 0.16, and 0.3, respectively. The sum of the values across the endpoints for all stations was normalized to 5. A similar procedure was followed for the percent reference envelope data points for each endpoint. The same weights were multiplied by the endpoint value divided by the reference envelope value (percent reference envelope), and the results were summed for all stations and normalized to 5. The control normalized and reference envelope sums were combined and further normalized to 10.

6.6.3 Decision Matrix-Benthic Data

A two step process was used to allocate the 10 points based on the degree of benthic alteration. First, weights of 0.457, 0.197, and 0.103 from the r^2 from of the models built to explain Species richness, Shannon-Weaver index, and HBI indices (Section 6.5.2) were applied to the appropriate data point. Next, the result was summed for all stations and the final values were normalized to 10.

7.0 Discussion

As previously indicated, the objective of this study was to answer the four questions below:

- What are the contaminants of concern (COC)?
- Is there a measurable response to this contamination? If so, are the contaminants causing the response?
- Which lakes or areas of the lakes are most impacted?
- What are the potential sources of the contaminants?

The answers to the four research questions posed above formed the framework for this investigation and the answers to them have important implications for future studies. Contamination in these lakes is associated with a variety of biological effects, including toxicity to multiple species and impaired benthic community structure. Generally, all three lines of evidence (sediment contamination, bioassay results, and benthic invertebrate community alteration) indicated that Lake Union is more impacted by chemical contamination than Lakes Washington and Sammamish. The highest contaminant concentrations were found in the near-shore areas of Lake Union. In all three lakes, PCBs were the chemical group that most frequently exceeded SQGs, followed by metals, PAHs, and phthalates. Toxicity, but not benthic invertebrate community alteration, was generally correlated with elevated contaminant concentrations, and the *C. tentans* growth endpoint was commonly the most responsive to contamination. Generally, the high degree of variability within the benthic community at each station made it difficult to characterize the differences between stations. This result has been confirmed by other research in the same study area (TPPS 1984). Comparing these three lakes to other freshwater lakes showed that Lake Union sediment is generally more toxic than other freshwater lakes, while Lakes Washington and Sammamish are similar to other lakes.

7.1 What are the contaminants of concern (COC)?

As mentioned above, in all three lakes, PCBs exceeded their SQGs most frequently (70% stations), followed by metals (50% stations), PAHs (23% stations), and phthalates (46% stations),. Individual chemical(s) within each chemical group that were frequently

detected and exceeded SQGs were Aroclor 1254, Zn, Cu, and Pb, Pyrene, and Bis(2-Ethylhexyl)Phthalate, respectively. Lake Union almost always had the highest contaminant concentrations and SQG exceedances. In particular, TBT, metals, PAH, and phthalate concentrations in Lake Union were markedly higher than in Lakes Washington and Sammamish. With the exception of stations located in close proximity to CSO's, stations in Lake Washington and Sammamish that had elevated sediment concentrations were generally those with fine-grained sediments and/or located at deep sampling sites. Contaminant concentrations in Lake Union were more affected by proximity to contaminant source (Gasworks Park, shipyards, etc), and typically less affected by grain size and depth.

7.2 Is there a measurable response to this contamination? If so, are contaminants causing the observed response?

This question was evaluated by assessing the results of the bioassay and benthic community data. Generally, this study showed weak to moderate correlation between the contaminant concentrations and the different biological responses. The discussion below details the bioassay endpoints most correlated with contaminant concentrations, as well as identifies specific areas in the lakes where contaminant concentration did not correlate with either observed toxicity or impaired benthic communities.

The three bioassays and the three benthic indices included in this assessment showed varying degrees of correlation with contaminant concentration. *C. tentans* growth was the most correlated with contaminant concentration, followed by *H. azteca* mortality and *C. tentans* mortality. The relative sensitivity of the growth endpoint may be due to the fact that it integrates numerous biochemical, physiological, and behavioral processes that are affected by contaminant exposure (Moore *et al.* 1993). The variation in the benthic indices was best explained by grain size and depth; however, the chemical PCs did explain some of the variation. Generally, the Species Richness was the most strongly correlated with chemical contamination, followed by Shannon-Weaver diversity index and HBI diversity index.

The potential additive and synergistic effects associated with complex mixtures make it difficult to identify particular contaminants responsible for observed toxicity and impaired benthos in a study such as this (Chapman *et al.* 1998). However, this assessment did identify relationships between particular contaminants and both the bioassay and benthic data. In the bootstrap analysis, which was used to validate relationships between chemicals and bioassay endpoints, the bioassay data were correlated with sediment concentrations of PCBs, phthalates, PAHs, metals and TBT. However, the correlation analysis only showed a significant relationship between the bioassay data and metal and PAH PCs. Conversely, neither the normalization of ΣSEM metal concentrations by AVS nor the OC normalization for excess ΣSEM improved the ability to predict toxicity (McGrath and DiToro 2002). For the benthic data, depth and percent fines explained much of the variability in the data, but the Butyl Tin PC was significant for the HBI and species richness indices, while the metals and PAH PCs were significant predictors of the Shannon-Weaver diversity index.

7.3 Which lakes or areas of the lakes are most impacted?

As discussed above, Lake Union sediments were more impacted than Lake Washington sediments and the Lake Washington exhibited a more negative response in all biologic responses (benthos and bioassays) than that observed for sediment from Lake Sammamish. Further, all of the 'C; and most of the 'B' stations were located in Lake Union. The near-shore areas in Lake Union had the highest contaminant concentrations. In particular, stations in the vicinity of the south and southwest shores, and along the western edge of the lake were the most impacted for all three LOE (stations 0568, 0569, and 0572, respectively). The most impacted sites in Lake Washington were located in close proximity to the Sayer Site*)* and Henderson CSO(stations 0864A and 4903B, respectively). The Henderson site is currently an active CSO (that will shortly be decommissioned). The Henderson CSO experienced one event (0.13 MG) in June of 2000 prior to sampling. During the 1999/2000 "CSO water year" (May- June) there were 2 events with a total discharge of 0.9 MG. The Sayer site is the location of the preparation area ("pit") used for the annual Seafair Hydroplane races, which likely partially explains the elevated concentrations of petroleum hydrocarbons at this site. In

addition, this is also the location of the Rainier CSO which is permitted to discharge at a frequency of less than once a year; however, there have been no CSO discharges at this location in the last few years prior to sampling. The same discharge also serves as an emergency bypass outfall for the Rainier Pump Station; however, there has never been an emergency discharge at this site. The City of Seattle also has at least one CSO/storm drain in the same general area. Based on the comparison to SQGs, both sites had only slightly elevated contaminant concentrations, however, the Sayer site exhibited statistically reduced *H. azteca* survival and the Henderson site exhibited statistically reduced *H. azteca* survival, *C. tentans* survival and *C. tentans* growth. However, at the Henderson site, the contamination appears limited to the immediate proximity of the outfall, since station 4903A which is less that 100 m from the Henderson Site (4903B) did not exhibit toxicity. It is interesting to note that since petroleum hydrocarbons do not have SQGs, petroleum hydrocarbons contamination would be included in the chemical component of the decision matrix analysis.

7.4 What are the potential contaminants sources?

Once metals (Zn, Pb, and Cu) and organics (PAHs, PCBs, TBT, and phthalates) were identified as contaminants of concern, the next step was to identify potential contamination sources.

7.4.1 Sources of Metal Contamination

The sediments of Lake Union had more metals at elevated concentrations, compared to Lakes Washington and Sammamish. Zn, Pb, and Cu were close to ubiquitous throughout the sediments of Lake Union. Since sediment Zn and Cu concentrations were highly correlated (r^2= 0.98) with each other, and to slightly lesser extent with Pb (r^2 =0.85), a common source of these metals in the watershed is implied. It is also significant that most metal concentrations correlated with grain size in uncontaminated sites, so grain size may be a mediating factor in the correlation between metals (Meador et al. 1994). Possible metal sources include human activities and weathering and erosion of parent materials. While information on background levels of sediment metal concentrations in the Puget Sound lowlands is sparse, one study showed concentrations of 31.3 and 84

PPM, respectively, in Puget Sound soil glacial deposits (Ames and Pyrch 1995). These two soil values represent approximately the 40th percentile of all of the sediment Cu and Zn data from the study presented here.

Anthropogenic activities, both historical and current, could have accounted for most of the metals observed at above-background concentrations. Potential watershed wide sources of Zn and Cu includes stormwater and CSOs (Metro, 1982, Kent, 1996). These trace metals are also linked to automobile usage and related road runoff (Andrews and Sutherland, 2004). Thus, local roadways and bridges may be responsible for some of the metals contamination found in these lakes. For example, recent research has shown elevated metals concentrations in runoff from local bridges and highways (WADOT 2004, Wilson, pers.comm.). In addition, Maltby et al. (1995) identified copper and zinc (as well as hydrocarbons) in runoff from a major roadway in the UK. Galvinized metal roofs have been identified as a source of Zn, which is released by rain and carried to surface waters by stormwater runoff (VanMetre and Mahler 2003). I would leave out the wastewater statement since there are no direct discharges of wastewater to the lakes). Within-lake sources of Cu and Zn, and TBT include anti-fouling agents in paint from Lake Union shipyards. Dibutyltin (DBT), a degradation byproduct of TBT was the chemical most strongly correlated with the *C. tentans* growth endpoint in this study. Di-n-Butyl tin is also used in the manufacturing of polyvinyl chloride (PVC). While TBT can cause acute and chronic toxicity to freshwater biota such as mussels, crustaceans, mollusks, and fish, DBT is believed to be more than 50 times less toxic than TBT (Day *et al.* 1998, Meador, NOAA, pers. comm).

Based on the results of the bootstrap analysis, organic contaminant concentrations were more closely correlated with the observed sediment toxicity than metal concentrations. One important aspect of organic contaminants is that they tend to occur in complex mixtures that can be toxic in an additive manner. Also, some compounds tend to co-occur due to common sources or pathways, such as PCBs and PAHs (Ingersoll *et al.* 1995). Thus, it is often quite difficult to identify the specific contaminant responsible for any observed toxicity or benthic community impairment.

Di-2-ethylhexyl-phthalate (DEHP) is a plasticizer with relatively low toxicity that has relatively ubiquitous distribution throughout the environment (Adams *et al.* 1995). PAHs are a class of stable organic compounds that originate from both point and non-point sources in these three lakes. As previously discussed, both Gasworks Park on north Lake Union and the Quendall/Baxter site on southeast Lake Washington are the most easily identifiable PAH point sources. Non-point PAH sources include incomplete combustion of organic materials, pyrolysis, and vaporization particular to industrial process, fuel combustion from stationary sources like power plants and residential heating, transportation sources such as gasoline and diesel-powered motors, combustion from forest fires and agricultural burning, and solid waste incineration (NRCC 1983). Vertebrate aquatic species and some invertebrates have an enzyme that enables them to metabolize PAHs. In particular, polychaetes worms, and to a lesser extent, crustaceans and mollusks are capable of metabolizing PAHs (NRCC 1983). While all PAHs are equipotent at a given tissue concentration, LPAHs accumulate faster in the critical body residue and thus appear to be more toxic. For chronic (higher) exposures, HPAHs can accumulate to higher tissue concentrations and would therefore be considered more toxic when expressed as a water or sediment concentrations (Meador, NOAA, pers. comm.). Further, LPAH are generally more bioavailable because they are more likely to be dissolved than HPAH, but HPAH have higher Kow, which can be further complicated if an organisms metabolizes PAHs (Lester, King County, pers. comm.). In this study, the majority of PAHs correlated with toxicity in the bootstrap analysis were LPAHs. However, it is important to note that many of the PAHs had high correlations with each other. To provide a temporal perspective, recent research by Wakeham et al. has shown a decrease in sediment Total PAH concentrations since the mid-1970's (Wakeham et al.2004).

7.5 Conclusion

Overall, the chemical contamination, toxicity, and benthic community observed in Lakes Washington and Sammamish are not dramatically compared to Lake Union. Lake Union, however, has elevated sediment contaminant concentrations, impaired benthic

communities, and statistically significant bioassays compared both to Lakes Washington and Sammamish. Toxicity was observed more frequently in Lake Union compared to other freshwater lakes. Additionally, there are also two sites in Lake Washington with low survival in multiple endpoints that possibly relate directly to the nearby outfalls, creak inputs, or storm drains. This analysis indicated that chemical concentrations co-varied with each other and, in turn, measures of field and laboratory adverse effects co-varied with sediment concentrations of PCBs, metals, PAHs, and phthalates. Further study will hopefully be able to establish stronger associations between particular contaminants and the biological response.

8.0 Uncertainty Analysis

8.1 Uncertainty for all Lines of Evidence

Evaluating and characterizing the potential risk posed by contaminated sediments to aquatic organisms poses technical challenges that underscore the importance of addressing uncertainty. Aquatic ecosystems in Lakes Sammamish, Washington, and Union are dynamic, involving complex interactions between biological, geochemical, physical and hydrologic factors. The benefit of having three partially redundant lines of evidence is that there is one indicator of the likelihood of biological stress (sediment chemistry) and two indicators of observed biological stress (toxicity and benthos), which overlap to reduce uncertainty. To evaluate uncertainty, Ingersoll *et al.* (1997) established criteria to support consistent assessments of the uncertainty associated with sediment characterizations. Table 20 details the criteria as they relate to measurements and analyses used in this study. The context of these criteria depends on the line of evidence being discussed.

Table 19. Uncertainty Associated with Individual Lines of Evidence (Ingersoll *et al.* 1997)

	Toxicity Tests			**Benthic Assessment**	**Sediment Guidelines**		**Sediment Chemistry**
Evaluation Criteria	Whole Sediment	Survival	Growth	Structure (indices)	SQG	SEM/ AVS	Bulk Sediment
Precision	1	1	1	2	1	2*	1
Ecological relevance	1	1	2*	1	2*	1	3
Causality: link	3	3	3	2*	1	1	1
Causality: source	1	1	2*	3*	1	1	2*
Sensitivity	1	1	2	2	2	2*	1*
Interference	2*	1*	2*	3*	2	2*	2*
Standardization	1	1	2	1	1	1	1*
Discrimination	1*	2	1	1	1	2	1
Bioavailability	1*	1	1	NA	1	1	2
Field validation	1*	1	2	1	2	2*	1

1 =low uncertainty (good); 2=moderate uncertainty; 3 =high (bad);*=lack of knowledge.

8.2 Sampling Design

For both Lakes Sammamish and Washington, many sampling locations were selected based on proximity to CSOs, storm drains, emergency bypass outfalls and creek/stream

inputs. Since the sampling design was not stratified random, it biased the data towards near shore areas and limited the ability to make inferences about each lake system. If future studies were developed based on a more random sampling design, it might be possible to make more robust statistical generalizations about the sediment quality in each lake.

The large number of replicates (5 for benthic data, 8 for bioassay data) used in this study enabled the characterization of both within and between station biological variability. The benthic communities naturally have high variability, and even the 5 replicates had such high variability, the between-station comparisons did not provide much information about sediment contamination. Conversely, the toxicity tests generally had high replicability and the 8 lab replicates were extremely useful in evaluating which stations were statistically different from the reference envelope and control.

8.3 Chemical Data

The primary sources of uncertainty for the bulk sediment chemistry are the laboratory methods used to measure concentrations and the analysis methods used to evaluate the chemical data (including sediment guidelines). The uncertainty associated with measuring sediment contaminant concentration is related to the precision and accuracy of the laboratory techniques. The precision of lab replicates was assessed by calculating the coefficient of variation (CV; standard deviation divided by the mean) for 13 metals and 19 PAHs for the 12 stations with lab replicates. The average metals and PAH CV were 8% and 19%, respectively, which is consistent with the high variability in organics concentrations, and the slightly lower variability in metals concentrations that is generally observed. Overall, this shows that the chemical analysis results were relatively reliable.

Analytical accuracy was assessed using standard reference materials (SRM), while the precision was evaluated using lab replicates. The SRM is a sediment sample with known chemical concentrations that have been measured and verified by multiple labs. The ability of the laboratory to recover contaminant concentrations within the QC limits of 80% to 120% of the actual amount certified to be in the sample is one test of a

laboratory's accuracy. In this study, the samples outside the QC range generally had recovery below the SRM, indicating that chemical concentrations reported in this study may have underestimated actual concentrations. However, SRM recovery was generally consistent between samples. In particular, the base neutral acid compounds (BNAs), and chlorinated pesticides had an overall CV of 30, while TBT had a CV of 23. Metals recovery was generally within the QC limits. Since these values were consistent with the accuracy of lab replicates (discussed above), no corrections were made on the individual samples.

Sediment quality guidelines are a controversial method for predicting adverse impacts from contaminated sediment (See Section 5.7). SQGs were used in this assessment to identify contaminants that exceeded guidelines, indicating potential causes of toxicity. However, since there is no group of SQGs that includes all chemicals, it is possible that chemicals responsible for any observed toxicity may not have been evaluated. The bootstrap analysis and PCA were another potential source of uncertainty in this analysis. In particular, reducing the chemical contaminant data from 30 parameters to four greatly streamlined the data analyses, but could have resulted in loss of information. However, in the present case, this loss was probably minimal, since the four PCs accounted for 92 of the variation in the chemical data set. One important limitation from the PCA is that Hg did not load onto any of the PCs and recent research (McIntyre 2004) has shown Hg bioaccumulation in Lake Washington fish (along with PCBs) to be considerable. However, Hg concentrations did not appear to be correlated with toxicity in the bootstrap analysis, perhaps due to the fact that the Hg-inducted toxicity is often caused by bioaccumulation, which was not measured in the acute toxicity tests used in this study. bootstrap analysis identified particular contaminants that were correlated with toxicity. While the chemistry to toxicity correlations alone had the potential for false positives, the bootstrap method allowed quantification of this affect. Therefore, this method is robust and has less uncertainty associated with it.

8.4 Bioassay Data

The three primary sources of uncertainty relating to biological data are; uncertainties related to the exposure media, the choice and ecological relevance of effects and exposure endpoints, uncertainties related to the cause of impairment, and uncertainties related to the statistical methods used to evaluate the data. The whole sediment tests used in this study are generally perceived to be precise (low intra- and interlaboratory variability), sensitive, ecologically relevant, well standardized across laboratories, and ecologically relevant when extrapolating to field conditions (Ingersoll *et al.* 1997). However, bioassay test conditions such as dissolved oxygen and temperature can affect the ability to extrapolate to field conditions. Additionally, different bioassay species are varied in their response to different contaminants. The choice of endpoint is also important, as mortality is generally an indication of serious impairment, with contaminant concentrations exceeding safe levels by approximately 2 orders of magnitude (Meador, NOAA, pers. comm.). Further, while the growth endpoint provides a more sensitive indication of impairment, it is not an accurate assessment tool for chemicals that cause altered behavior, immunosuppression, reproductive abnormalities, or other adverse effects. To more accurately assess the biological effects of contaminants, additional, more sensitive bioassays could be conducted.

Both the survival and growth endpoints are generally very reproducible and amphipod survival and *C. tentans* growth have previously been correlated with *in situ* benthic community structure (Ingersoll *et al.* 1997). One very important aspect of bioassays are that they rarely provide clear indications as to which contaminants were responsible for any observed toxicity. This may be because the contaminants which actually caused toxicity were not measured or that multiple contaminants acted in an additive manner (Ingersoll *et al.* 1997). In to this study, uncertainty could have been caused by the statistical methods used to evaluate toxicity, and the reference envelope method used to create numerical criteria for comparisons between stations.

8.5 Benthic Community Structure and Abundance Data

A key uncertainty related to the benthic invertebrate assessment is that, given the high natural variability in benthic communities, it is difficult to know what 'normal' conditions are in order to identify when benthic communities have deviated from this state. Also, there was not any historical data to compare the existing data to. Additional uncertainty comes from the benthic indices that were utilized and the methods used to evaluate these indices. The field and laboratory methods used in this study were conservative, and the five field replicates taken at each station and the QA/QC procedures utilized to identify taxa minimized field and laboratory related uncertainty. However, variations in ambient concentrations during the three years of sampling could lead to uncertainty in the results. In addition, additional uncertainty could have stemmed from the fact that some benthic organisms were not identified to the species level.

Three benthic indices were utilized in this study, and there is uncertainty associated with the correlation method used to select the metrics, the ecological relevance of the metrics, and the assumptions inherent in metrics. For example, the Shannon-Weaver index (Shannon 1948) is one of the more widely used benthic metrics. The primary assumption of this index, that high diversity represents a more pristine benthic community, has caused this and other diversity indices to be the subject of much debate. Using only three of the many available indices, and excluding the taxa abundance data could have caused valuable information to be excluded from this analysis. Also, while there were five stations over 30 m, the deepest reference station was 21 m, and the comparison between the two stations might not be that ecologically sound since the 10 m depth difference between these sites (and related changes in incident light, DO, grain size, and temperature) could result in naturally different benthic communities. The attempt in Section 6.5.3 at calculating total station biomass was not successful at identifying stations with statistically reduced biomass, perhaps due to uncertainties in the biomass of each species.

8.6 Uncertainty in Decision Matrix.

Correlating and aggregating multiple lines of evidence created additional uncertainty in this analysis. In particular, while the decision matrix enabled the spatial representation of

large amounts of information, it may have resulted in a loss of important information. However, the synthesis of multiple lines of evidence resulted in the identification of contaminants correlated with adverse biological effects, specific sites that were more impacted than others, and sites suitable for further study due to toxicity or impaired benthic communities not explained by elevated chemical concentrations. Hopefully, further study will validate these findings and reduce the uncertainty associated with them.

8.7 Uncertainty and Study Results

As discussed above, there are many sources of uncertainty that could affect the results and conclusions of this study. The sampling, analysis, and synthesis of each individual LOE, and the relationship between the LOEs have uncertainties associated with them. However, it is likely that the uncertainties discussed above will cause only cause minor inaccuracies in the outcome of the chemistry, bioassay, or benthic community structure analysis at each station due to the fact that all data types provide information about the degree of impairment. For example, if the degree of impairment in one LOE is under or overestimated by this analysis, it is probably that a different LOE at another station is more accurately described, thus balancing out the overall story from the station.

9.0 Recommendations

This study made important headway in characterizing sediment quality in Lakes Sammamish, Washington, and Union and raised important questions that should benefit further studies. In particular, the decision matrix aided in identifying specific sites that could benefit from further evaluation. Additionally, specific contaminants of concern and the most sensitive bioassays were identified. The decision matrix (Section 6.6.1) established three groups of stations; a relatively unimpaired group ('A'), a group with either impaired toxicity or benthos ('B') and a group with elevated contaminant concentrations and toxicity and/or benthos ('C'). While these groupings are subjective, they do form a convenient basis to suggest possible areas that would benefit from future monitoring. Stations in group 'A' would benefit from continued, low-level monitoring, perhaps on the order of every 5 to 10 years. Stations in group 'B' could be further investigated to determine the cause of the observed toxicity. Stations in group 'C' could be further assessed to determine which contaminants are responsible for the observed toxicity.

The bioassays evaluated in this study suggest several directions for further study. The Microtox® bioassay was not very useful in these analyses and its use could be discontinued. Since the *C. tentans* growth endpoint was the most responsive to contaminant concentrations, other bioassays with sub-lethal endpoints such as reproduction could also be evaluated, particularly for the more heavily contaminated areas. It is also important to better understand the chronic effects that contaminants are having on the entire aquatic ecosystem. Particular contaminant groups (PCBs, PAHs, metals, and phthalates) were identified as related to the observed toxicity, and warrant further study. In addition, recent research has shown that PCBs and Hg are present in high concentrations in Lake Washington fish (McIntyre 2004). This is particularly interesting given the relatively low sediment Hg concentrations. Also, it would be useful to evaluate individual PCB congers rather than Aroclor® concentrations. This would help to identify specific congers that make up the total PCB load and thus have a more precise understanding of which PCBs may be causing impaired biological communities.

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Appendix A: Annotated Bibliography

Table A-1. Annotated Bibliography

Study Originator	Lake	Citation	Peer Reviewed	Published	Results
Ecology	Wash	Bennett and Cubbage, 1992	N	Y	Evaluated toxicity of PAH-contaminated sediments adjacent to the Quendall and Baxter sites. Sediments showed a significant reduction in *Hyalella azteca* survival and decreases in luminescence for one Microtox® sample corresponding to elevated PAH concentrations.
	Wash	Crecelius and Piper, 1973	Y	Y	Elevated concentrations of lead, antimony, mercury, arsenic, and copper in Lake Washington sediment were possibly caused by the ASARCO copper smelter located near Tacoma, WA.
	Wash	Crecelius, 1975	Y	Y	Arsenic is present in 15-210 PPM in the sediment, with peaks beginning at a depth of 25 cm, probably coinciding to the initiation of smelting in 1890
EPA	Many Locs	Crecelius et al., 1989	N	Y	High levels of dieldrin in edible fish tissue. As a result, Lake Union is on the 1996 and 1998 303(d) list for dieldrin.
Ecology	Union	Cubbage, 1992	N	Y	Lake Union has reduced water quality due to commercial and industrial use as well as from stormwater runoff. Thus study found elevated concentrations of PAHs near GWP, PCBs near the Seattle City Light Steam Plant, and metals ubiquitous throughout the lake. *H. azteca* and *Daphnia magna* exhibited statistically significant responses to the sediment. This report led to Lake Union & Lake Washington ship canal being added to the Washington State 303(d) list for 1996 and 1998 for sediment bioassays.
cology	NA	Cubbage *et al.*, 1997	N	Y	Describes the development of Draft Washington State freshwater sediment guidelines

Table A-1 (continued)

Study Originator	Lake	Citation	Peer Reviewed	Published	Results
Ecology	Wash	Ecology, 2001	N	Y	Telephone and power poles have been treated with pentachlorophenol (PCP) from 1960s until the early 1980's at the J.H. Baxter site. PCP spills of 1,400, 200, and 2,000, gallons occurred at the Baxter-site in 1981, 1989, and 1990 respectively.
Ecology	Wash	Ecology, 2002	N	Y	Baxter/Quendall were responsible for contaminating the nearby soil, groundwater, surface water, and sediments with polycyclic aromatic hydrocarbons (PAHs) and the volatile organic compounds (VOCs) benzene, toluene, ethylbenzene, and xylene (BTEX). At the Quendall Site, a "Remedial Investigation/Risk Assessment/Feasibility Study" is ongoing,
UW	Wash	Edmondson and Allison, 1970	Y	Y	The sedimentation rate has been estimated at 3.1 mm/yr
UW	Wash	Edmondson, 1994	Y	Y	Sixty years of Lake Washington
Ecology	Union	Jack, 2003	N	Y	Wastes found at GWP include solvent-soaked wood chips, slag, lampblack carbon, coal by-products, and tar
KCDNR	Samm	KC, 1999-2001	N	Online	SAPs for Lakes Sammamish, Washington, and Union sampling. Contain background information on the 3 lakes.
	All	Leisch, 1963	Y	Y	Geology and Ground-Water Resources of Northwestern King County, Washington

Table A-1 (continued)

Study Originator	Lake	Citation	Peer Reviewed	Published	Results
	Samm	Metro and KC, 1995	N	Y	Lake Sammamish Total Phosphorus Model document. Geologic/Hydraulic history of lakes.
UW	Samm	Moon, 1973	N	N	Tibbetts Creek to the south and Pine Lake drainage to the east contribute about 6 and 3 respectively of the surface water
Ecology	Wash	Norton, 1991	N	N	Norton analyzed Total PAH concentrations in sediments at 18 sites near Quendall/Baxter. PAH sediment concentrations ranged from 0.8-7300 PPM (dw), reduced benthic macroinvertebrate diversity, and toxicity to *Hyalella azteca*
Ecology	Wash	Norton, 1992	N	N	At Baxter site, PAH concentrations between 43-33,000 PPM, as well as significant toxicity exhibited in Microtox and *H. azteca* bioassays
Ecology	Salmon Bay	Serder and Cubbage, 1996	N	Y	Bioassays of *H. azteca* survival, *C. tentans* growth & survival, and Microtox in Salmon Bay showed toxicity to least one organism in over 90 of the samples. Contaminant distribution in Salmon Bay characterized by nearshore hot-spots, with cleaner sediments found toward the main channel. Comparisons to FSQVs showed that organic contaminants are assumed are responsible for this toxicity.
USACOE	Wash	USACOE, 1992	N	N	Water Control Manual for the Lake Washington Ship Canal

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Appendix B: Chemical Analysis Methods and Detection Limits

Table B-1. Analysis Methods and Detection Limits

Analysis/Method	Method Summary	Lake Washington MDL	Lake Washington RDL	Lakes Sammamish and Union MDL	Lakes Sammamish and Union RDL
AVS EPA, 1991[1]/EPA 6010	Acidification with Purge and Trap	5 mg/Kg	5 mg/Kg	See ICP MDLs	See ICP RDLs
Ammonia SM 4500-NH_3	KCl Extraction with Nutrient Autoanalyzer	0.1 mg/Kg	0.2 mg/Kg	0.25 mg/Kg	0.5 mg/Kg
Particle Size Distribution ASTM D422	Sieve/Hydrometer	0.10	0.10	0.10	0.10
Percent Solids SM 2540-G	Gravimetric	0.01	0.01	0.01	0.01
Total Volatile Solids SM 2540-E	Gravimetric	0.01	0.01		
Phosphorous (Extractable) SM 4500-P	KCl or $CaCO_3$ Extraction[2] with Nutrient Autoanalyzer	0.20 mg/Kg	0.50 mg/Kg	0.50 mg/Kg	1.25mg/Kg
Phosphorous (Total) EPA 3050A/SM 4500-P	Acid Digestion with Nutrient Autoanalyzer	6.25 mg/Kg	12.5 mg/Kg	0.25 mg/Kg	0.63mg/Kg
Sulfide (Total) EPA 9030	Distillation with Ion Selective Electrode	10 mg/Kg	10 mg/Kg	5 mg/Kg	5 mg/Kg
Total Organic Carbon SM 5310-B	High Temp. Combustion with Infrared Spectroscopy	5 mg/Kg	10 mg/Kg	5 mg/Kg	10 mg/Kg
Antimony - EPA 3050A	ICP-OES (EPA 6010B)	1.5	7.5	1.5	7.5
	ICP-MS (EPA 6020)	0.1	0.5	0.1	0.5
Arsenic - EPA 3050A	ICP-OES (EPA 6010B)	2.5	12.5	2.5	12.5
	ICP-MS (EPA 6020)	0.1	0.5	0.1	0.5
Beryllium - EPA 3050A	ICP-OES (EPA 6010B)	0.05	0.25	0.05	0.25
	ICP-MS (EPA 6020)	0.04	0.2	0.04	0.2
Cadmium - EPA 3050A	ICP-OES (EPA 6010B)	0.15	0.75	0.15	0.75
	ICP-MS (EPA 6020)	0.02	0.1	0.02	0.1
Chromium - EPA 3050A	ICP-OES (EPA 6010B)	0.25	1.25	0.25	1.25
	ICP-MS (EPA 6020)	0.08	0.4	0.08	0.4
Copper - EPA 3050A	ICP-OES (EPA 6010B)	0.2	1	0.2	1
	ICP-MS (EPA 6020)	0.08	0.4	0.08	0.4

Table B-1 (continued)

Analysis/Method	Method Summary	Lake Washington MDL	Lake Washington RDL	Lakes Sammamish and Union MDL	Lakes Sammamish and Union RDL
Manganese - EPA 3050A	ICP-OES (EPA 6010B)	0.1	0.5	0.1	0.5
	ICP-MS (EPA 6020)	0.04	0.2	0.04	0.2
	ICP-OES (EPA 6010B)	1.5	7.5	1.5	7.5
Lead - EPA 3050A	ICP-MS (EPA 6020)	0.04	0.2	0.04	0.2
	Cold Vapor Atomic Absorption Spectroscopy				
Mercury - EPA 245.5	ICP-OES (EPA 6010B)	0.02	0.2	0.02	0.2
		1	5	1	5
Nickel – EPA 3050A	ICP-MS (EPA 6020)	0.06	0.3	0.06	0.3
	ICP-OES (EPA 6010B)	2.5	12.5	2.5	12.5
Selenium - EPA 3050A	ICP-MS (EPA 6020)	0.3	1.5	0.3	1.5
	ICP-OES (EPA 6010B)	0.2	1	0.2	1
Silver – EPA 3050A	ICP-MS (EPA 6020)	0.04	0.2	0.04	0.2
	ICP-OES (EPA 6010B)	10	50	10	50
Thallium - EPA 3050A	ICP-MS (EPA 6020)	0.04	0.2	0.04	0.2
	ICP-OES (EPA 6010B)	0.25	1.25	0.25	1.25
Zinc – EPA 3050A	ICP-MS (EPA 6020)	0.1	0.5	0.1	0.5
	ICP-OES (EPA 200.7)	0.075	0.375		
Cadmium	ICP-OES (EPA 200.7)	0.1	0.5		
Copper	ICP-OES (EPA 200.7)	0.75	3.75		
Lead	Cold Vapor Atomic Absorption Spectroscopy	0.005	0.05		
Mercury	ICP-OES (EPA 200.7)	0.5	2.5		
Nickel	ICP-OES (EPA 200.7)	0.125	0.625		
Zinc	Gas Chromatography with Mass Spectroscopy	11 to 640	16 to 1,280	11 to 640	16 to 1,280

Table B-1 (continued)

Analysis/Method	Method Summary	Lake Washington MDL	Lake Washington RDL	Lakes Sammamish and Union MDL	Lakes Sammamish and Union RDL
BNAs EPA 8270	Gas Chromatography with Mass Spectroscopy (SIM[2])	0.35 to 1.7	0.7 to 3.4	0.35 to 1.7	0.7 to 3.4
Butyltin Isomers KCEL SOP[1]	Gas Chromatography with Mass Spectroscopy (SIM[2])	0.7	1.4	0.7	1.4
Chlorobenzenes EPA 8270 (Modified by SIM)	Gas Chromatography with Electron Capture Detector	1.3 to 13	2.7 to 27	1.3 to 13	2.7 to 27
Chlorinated Pesticides/PCBs EPA 8081A/8082	Gas Chromatography with Mass Spectroscopy (SIM[2])	10	10	10	10
Chlorinated Herbicides EPA 8151	Gas Chromatography with Mass Spectroscopy (SIM[2])	20 to 53	33 to 100	20 to 53	33 to 100
Organophosphorous Pesticides EPA 8141A	Gas Chromatography with Flame Ionization Detector	20 to 200 mg/Kg	20 to 200 mg/Kg	20 to 200 mg/Kg	20 to 200 mg/Kg
Petroleum Hydrocarbons NWTPH-HCID (Identification)[3]	Gas Chromatography with Flame Ionization Detector	5 to 10 mg/Kg	5 to 10 mg/Kg	5 to 10 mg/Kg	5 to 10 mg/Kg
Petroleum Hydrocarbons NWTPH-G (Gasoline)[3]	Gas Chromatography with Flame Ionization Detector	25 to 50 mg/Kg	25 to 50 mg/Kg	25 to 50 mg/Kg	25 to 50 mg/Kg
Petroleum Hydrocarbons NWTPH-Dx (Diesel Extended)[3]					

Appendix C Chemistry Data Tables

Table C-1. All chemicals and Number of times measured/detected

Parameter	Parameter Group	Number of Detections	Number of Samples	Evaluated in this analysis?
Acid Volatile Sulfides	Conventional	65	84	Yes
2,4,5-T	Chlorinated Herbicide	1	84	No
2,4,5-TP (Silvex)	Chlorinated Herbicide	1	84	No
2,4-D	Chlorinated Herbicide	1	84	No
2,4-DB	Chlorinated Herbicide	1	84	No
Dalapon	Chlorinated Herbicide	1	84	No
Dicamba	Chlorinated Herbicide	1	84	No
Dichloroprop	Chlorinated Herbicide	1	84	No
Dinoseb	Chlorinated Herbicide	1	84	No
MCPA	Chlorinated Herbicide	1	84	No
MCPP	Chlorinated Herbicide	1	84	No
Extractable Phosphorus	Conventional	85	84	Yes
pH, Field	Conventional	47	47	Yes
Total Organic Carbon	Conventional	84	85	Yes
Total Solids	Conventional	86	85	Yes
Total Sulfide	Conventional	59	85	Yes
Diesel Range (>C12 - C24)	Gas/Fuel	14	71	No
Diesel Range (>C12-C24)	Gas/Fuel	14	14	No
Gasoline Range (C7 - C12)	Gas/Fuel	15	70	No
Lube Oil Range (>C24)	Gas/Fuel	82	50	No
Antimony	Metal	20	67	No
Arsenic	Metal	84	84	Yes
Beryllium	Metal	71	84	No
Cadmium, Extractable, SEM	Metal	78	84	Yes
Cadmium	Metal	81	84	Yes
Chromium	Metal	84	84	Yes
Copper, Extractable, SEM	Metal	84	84	Yes
Copper	Metal	84	84	Yes
Lead, Extractable, SEM	Metal	83	84	Yes
Lead	Metal	84	84	Yes
Manganese	Metal	84	84	Yes
Mercury, Extractable, SEM	Metal	8	84	Yes
Mercury	Metal	68	84	Yes
Nickel, Extractable, SEM	Metal	80	84	Yes
Nickel	Metal	84	84	Yes
Selenium	Metal	11	84	No
Silver	Metal	69	84	Yes
Thallium	Metal	19	84	No
Zinc, Extractable, SEM	Metal	84	84	Yes
Zinc	Metal	84	84	Yes
Ammonia Nitrogen	Nutrient	84	85	Yes

Table C-1 (continued)

Parameter	Parameter Group	Number of Detects	Number of Samples	Evaluated in this analysis?
Total Phosphorus	Nutrients	85	84	Yes
Parathion-Ethyl	Organochlorine Pesticide	1	84	No
Parathion-Methyl	Organochlorine Pesticide	1	84	No
Aldrin	Organochlorine Pesticide	1	84	No
Chlordane	Organochlorine Pesticide	25	84	Yes
Chlorpyrifos	Organochlorine Pesticide	1	84	No
Delta-BHC	Organochlorine Pesticide	1	84	No
Diazinon	Organochlorine Pesticide	1	84	No
Dieldrin	Organochlorine Pesticide	0	84	Yes
Endosulfan I	Organochlorine Pesticide	1	84	No
Endosulfan II	Organochlorine Pesticide	1	84	No
Endosulfan Sulfate	Organochlorine Pesticide	1	84	No
Endrin	Organochlorine Pesticide	0	84	Yes
Endrin Aldehyde	Organochlorine Pesticide	1	84	No
Gamma-BHC (Lindane)	Organochlorine Pesticide	0	84	Yes
Heptachlor	Organochlorine Pesticide	1	84	No
Heptachlor Epoxide	Organochlorine Pesticide	0	84	Yes
Hexachlorobenzene	Organochlorine Pesticide	8	85	No
Methoxychlor	Organochlorine Pesticide	1	84	No
Toxaphene	Organochlorine Pesticide	1	84	No
Disulfoton	Organophosphate Pest.	1	84	No
Malathion	Organophosphate Pest.	1	84	No
Phorate	Organophosphate Pest.	1	84	No
Aroclor 1016	PCB	1	84	No
Aroclor 1221	PCB	0	84	No
Aroclor 1232	PCB	0	84	No
Aroclor 1242	PCB	0	84	No
Aroclor 1248	PCB	15	84	No
Aroclor 1254	PCB	34	84	Yes
Aroclor 1260	PCB	19	84	No
4,4'-DDD	Pesticide	41	84	Yes
4,4'-DDE	Pesticide	36	84	Yes
4,4'-DDT	Pesticide	8	84	Yes
Alpha-BHC	Pesticide	1	84	No
Beta-BHC	Pesticide	1	84	No
2,4,5-Trichlorophenol	Phenol	1	84	No
2,4,6-Trichlorophenol	Phenol	1	84	No
2,4-Dichlorophenol	Phenol	1	84	No
2,4-Dimethylphenol	Phenol	1	84	No
2,4-Dinitrophenol	Phenol	1	62	No
2-Chlorophenol	Phenol	1	84	No
2-Methylphenol	Phenol	1	84	No

Table C-1 (continued)

Parameter	Parameter Group	Number of Detects	Number of Samples	Evaluated in this analysis?
2-Nitrophenol	Phenol	1	84	No
4,6-Dinitro-O-Cresol	Phenol	1	62	No
4-Chloro-3-Methylphenol	Phenol	1	62	No
4-Methylphenol	Phenol	3	84	No
4-Nitrophenol	Phenol	1	62	No
Phenol	Phenol	2	82	No
Benzyl Butyl Phthalate	Phthalate Ester	25	84	Yes
Di-N-Butyl Phthalate	Phthalate Ester	18	84	Yes
Di-N-Octyl Phthalate	Phthalate Ester	0	84	Yes
1,2,4-Trichlorobenzene	Semi-volatile	0	85	No
1,2-Dichlorobenzene	Semi-volatile	1	85	No
2,4-Dinitrotoluene	Semi-volatile	0	84	No
2,6-Dinitrotoluene	Semi-volatile	0	84	No
Bis(2-Chloroethyl)Ether	Semi-volatile	0	84	No
Bis(2-Chloroisopropyl)Ether	Semi-volatile	0	84	No
Bis(2-Ethylhexyl)Phthalate	Semi-volatile	74	84	Yes
1,2-Diphenylhydrazine	Semi-Volatile Organic	0	84	No
2-Chloronaphthalene	Semi-Volatile Organic	0	84	No
2-Nitroaniline	Semi-Volatile Organic	0	62	No
3,3'-Dichlorobenzidine	Semi-Volatile Organic	0	37	No
3-Nitroaniline	Semi-Volatile Organic	0	54	No
4-Bromophenyl Phenyl Ether	Semi-Volatile Organic	0	84	No
4-Chloroaniline	Semi-Volatile Organic	0	17	No
4-Chlorophenyl Phenyl Ether	Semi-Volatile Organic	0	84	No
4-Nitroaniline	Semi-Volatile Organic	0	54	No
Aniline	Semi-Volatile Organic	1	0	No
Benzidine	Semi-Volatile Organic	0	0	No
Benzoic Acid	Semi-Volatile Organic	31	84	No
Benzyl Alcohol	Semi-Volatile Organic	0	84	No
Bis(2-Chloroethoxy)Methane	Semi-Volatile Organic	0	84	No
Carbazole	Semi-Volatile Organic	26	82	No
Dibenzofuran	Semi-Volatile Organic	11	84	Yes
Diethyl Phthalate	Semi-Volatile Organic	0	84	No
Dimethyl Phthalate	Semi-Volatile Organic	8	84	Yes
Hexachlorobutadiene	Semi-Volatile Organic	0	84	No
Hexachlorocyclopentadiene	Semi-Volatile Organic	0	54	No
Hexachloroethane	Semi-Volatile Organic	0	84	No
Isophorone	Semi-Volatile Organic	0	84	No
Nitrobenzene	Semi-Volatile Organic	0	84	No
N-Nitrosodimethylamine	Semi-Volatile Organic	0	54	No
N-Nitrosodi-N-Propylamine	Semi-Volatile Organic	0	84	No
N-Nitrosodiphenylamine	Semi-Volatile Organic	0	84	No

Table C-1 (continued)

Parameter	Parameter Group	Number of Detects	Number of Samples	Evaluated in this analysis?
Pentachlorophenol	Semi-Volatile Organic	0	84	No
1,3-Dichlorobenzene	PAH	0	85	No
1,4-Dichlorobenzene	PAH	4	85	No
2-Methylnaphthalene	PAH	5	84	Yes
Acenaphthene	PAH	25	82	Yes
Acenaphthylene	PAH	9	84	Yes
Anthracene	PAH	32	82	Yes
Benzo(a)anthracene	PAH	62	83	Yes
Benzo(a)pyrene	PAH	53	84	Yes
Benzo(b)fluoranthene	PAH	50	82	Yes
Benzo(g,h,i)perylene	PAH	48	82	Yes
Benzo(k)fluoranthene	PAH	37	82	Yes
Chrysene	PAH	64	83	Yes
Dibenzo(a,h)anthracene	PAH	26	82	Yes
Fluoranthene	PAH	69	83	Yes
Fluorene	PAH	23	82	Yes
Indeno(1,2,3-Cd)Pyrene	PAH	47	82	Yes
Naphthalene	PAH	12	84	Yes
Phenanthrene	PAH	54	83	Yes
Pyrene	PAH	71	83	Yes
Di-n-Butyltin	TBT	57	84	Yes
Mono-n-Butyltin	TBT	81	84	Yes
Tetra-n-Butyltin	TBT	9	84	No
Tri-n-Butyltin	TBT	65	84	Yes

Table C-2. Chemicals included in Chemical Sums

Parameter	Sum Group	Molar Sum
2-Methylnaphthalene	Total PAHs	Yes
Acenaphthene	Total PAHs	Yes
Acenaphthylene	Total PAHs	Yes
Anthracene	Total PAHs	Yes
Benzo(a)anthracene	Total PAHs	Yes
Benzo(a)pyrene	Total PAHs	Yes
Benzo(b)fluoranthene	Total PAHs	Yes
Benzo(g,h,i)perylene	Total PAHs	Yes
Benzo(k)fluoranthene	Total PAHs	Yes
Chrysene	Total PAHs	Yes
Dibenzo(a,h)anthracene	Total PAHs	Yes
Fluoranthene	Total PAHs	Yes
Fluorene	Total PAHs	Yes
Indeno(1,2,3-Cd)Pyrene	Total PAHs	Yes
Naphthalene	Total PAHs	Yes
Phenanthrene	Total PAHs	Yes
Pyrene	Total PAHs	Yes
Aroclor 1016	Total PCBs	
Aroclor 1221	Total PCBs	
Aroclor 1232	Total PCBs	
Aroclor 1242	Total PCBs	
Aroclor 1248	Total PCBs	
Aroclor 1254	Total PCBs	
Aroclor 1260	Total PCBs	
Benzo(b)fluoranthene	Total Benzofluoranthenes	
Benzo(k)fluoranthene	Total Benzofluoranthenes	
4,4'-DDD	Total DDT	
4,4'-DDE	Total DDT	
4,4'-DDT	Total DDT	
Cadmium, Extractable, SEM	Sum SEM	Yes
Copper, Extractable, SEM	Sum SEM	Yes
Lead, Extractable, SEM	Sum SEM	Yes
Mercury, Extractable, SEM	Sum SEM	Yes
Nickel, Extractable, SEM	Sum SEM	Yes
Zinc, Extractable, SEM	Sum SEM	Yes

Table C-3. Floating Percentile and PEL/TEL Sediment Quality Guidelines

		Floating Percentile Guidelines			Freshwater Effects Levels	
Chemical Group	**Parameter**	**Statistical Effects Level**	**SQS Effects Level**	**CSL Effects Level**	**PEL**	**TEL**
Metals	Antimony	0.4	0.4	0.6		
Metals	Arsenic	20	20	51	17	5.9
Metals	Cadmium	0.6	0.6	1	3.53	0.596
Metals	Chromium	95	95	100	90	37.3
Metals	Copper	50	80	830	196.6	35.7
Metals	Lead	335	335	430	91.3	35
Metals	Mercury	0.5	0.5	0.75	0.486	0.174
Metals	Nickel	55	60	70	35.9	18
Metals	Silver	0.55	2	2.5		
Metals	Zinc	140	140	160	314.8	123.1
Organochlor. Pest	Chlordane				8.9	4.5
Organochlor. Pest	Dieldrin				6.67	2.85
Organochlor. Pest	Endrin				62.4	2.67
Organochlor. Pest	Gamma-BHC (Lindane)				1.38	0.94
Organochlor. Pest	Heptachlor Epoxide				2.74	0.6
PCB	Aroclor 1254	230	230	340		
PCB	Aroclor 1260	140	140	140		
PCB Sum	Total PCBs	60	60	120	277.2	34.1
Pesticides	4,4'-DDD				8.51	3.54
Pesticides	4,4'-DDE				6.75	1.42
Phthalate	Di-N-Octyl Phthalate	26	26	45		
Phthalate	Bis(2-Ethylhexyl)Phthalate	230	230	320		
Phthalate	Dimethyl Phthalate	46	46	440		
SemiVolatile Organ.	Dibenzofuran	400	400	440		
PAHs	2-Methylnaphthalene	470	470	555		
PAHs	Acenaphthene	1060	1060	1320		
PAHs	Acenaphthylene	470	470	640		
PAHs	Anthracene	600	1200	1580		
PAHs	Benzo(a)anthracene	4260	4260	5800	384.7	31.7
PAHs	Benzo(a)pyrene	3300	3300	4810	782	31.9
PAHs	Benzo(b)fluoranthene					
PAHs	Benzo(g,h,i)perylene	4020	4020	5200		
PAHs	Benzo(k)fluoranthene					
PAHs	Chrysene	5940	5940	6400	861.7	57.1
PAHs	Dibenzo(a,h)anthracene	300	800	840		
PAHs	Fluoranthene	5000	11000	15000	2354.9	111.3
PAHs	Fluorene	200	1000	3000		

Table C-3 (continued)

		Floating Percentile Guidelines			Floating Percentile Guidelines	
Chemical Group	**Parameter**	**Statistical Effects Level**	**SQS Effects Level**	**CSL Effects Level**	**PEL**	**TEL**
PAHs	Indeno(1,2,3-Cd)Pyrene	4120	4120	5300		
PAHs	Naphthalene	100	500	1310		
PAHs	Phenanthrene	6100	6100	7600	514.9	41.9
PAHs	Pyrene	3000	8800	16000	875	53
PAH Sum	Total PAHs (Molar Sum)	14	15	50		
Benzofluoranthenes	Total Benzofluoranthenes	450	11000	13800		
TBT	Tri-n-Butyltin	75	75	75		

Table C-4. Floating Percentile and TEL/PEL HQ Values for All Stations

		Station Average			Metals			PAHs			PCBs			Phthalates			TBT			Dibenzofuran			Semi-Volatile		
Lake	Locator	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL
Samm	0600REFNE	0.09	0.08	0.05	0.08	0.07	0.05	0.04	0.02	0.01	0.97	0.97	0.49	0.2	0.2	0.1	0.00	0.00	0.00	0.12	0.01	0	0.04	0.04	0.04
Samm	0600REFSE	0.22	0.19	0.12	0.3	0.26	0.17	0.04	0.03	0.02	2.26	2.26	1.13	0.32	0.32	0.17	0.01	0.01	0.01	0.28	0.01	0.01	0.1	0.1	0.09
Samm	602	0.35	0.28	0.19	0.35	0.31	0.22	0.11	0.06	0.04	1.52	1.52	0.76	1.67	1.67	0.87	0.02	0.02	0.02						
Samm	610	0.4	0.34	0.2	0.72	0.64	0.4	0.07	0.05	0.03	3.33	3.33	1.66	0.3	0.3	0.14	0.01	0.01	0.01						
Samm	0611A	0.54	0.47	0.28	0.78	0.69	0.42	0.11	0.07	0.05	3.7	3.7	1.85	1.51	1.51	0.58	0.04	0.04	0.04	0.64	0.03	0.02	0.18	0.18	0.17
Samm	612	0.47	0.4	0.23	0.8	0.7	0.42	0.08	0.06	0.04	3.94	3.94	1.97	0.67	0.67	0.27	0.03	0.03	0.03						
Samm	614	0.19	0.16	0.11	0.27	0.24	0.16	0.06	0.02	0.02	1.58	1.58	0.79	0.38	0.38	0.26	0.01	0.01	0.01						
Samm	615	0.21	0.18	0.11	0.27	0.23	0.15	0.06	0.03	0.02	1.63	1.63	0.82	0.58	0.58	0.35	0.04	0.04	0.04						
Samm	617	0.15	0.12	0.08	0.21	0.17	0.11	0.06	0.02	0.02	1.46	1.46	0.73	0.18	0.18	0.15	0.00	0.00	0.00						
Samm	618	0.52	0.27	0.19	0.24	0.21	0.14	0.33	0.19	0.14	1.38	1.38	0.69	1.08	1.08	0.58	0.00	0.00	0.00						
Samm	622	0.14	0.12	0.07	0.23	0.19	0.12	0.04	0.02	0.01	1.11	1.11	0.55	0.22	0.22	0.1	0.00	0.00	0.00						
Samm	623	0.25	0.21	0.14	0.38	0.33	0.23	0.07	0.03	0.02	1.34	1.34	0.67	0.81	0.81	0.45	0.00	0.00	0.00						
Samm	624	0.4	0.35	0.21	0.48	0.4	0.25	0.06	0.04	0.03	2.68	2.68	1.34	1.89	1.89	1.23	0.03	0.03	0.03						
Samm	625	0.35	0.3	0.17	0.31	0.27	0.14	0.09	0.07	0.05	4.61	4.61	2.3	0.52	0.52	0.23	0.01	0.01	0.01						
Samm	626	0.33	0.23	0.14	0.29	0.26	0.19	0.15	0.08	0.06	1.34	1.34	0.67	1.12	1.12	0.44	0.03	0.03	0.03						
Samm	M621	0.16	0.13	0.09	0.25	0.23	0.16	0.05	0.02	0.01	1.4	1.4	0.7	0.14	0.14	0.13	0.00	0.00	0.00	0.17	0.01	0.01	0.06	0.06	0.06
Union	513	1.49	1.07	0.7	1.12	0.94	0.6	1.41	0.82	0.58	4.76	4.76	2.38	1.01	1.01	0.42	0.63	0.63	0.63						
Union	539	0.74	0.65	0.41	1.38	1.2	0.77	0.12	0.06	0.04	4.38	4.38	2.19	0.72	0.72	0.3	0.79	0.79	0.79						
Union	563	0.19	0.15	0.1	0.36	0.31	0.21	0.07	0.03	0.02	0.65	0.65	0.32	0.28	0.28	0.14	0.09	0.09	0.09						
Union	564	1.16	1.03	0.72	1.34	1.18	0.73	0.25	0.15	0.11	3.95	3.95	1.97	6.57	6.57	2.42	6.29	6.29	6.29						
Union	565	2.02	1.81	1.31	1.77	1.48	0.89	0.36	0.22	0.16	4.72	4.72	2.36	7.59	7.59	4.32	18.40	18.40	18.40						
Union	566	4.69	4.32	3.31	4.05	3.4	2.03	0.74	0.4	0.29	14.69	14.69	7.34	12.5	12.5	7.81	55.07	55.07	55.07						
Union	567	2.09	1.83	1.29	2.63	2.2	1.31	0.6	0.32	0.23	5.42	5.42	2.71	3.29	3.29	1.28	18.13	18.13	18.13						
Union	568	7.44	6.8	4.84	4.01	3.02	1.88	0.63	0.39	0.29	26.77	26.77	13.38	85.2	85.2	30.74	42.00	42.00	42.00						
Union	569	11.8	9.5	5.95	13.46	8.52	4.37	1.61	1.15	1.26	63.36	63.36	31.68	53.3	53.3	19.31	32.44	32.44	32.44						
Union	570	3.73	3.39	2.6	3.51	2.91	1.75	0.67	0.43	0.31	11.91	11.91	5.95	6.16	6.16	3.39	45.47	45.47	45.47						
Union	572	9.38	5.94	4.03	3.28	2.62	1.52	12.03	7.56	5.33	10.28	10.28	5.14	2.67	2.67	1.09	11.89	11.89	11.89						
Union	573	0.16	0.13	0.08	0.34	0.3	0.2	0.05	0.02	0.01	0.36	0.36	0.18	0.17	0.17	0.08	0.06	0.06	0.06						

Table C-4 (Continued)

		Station Average			Metals			PAHs			PCBs			Phthalates			TBT			Dibenzofuran			Semi-Volatile		
Lake	Locator	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL
Union	574	0.43	0.36	0.24	0.74	0.62	0.39	0.14	0.06	0.04	1.08	1.08	0.54	0.61	0.61	0.36	1.41	1.41	1.41						
Union	575	3.95	3.59	2.58	3.04	2.29	1.39	0.63	0.31	0.21	14.55	14.55	7.27	16.2	16.2	11.1	23.73	23.73	23.73						
Union	580	0.52	0.43	0.24	0.99	0.78	0.34	0.13	0.07	0.05	0.64	0.64	0.32	0.72	0.72	0.29	2.28	2.28	2.28						
Wash	0425A	0.26	0.22	0.14	0.45	0.39	0.27	0.07	0.03	0.02	1.69	1.69	0.85	0.36	0.36	0.25	0.11	0.11	0.11	0.22	0.01	0.01	0.08	0.08	0.07
Wash	544	0.15	0.13	0.08	0.22	0.2	0.13	0.05	0.02	0.01	0.79	0.79	0.39	0.15	0.15	0.09	0.04	0.04	0.04	0.17	0.01	0.01	0.08	0.08	0.07
Wash	560	0.29	0.24	0.16	0.55	0.47	0.32	0.07	0.03	0.02	1.69	1.69	0.85	0.37	0.37	0.25	0.10	0.10	0.10	0.27	0.01	0.01	0.08	0.08	0.07
Wash	562	0.69	0.62	0.41	0.75	0.66	0.55	0.13	0.1	0.07	1.54	1.54	0.77	6.17	6.17	2.32	0.85	0.85	0.85						
Wash	0801A	0.34	0.3	0.19	0.39	0.35	0.24	0.06	0.04	0.03	2.22	2.22	1.11	1.43	1.43	0.92	0.13	0.13	0.13	0.49	0.02	0.02	0.1	0.1	0.09
Wash	804	0.56	0.47	0.31	0.65	0.58	0.39	0.08	0.06	0.04	2.51	2.51	1.25	2.78	2.78	1.88	0.36	0.36	0.36	1.39	0.06	0.05	0.11	0.11	0.1
Wash	0807A	0.27	0.22	0.15	0.49	0.41	0.28	0.04	0.03	0.02	1.87	1.87	0.93	0.28	0.28	0.22	0.12	0.12	0.12	0.24	0.01	0.01	0.08	0.08	0.08
Wash	0814B	0.43	0.37	0.24	0.79	0.68	0.45	0.07	0.05	0.04	3.27	3.27	1.63	0.43	0.43	0.18	0.33	0.33	0.33	0.42	0.02	0.01	0.15	0.15	0.14
Wash	0817A	0.95	0.82	0.53	1.13	0.97	0.66	0.09	0.07	0.05	7.23	7.23	3.61	3.31	3.31	2.27	0.43	0.43	0.43	1.62	0.07	0.05	0.1	0.1	0.09
Wash	826	0.74	0.59	0.36	1.64	1.31	0.79	0.09	0.07	0.05	3.79	3.79	1.9	0.75	0.75	0.3	0.18	0.18	0.18	0.69	0.03	0.02	0.18	0.18	0.16
Wash	0829A	0.23	0.19	0.13	0.36	0.31	0.2	0.07	0.03	0.02	1.81	1.81	0.9	0.41	0.41	0.28	0.00	0.00	0.00	0.22	0.01	0.01	0.08	0.08	0.07
Wash	831	0.31	0.26	0.16	0.49	0.41	0.27	0.05	0.04	0.03	2.28	2.28	1.14	0.56	0.56	0.29	0.25	0.25	0.25	0.29	0.01	0.01	0.1	0.1	0.09
Wash	832	0.35	0.28	0.18	0.4	0.34	0.22	0.13	0.06	0.05	1.69	1.69	0.85	1.41	1.41	0.75	0.00	0.00	0.00	1.07	0.04	0.03	0.08	0.08	0.07
Wash	834	0.88	0.7	0.44	1.25	0.98	0.64	0.13	0.09	0.07	6.17	6.17	3.08	2.74	2.74	1.02	1.36	1.36	1.36	1.99	0.08	0.06	0.19	0.19	0.17
Wash	840	0.7	0.52	0.32	1.31	0.93	0.58	0.09	0.07	0.05	4.67	4.67	2.33	0.66	0.66	0.32	0.50	0.50	0.50	1.33	0.05	0.04	0.14	0.14	0.13
Wash	852	1.01	0.77	0.46	1.55	1.25	0.77	0.33	0.2	0.14	6.78	6.78	3.39	0.98	0.98	0.39	0.47	0.47	0.47	2.3	0.09	0.08	0.19	0.19	0.17
Wash	861	0.86	0.7	0.41	1.78	1.43	0.88	0.1	0.08	0.05	5.78	5.78	2.89	0.64	0.64	0.26	0.13	0.13	0.13	0.82	0.03	0.03	0.18	0.18	0.16
Wash	862	0.13	0.11	0.07	0.16	0.15	0.1	0.04	0.02	0.01	1.11	1.11	0.55	0.24	0.24	0.12	0.01	0.01	0.01	0.14	0.01	0	0.05	0.05	0.04
Wash	0864A	2.58	2.35	1.59	1.13	0.99	0.65	0.19	0.12	0.09	10.99	10.99	5.5	24.7	24.7	11.93	3.48	3.48	3.48	3.14	0.13	0.1	0.09	0.09	0.08
Wash	0864B	0.3	0.26	0.17	0.47	0.41	0.27	0.08	0.03	0.02	1.75	1.75	0.88	0.6	0.6	0.37	0.65	0.65	0.65	0.22	0.01	0.01	0.08	0.08	0.07
Wash	890	0.84	0.69	0.41	1.72	1.38	0.85	0.09	0.07	0.05	5.58	5.58	2.79	1.02	1.02	0.4	0.30	0.30	0.30	0.57	0.02	0.02	0.18	0.18	0.16
Wash	4901A	0.28	0.24	0.16	0.47	0.39	0.27	0.05	0.04	0.02	1.87	1.87	0.93	0.52	0.52	0.34	0.14	0.14	0.14	0.33	0.01	0.01	0.08	0.08	0.08
Wash	4903A	2.37	1.66	1.11	0.88	0.78	0.51	0.9	0.5	0.36	7.53	7.53	3.77	14.4	14.4	6.96	0.80	0.80	0.80	16.71	0.68	0.54	0.49	0.49	0.45

Table C-4 (Continued)

		Station Average			Metals			PAHs			PCBs			Phthalates			TBT			Dibenzofuran			Semi-Volatile		
Lake	Locator	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL	Stat	SQS	CSL
Wash	4903B	2.67	2.18	1.53	0.85	0.74	0.49	0.6	0.33	0.24	6.19	6.19	3.09	24.8	24.8	17.68	2.17	2.17	2.17	9.51	0.39	0.31	0.35	0.35	0.32
Wash	A422A	0.46	0.37	0.24	0.94	0.74	0.48	0.07	0.05	0.04	2.92	2.92	1.46	0.48	0.48	0.21	0.18	0.18	0.18	0.4	0.02	0.01	0.13	0.13	0.11
Wash	S0025	0.23	0.19	0.12	0.37	0.32	0.22	0.07	0.03	0.02	1.75	1.75	0.88	0.26	0.26	0.2	0.06	0.06	0.06	0.22	0.01	0.01	0.08	0.08	0.07
Wash	SD007A	0.24	0.2	0.13	0.39	0.33	0.23	0.06	0.03	0.02	1.54	1.54	0.77	0.43	0.43	0.28	0.09	0.09	0.09	0.2	0.01	0.01	0.07	0.07	0.06
Wash	SD007B	0.36	0.3	0.2	0.4	0.35	0.24	0.08	0.04	0.03	2.36	2.36	1.18	1.44	1.44	0.77	0.12	0.12	0.12	0.71	0.03	0.02	0.07	0.07	0.06
Wash	SD017A	0.42	0.36	0.24	0.63	0.54	0.37	0.05	0.04	0.03	3.21	3.21	1.61	0.87	0.87	0.5	0.24	0.24	0.24	0.42	0.02	0.01	0.09	0.09	0.08

Appendix D: Bioassay Tables and Figures

Table D-1. Results of Bioassay Statistical Tests. Biological hits are indicated with a 'Yes'

Lake	Locator	Description	Sample ID	*C. tentans* Growth	*C. tentans* Mortality	*H. azteca* Mortality	Microtox Lum.
Samm	0600REFNE	Lake Sammamish: North (Coarse)	L16428-1				Yes
Samm	0600REFSE	Lake Sammamish: South (Fine)	L16245-1				Yes
Samm	0600REFSE	Lake Sammamish: South (Fine)	L16428-27				Yes
Samm	0602	Idylwood	L16428-5				Yes
Samm	0610	Timberline	L16428-6				Yes
Samm	0611A	Lake Sann, fine ref	L16245-4		Yes		Yes
Samm	0611A	Lake Samm, fine ref	L18493-11			Yes	
Samm	0611A	Lake Samm reference	L21645-11				Yes
Samm	0612	Middle Deep	L16245-2				Yes
Samm	0614	Issaquah River	L16245-6				Yes
Samm	0615	Tibbets Creek	L16245-7				Yes
Samm	0617	Lewis Creek	L16428-4				
Samm	0618	Sunset Pump	L16428-10				
Samm	0622	Eden Creek	L16428-3				
Samm	0623	Rosemont	L16428-2				
Samm	0624	Issaquah Interceptor EBO	L16428-9			Yes	
Samm	0625	Mouth of Slough	L16245-8	Yes			Yes
Samm	0626	Lake Hills SD	L16428-8				Yes
Samm	M621	'New' Pine Lake Ctr	L16428-7			Yes	
Union	0513	South End Ship Canal	L21689-7				
Union	0539	Portage Bay	L21645-2				
Union	0563	Portage Bay, Brooklyn	L21645-3				
Union	0564	L. Union East Arm, Eastern Ave	L21645-4	Yes			
Union	0565	L. Union East Arm, Hamlin St	L21645-5	Yes			
Union	0566	Lake Union East, Lynn St	L21645-6				
Union	0567	Lake Union SE Nelson St	L21645-7				Yes
Union	0568	South Lake Union, Minor	L21689-1	Yes			

Table C-3 (continued)

Lake	Locator	Description	Sample ID	*C. tentans* Growth	*C. tentans* Mortality	*H. azteca* Mortality	Microtox Lum.
Union	0569	Dexter Ave	L21689-2	Yes	Yes	Yes	
Union	0570	Lake Union Center	L21645-8				
Union	0572	W. L. Union Arm, Stone Way	L21689-6	Yes	Yes		
Union	0574	Fishermen's Terminal	L21689-9				
Union	0575	McGraw St	L21689-5				
Union	0580	West Salmon Bay	L21689-10				
Wash	0425A	Denny Creek	L18656-2				
Wash	0544	Webster Point (ref)	L18812-1				
Wash	0544	Webster Point (ref)	L21689-11		Yes		
Wash	0560	Wolf Bay	L18812-2				
Wash	0562	Union Bay, 41st St.	L21645-1	Yes			
Wash	0801A	N.End Lake WA	L18493-1				
Wash	0804	N.End Deep off Samm. Slough	L18493-2				
Wash	0807A	Juanita Bay	L18656-5				
Wash	0814B	Outer Cozy Cove	L18656-10				
Wash	0817A	Thornton Creek	L18656-3				
Wash	0826	N.End off Sand Pt.	L18493-3				
Wash	0829A	New Cedar R. Site	L18812-6				
Wash	0831	S. Lake WA Deep	L18493-9				
Wash	0832	Coal Creek	L18812-5				
Wash	0834	Meydenbauer Bay	L18812-3				Yes
Wash	0840	Lk WA Deep N. of M.I. Spill Site	L18493-10				
Wash	0852	Madison Park	L18493-4				
Wash	0861	Medina City Hall	L18493-5				
Wash	0862	Sand Point –Rep	L18656-6				
Wash	0862	Sand Point –Rep	L18656-7				
Wash	0864A	Sayer CSO	L18862-2			Yes	
Wash	0890	Seward Park	L18493-7				
Wash	4901A	Pontiac Bay	L18656-4				
Wash	4903A	Henderson CSO	L18862-5				
Wash	4903B	Henderson CSO mouth	L18862-8	Yes		Yes	
Wash	A422A	Kirkland	L18656-9		Yes		
Wash	S0025	Lake City	L18656-1		Yes		
Wash	SD007A	Pine St. CSO	L18862-1				
Wash	SD017A	N. Mercer Is. SD 17	L18812-4		Yes		
Wash	4903B	Henderson CSO mouth	L18862-8	Yes		Yes	

Appendix E: Benthic Data Analysis Tables

Table E-1. Shannon-Weaver Benthic Model

Shannon-Weaver = 2.3-.25*ln(Depth)-0. 36*ln(PC2+1) -0. 315*ln(PC1+1)

	Sum of Squares	df	Mean Square	F	Sig.
Regression	3.32	3	1.108	5.66	0.002
Residual	10.56	54	0.196		
Total	13.88	57			

Predictor Variables: Depth, ln(PC2)

Table E-2. Species Richness Benthic Model

Species Richness= 3.8-0.36*ln(Depth)-0. 21*ln(PC4+1) -
.0056*PercentFines

	Sum of Squares	df	Mean Square	F	Sig.
Regression	8.67	3	2.89	17.01	.000
Residual	9.17	54	0.17		
Total	17.83	57			

Predictor Variables: ln(Depth), Percent Fines, ln(PC4)

Table E-3. HBI Benthic Model

HBI = 2.0-0.035*ln(Depth)-0. 0475*ln(PC4+1)

	Sum of Squares	df	Mean Square	F	Sig.
Regression	0.11	2	0.05	4.27	0.01881
Residual	0.70	55	0.01		
Total	0.81	57			

Predictor Variables: ln(Depth), ln(PC4)

Table E-4. Chironomid Richness Benthic Model

Chironomid Richness = 3.030-.416*Depth-.403*PC4-.009*PercentFines

	Sum of Squares	df	Mean Square	F	Sig.
Regression	16.36	3	5.45	9.24	4.9E-05
Residual	31.85	54	0.59		
Total	48.21	57			

Predictor Variables: ln(Depth), ln(PC4), Percent Fines

Table E-5. Benthic Analysis Statistical Results

Lake	Locator	Reference	Depth (m)	Group	Species Richness	HBI	Shannon-Weaver	Benthic Biomass
SAMM	0602		8	Fine-Shallow				
SAMM	0610		18	Fine-Shallow	Yes (0.000)		Yes (0.000)	
SAMM	0611	Yes	21	Fine-Deep	NA	NA	NA	NA
SAMM	0612		28	Fine-Deep	Yes (0.018)			
SAMM	0614		10	Fine-Shallow		Yes (0.017)	Yes (0.021)	
SAMM	0615		5	Fine-Shallow				
SAMM	0617		8	Coarse-Shallow		Yes (0.000)		
SAMM	0618		8	Coarse-Shallow				
SAMM	0622		11	Coarse-Shallow				
SAMM	0623		18	Fine-Shallow	Yes (0.000)	Yes (0.001)	Yes (0.000)	
SAMM	0624		4	Fine-Shallow				
SAMM	0625		2	Fine-Shallow				
SAMM	0626		8	Coarse-Shallow				
SAMM	0600REFNE	Yes	4	Coarse-Shallow	NA	NA	NA	NA
SAMM	0600REFSE	Yes	4	Fine-Shallow	NA	NA	NA	NA
SAMM	M621		10	Fine-Shallow				
UNION	0513		12	Fine-Shallow	Yes (0.000)	Yes (0.000)	Yes (0.000)	
UNION	0539		4	Fine-Shallow				
UNION	0563		8	Fine-Shallow	Yes (0.000)		Yes (0.001)	
UNION	0564		10	Coarse-Shallow				
UNION	0565		14	Coarse-Shallow	Yes (0.008)	Yes (0.000)	Yes (0.028)	
UNION	0566		15	Fine-Shallow	Yes (0.000)	Yes (0.000)	Yes (0.000)	
UNION	0567		8	Fine-Shallow	Yes (0.005)		Yes (0.032)	
UNION	0568		12	Fine-Shallow	Yes (0.000)	Yes (0.000)	Yes (0.000)	
UNION	0569		15	Fine-Shallow	Yes (0.000)	Yes (0.001)	Yes (0.000)	

Table E-5 (Continued)

Lake	Locator	Reference	Depth (m)	Group	Species Richness	HBI	Shannon-Weaver	Benthic Biomass
UNION	0570		13	Fine-Shallow	Yes (0.000)	Yes (0.002)	Yes (0.000)	
UNION	0572		13	Fine-Shallow	Yes (0.000)		Yes (0.000)	
UNION	0573		7	Fine-Shallow				
UNION	0574		6	Fine-Shallow	Yes (0.001)	Yes (0.001)	Yes (0.000)	
UNION	0575		15	Fine-Shallow	Yes (0.000)	Yes (0.000)	Yes (0.000)	
UNION	0580		8	Coarse-Shallow		Yes (0.000)	Yes (0.002)	
WASH	0544	Yes	12	Coarse-Shallow	NA	NA	NA	NA
WASH	0560		12	Fine-Shallow				
WASH	0562		2.5	Coarse-Shallow				
WASH	0804		9	Fine-Shallow				
WASH	0826		49	Fine-Deep				Yes (0.012)
WASH	0831		28	Fine-Deep				Marginal (0.059)
WASH	0832		7	Fine-Shallow				
WASH	0834		9	Fine-Shallow				
WASH	0840		27	Fine-Deep				
WASH	0852		65	Fine-Deep				Yes (0.000)
WASH	0861		58	Fine-Deep				Yes (0.000)
WASH	0862		10	Coarse-Shallow				
WASH	0890		53	Fine-Deep				Yes (0.035)
WASH	0425A		35	Fine-Deep				Yes (0.000)
WASH	0801A		2.5	Coarse-Shallow				
WASH	0807A		10	Fine-Shallow				
WASH	0814B		8	Fine-Shallow			Yes (0.000)	
WASH	0817A		12	Fine-Shallow				
WASH	0829A		20	Fine-Shallow	Yes (0.045)			
WASH	0864A		9	Fine-Shallow		Yes (0.003)	Yes (0.000)	
WASH	4901A		10	Fine-Shallow				
WASH	4903A		2	Coarse-Shallow			Yes (0.001)	

Table E-5 (Continued)

Lake	Locator	Reference	Depth (m)	Group	Species Richness	HBI	Shannon-Weaver	Benthic Biomass
WASH	4903B		1	Coarse-Shallow		Yes (0.000)		
WASH	A422A		13	Fine-Shallow	Yes (0.018)			
WASH	S0025		13	Coarse-Shallow				
WASH	SD007A		12	Coarse-Shallow				
WASH	SD017A		11	Fine-Shallow				

Appendix F: Summarizing Three Lines of Evidence

Table F-1 Chemicals PCs, Benthic Metrics, and Bioassay tests results. If a station was sampled multiple times, the value below is an average.

Locator	PC1	PC2	PC3	PC4	HBI	Shannon-Weaver H' (log e)	Species Rich.	*C. tentans* Growth	*C. tentans* Survival	*H. azteca* Survival
0425A	-0.21	-0.24	-0.13	-0.36	7.17	2.00	14.20	2.81	0.75	0.96
0513	0.53	-0.35	0.82	-0.53	9.93	0.55	7.20	2.27	0.81	0.94
0539	-0.42	-0.62	2.38	-0.72	7.17	2.58	21.80	2.80	0.59	0.96
0544	0.34	-0.29	-0.75	-0.22	7.74	2.62	25.20	2.53	0.75	0.92
0560	-0.29	-0.45	0.67	-0.52	7.14	2.12	22.60	3.08	0.66	0.98
0562	-0.20	-0.28	-0.06	-0.04	6.40	2.36	21.80	2.33	0.84	0.85
0563	-0.21	-0.38	0.01	-0.31	7.65	1.74	8.20	2.23	0.76	0.95
0564	-0.15	-0.21	0.24	0.44	7.86	1.92	13.40	2.56	0.76	0.93
0565	-0.08	-0.15	-0.05	1.80	8.91	1.53	6.40	2.67	0.73	0.88
0566	-0.22	-0.43	2.56	3.59	9.51	1.40	6.20	2.59	0.55	0.86
0567	0.08	0.12	-0.02	1.40	7.33	1.93	14.60	2.23	0.59	0.85
0568	-0.09	1.51	0.46	2.32	9.78	1.24	7.20	1.56	0.88	0.78
0569	0.23	5.60	-1.27	0.44	9.37	1.02	5.20	0.48	0.58	0.54
0570	0.03	-0.25	0.32	4.59	9.24	1.41	5.40	2.58	0.63	0.91
0572	8.64	-0.70	0.94	-0.29	8.74	1.57	7.80	1.11	0.56	0.89
0573	-0.20	-0.31	-0.22	-0.33	7.63	2.41	18.60	3.65	0.59	0.91
0574	-0.20	-0.23	0.10	-0.19	9.31	1.65	13.80	3.01	0.69	0.74
0575	-0.26	-0.01	1.48	2.48	9.48	1.47	6.20	2.05	0.85	0.94
0580	-0.10	-0.12	-0.75	0.02	9.58	1.33	11.40	3.09	0.75	0.85
0600REFNE	-0.10	-0.24	-0.98	-0.08	7.60	1.71	10.20	3.09	0.70	1.00
0600REFSE	-0.16	-0.25	-0.47	-0.26	7.94	2.45	29.00	2.65	0.83	0.89
0602	-0.14	-0.21	-0.33	-0.32	8.33	2.27	27.60	2.72	0.89	0.89
0610	-0.24	-0.21	0.10	-0.38	8.38	1.04	8.60	2.28	0.89	0.96
0611A	-0.23	-0.21	0.05	-0.29	9.79	0.98	7.20	2.54	0.71	0.95
0612	-0.24	-0.26	0.14	-0.27	9.95	1.03	5.20	3.14	0.63	0.83
0614	-0.16	-0.23	-0.52	-0.27	9.01	1.90	24.60	2.99	0.83	0.84
0615	-0.13	-0.21	-0.72	-0.19	7.02	1.96	20.20	3.13	0.71	0.84
0617	-0.12	-0.20	-0.87	-0.16	9.32	1.84	22.00	2.71	0.70	0.95
0618	0.20	-0.22	-0.85	-0.16	7.45	1.71	22.00	2.95	0.80	0.95
0622	-0.13	-0.25	-0.72	-0.19	6.92	2.08	24.80	3.00	0.63	0.99
0623	-0.19	-0.24	-0.18	-0.38	9.38	1.18	7.20	3.24	0.89	0.91

Table F-1 (Continued)

Locator	PC1	PC2	PC3	PC4	HBI	Shannon-Weaver H' (log e)	Species Rich.	*C. tentans* Growth	*C. tentans* Survival	*H. azteca* Survival
0624	-0.18	-0.21	-0.42	-0.21	8.02	2.54	35.40	2.10	0.94	0.86
0625	-0.15	-0.34	-0.50	-0.09	6.86	1.99	22.20	2.16	0.75	0.94
0626	-0.10	-0.24	-0.44	-0.25	7.85	2.17	18.40	2.86	0.70	0.99
0801A	-0.15	-0.19	-0.50	-0.23	7.63	2.46	41.40	2.74	0.71	0.95
0804	-0.19	-0.08	-0.25	-0.25	7.38	2.48	22.40	2.73	0.79	0.99
0807A	-0.19	-0.17	-0.32	-0.31	6.53	1.98	18.00	3.01	0.73	1.00
0814B	-0.22	-0.13	0.00	-0.33	7.53	1.69	18.00	2.57	0.80	1.00
0817A	-0.47	-0.56	2.68	-0.99	7.59	2.19	17.40	2.82	0.76	1.00
0826	-0.47	-0.68	2.63	-0.86	7.98	1.60	10.00	2.67	0.76	0.99
0829A	-0.17	-0.24	-0.42	-0.28	7.31	2.31	16.80	2.59	0.84	0.99
0831	-0.18	-0.21	-0.35	-0.25	7.89	1.97	14.60	2.81	0.85	0.96
0832	-0.11	-0.21	-0.54	-0.21	7.82	2.27	29.00	2.21	0.50	0.96
0834	-0.26	-0.10	0.54	-0.26	7.67	2.47	18.20	2.52	0.74	0.93
0840	-0.27	-0.11	0.44	-0.42	7.70	1.66	12.80	3.14	0.76	0.99
0852	-0.26	-0.51	2.15	-0.72	7.96	1.78	10.60	2.71	0.75	0.95
0861	-0.42	-0.41	2.11	-0.77	7.65	1.26	6.60	2.91	0.76	0.94
0862	-0.11	-0.21	-0.93	-0.14	6.98	2.53	23.00	2.65	0.84	0.95
0864A	-0.16	0.47	-0.02	0.03	9.20	1.61	24.40	1.42	0.69	0.71
0890	-0.35	-0.14	0.97	-0.44	7.91	1.59	8.20	2.91	0.75	0.99
4901A	-0.19	-0.22	-0.28	-0.29	7.31	2.39	18.40	2.83	0.73	0.99
4903A	0.69	0.26	-0.94	0.09	7.70	1.31	30.00	2.09	0.56	0.85
4903B	0.23	0.41	-0.88	0.29	8.94	1.67	23.20	0.66	0.35	0.59
A422A	-0.24	-0.14	0.18	-0.42	6.74	2.05	15.60	3.68	0.54	1.00
M621	-0.18	-0.27	-0.32	-0.33	6.93	2.42	32.67	2.44	1.00	0.88
S0025	-0.18	-0.15	-0.59	-0.24	7.51	2.42	18.40	2.90	0.64	0.96
SD007A	-0.16	-0.15	-0.73	-0.20	7.29	2.45	28.80	2.83	0.80	0.95
SD017A	-0.22	-0.11	-0.22	-0.30	6.72	2.19	18.80	3.09	0.68	0.98

Table F- 2. Decision Matrix Results.

Station	Lake	Grade	Benthic	Toxicity	Chemistry	Sum
0600REFNE	SAMM	A	0.00	0.00	0.08	0.08
0600REFSE	SAMM	A	0.00	2.00	0.19	2.19
0602	SAMM	A	0.00	1.29	0.32	1.61
0610	SAMM	B	8.55	0.00	0.34	8.89
0611A	SAMM	A	0.00	3.29	0.47	3.76
0612	SAMM	A	6.42	2.69	0.39	9.50
0614	SAMM	A	3.58	0.00	0.18	3.76
0615	SAMM	A	0.00	2.00	0.19	2.19
0617	SAMM	A	1.45	0.00	0.13	1.58
0618	SAMM	A	0.00	0.00	0.31	0.31
0622	SAMM	A	0.00	0.00	0.11	0.11
0623	SAMM	B	10.00	0.00	0.24	10.24
0624	SAMM	A	0.00	1.29	0.36	1.65
0625	SAMM	A	0.00	2.00	0.28	2.28
0626	SAMM	A	0.00	0.00	0.24	0.24
M621	SAMM	A	0.00	1.29	0.15	1.44
0513	UNION	B	10.00	0.00	1.18	11.18
0539	UNION	A	0.00	2.00	0.69	2.69
0563	UNION	B	8.55	0.00	0.17	8.72
0564	UNION	B	0.00	2.02	1.21	3.23
0565	UNION	C	10.00	2.02	2.21	14.23
0566	UNION	C	10.00	3.29	5.56	18.85
0567	UNION	B	8.55	3.29	2.17	14.01
0568	UNION	C	10.00	4.02	8.13	22.15
0569	UNION	C	10.00	10.00	10.00	30.00
0570	UNION	C	10.00	2.00	4.37	16.37
0572	UNION	C	8.55	6.71	6.77	22.03
0573	UNION	A	0.00	2.69	0.14	2.83
0574	UNION	B	10.00	5.29	0.41	15.70
0575	UNION	C	10.00	0.00	4.33	14.33
0580	UNION	A	3.58	0.00	0.40	3.98
0425A	WASH	A	0.00	2.00	0.24	2.24
0544	WASH	A	0.00	0.00	0.14	0.14
0560	WASH	A	0.00	2.69	0.27	2.96
0562	WASH	A	0.00	2.02	0.69	2.71
0801A	WASH	A	0.00	0.00	0.32	0.32
0804	WASH	A	0.00	0.00	0.52	0.52
0807A	WASH	A	0.00	2.00	0.25	2.25
0814B	WASH	A	2.13	0.00	0.40	2.53
0817A	WASH	A	0.00	0.00	0.89	0.89
0826	WASH	A	0.00	0.00	0.60	0.60
0829A	WASH	A	6.42	0.00	0.21	6.63
0831	WASH	A	0.00	0.00	0.27	0.27

Table F-2 (Continued)

Station	Lake	Grade	Benthic	Toxicity	Chemistry	Sum
0832	WASH	A	0.00	2.00	0.31	2.31
0834	WASH	A	0.00	2.00	0.74	2.74
0840	WASH	A	0.00	0.00	0.54	0.54
0852	WASH	A	0.00	2.00	0.78	2.78
0861	WASH	A	0.00	1.29	0.69	1.98
0862	WASH	A	0.00	0.00	0.11	0.11
0864A	WASH	B	3.58	7.29	2.66	13.54
0890	WASH	A	0.00	2.00	0.69	2.69
4901A	WASH	A	0.00	2.00	0.26	2.26
4903A	WASH	A	2.13	0.00	1.87	4.00
4903B	WASH	B	1.45	10.00	2.57	14.02
A422A	WASH	A	6.42	2.69	0.40	9.50
S0025	WASH	A	0.00	0.69	0.21	0.90
SD007A	WASH	A	0.00	0.00	0.22	0.22
SD017A	WASH	A	0.00	2.00	0.40	2.40