Assessment of Aquatic Toxicity in North Creek, Gig Harbor

DEPARTMENT OF ECOLOGY
State of Washington

April 2011
Publication No. 11-03-022

Publication and Contact Information

This report is available on the Department of Ecology's website at
www.ecy.wa.gov/biblio/1103022.html

Data for this project are available at Ecology's Environmental Information Management (EIM) website www.ecy.wa.gov/eim/index.htm. Search User Study ID, BERA0007.

The Activity Tracker Code for this study is 10-123.

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Cover photo: North Creek at the upstream monitoring site.

Assessment of Aquatic Toxicity in North Creek, Gig Harbor

by

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North Creek does not have a Waterbody Number.
LLID: 1225993473445; Lower Route: 0.158; Upper Route: 1.393
Water Assessment Quality Listing ID: 12585

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Table of Contents

List of Figures and Tables

Page

Figures

Tables

.

Abstract

From December 2009 through March 2010, the Washington State Department of Ecology (Ecology) conducted a study of toxicity in North Creek, near Gig Harbor, Washington. The purpose of the study was to determine if elevated levels of lead and copper were causing acute toxicity to the living organisms of North Creek. Previous studies by Ecology and others have determined that dissolved lead levels are elevated in North Creek and that the Gig Harbor Sportsman's Club is a major source of lead to the creek.

Based on the results of bioassay testing using daphnids and trout in the 2009-10 study, North Creek does not appear to be acutely toxic; however, further research into chronic effects is needed before ruling out chronic toxicity. High dissolved organic carbon concentrations from decaying leaves and plant matter in North Creek are likely buffering lead toxicity in North Creek.

Analysis of periphyton native to North Creek revealed that the periphyton community in the creek is impacted downstream of the Sportsman's Club. Several periphyton community metrics point to the possibility that lead may be impacting periphyton community health.

Dissolved lead concentrations in North Creek below the Sportsman's Club continue to be extremely elevated, exceeding Washington State aquatic life chronic criteria by a factor of over 1,000. Dissolved lead concentrations above the Sportsman's Club are much lower, exceeding the chronic criteria by a factor of only 3.

Dissolved copper concentrations continue to be moderate, exceeding the chronic criteria by a factor of approximately 1.2, both above and below the Sportsman's Club. The Sportsman's Club is not the likely source of copper to North Creek.

Acknowledgements

The authors of this report thank the following people for their contribution to this study:

- Nautilus Environmental for bioassay work.
- Rhithron Associates Inc. for diatom taxonomic identification.
- Leska Fore for guidance on statistical analysis for periphyton assessment.
- Washington State Department of Ecology staff:
  - Tanya Roberts for field assistance.
  - Sally Lawrence, Dale Norton, Callie Meredith, and Karen Adams for reviewing the report.
  - Paul Anderson and James Kardouni for technical assistance with field sampling equipment and methods.
  - Dean Momohara and others at Manchester Environmental Laboratory.
  - Randy Coots for reconnaissance information on North Creek.
  - Steve Golding for assistance with planning the project.

Introduction

Previous studies by the Tacoma-Pierce County Health Department (TPCHD) and by the Washington State Department of Ecology (Ecology) have shown that North Creek contains elevated levels of lead and copper (Bell, 2002; Golding, 2008). North Creek is currently listed on the federal Clean Water Act Section 303(d) list for elevated concentrations of lead. Total and dissolved lead concentrations in North Creek are the highest that have been found in surface waters of Washington State. Copper concentrations are relatively low, only slightly above State water quality standards.

The Gig Harbor Sportsman's Club (Club) was identified as the likely source of lead to North Creek based on the sampling efforts by TPCHD in 2002 and by Ecology in 2008. The 2008 Ecology study recommended a bioassessment study of North Creek below the Club boundary to help determine whether adverse ecological impacts are occurring due to high lead concentrations.

Because the Club has been identified as the likely source of lead to North Creek, TPCHD in conjunction with Ecology's Toxics Cleanup Program recently completed a site hazard assessment for the Club. The site received a high priority ranking of 1 and is currently awaiting future clean-up action by Ecology (Cris Matthews, personal communication).

The purpose of the current 2009-10 study was to assess the potential for adverse biological effects in North Creek due to elevated levels of lead and copper. Bioassay tests and periphyton were used to evaluate aquatic toxicity. Total and dissolved concentrations of lead and copper were also measured in the surface water of North Creek. This work was conducted following a published Quality Assurance (QA) Project Plan (Era-Miller, 2009).

Site Description

North Creek drains a watershed of approximately 0.2 square miles (130 acres) made up of mixed forest, family residences, a shooting range, athletic fields, and a business park. The creek is located in Pierce County, within Water Resource Inventory Area (WRIA) 15. The creek flows north to south and discharges to Donkey Creek, a salmon-bearing stream which flows through the city of Gig Harbor and into Puget Sound (Golding, 2008). The North Creek watershed is shown in Figure 1.

North Creek is an intermittent stream. It maintains a very low but constant flow during the wet winter months and tends to dry up during the summer.

The headwaters of the creek originate in the Bellingham soil type, a soil type that is characterized by a seasonally high water table and poor drainage. The creek then travels through the Harstine soil type, which is strongly acidic (Sally Lawrence and Mike Woodall, personal communication).

North Creek flows across the Gig Harbor Sportsman's Club property. The headwaters of the creek are within one mile of the Club. The Club is an active shooting range located off Burnham Drive in Gig Harbor. It has operated since the 1940s. Club property consists of a shotgun range with seven regulation trap fields and a rifle and pistol range (Golding, 2008).

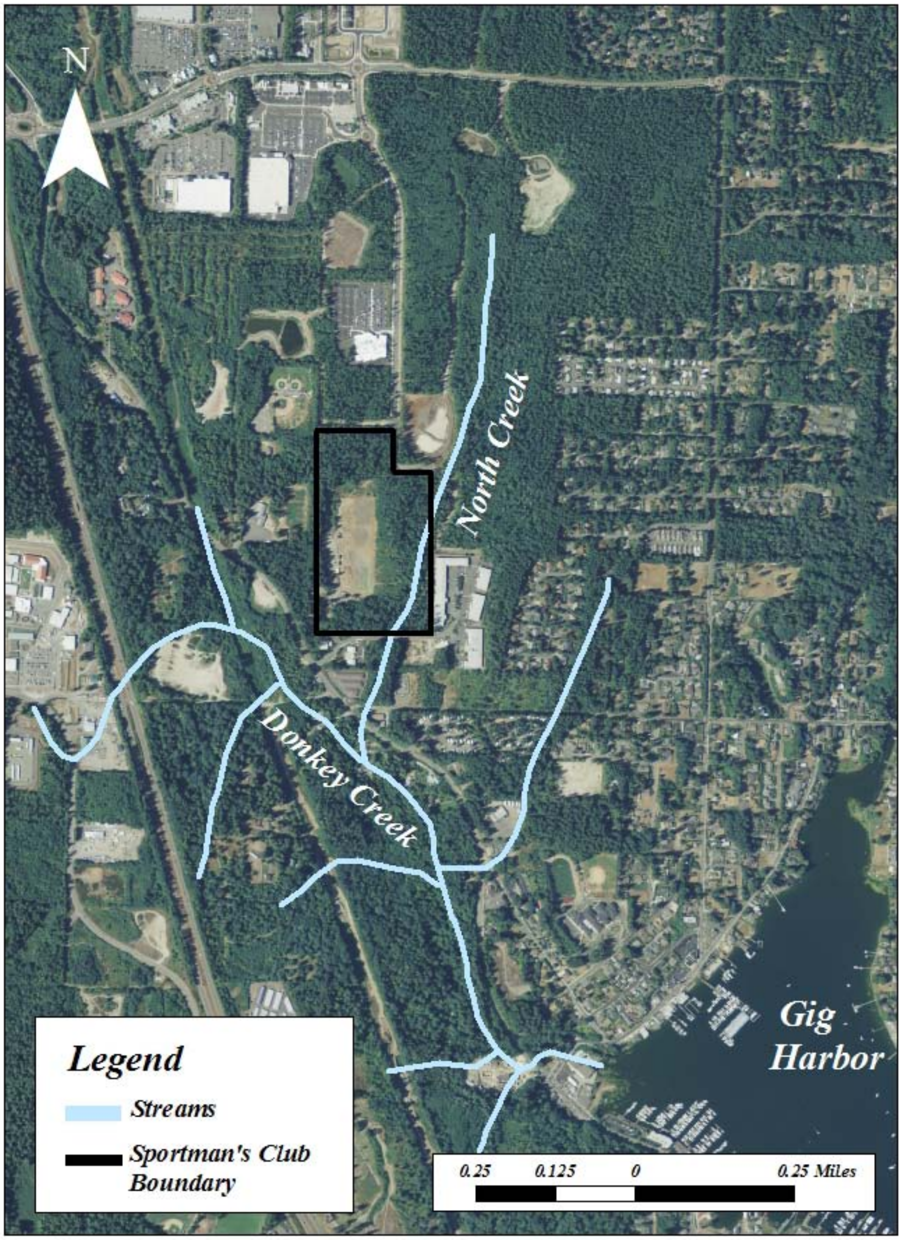

Figure 1. North Creek Watershed (photo from 2009).

Previous Ecology Data

In the spring of 2008, sampling conducted by Ecology showed that lead concentrations below the Club property were the highest found in surface waters of Washington State (Golding, 2008). Table 1 shows how dissolved lead concentrations below the Club property boundary increased by three orders of magnitude compared to levels just above the Club property boundary. Water quality criteria for dissolved lead were grossly exceeded (not met) below the Club property boundary.

Table 1. Lead Concentrations in North Creek Above and Below the Sportsman's Club.

Station	Lead (ug/L)		Hardness (mg/L)	Water Quality Criteria*	
	Total Recoverable	Dissolved		Acute	Chronic
Collected 4/10/2008					
N Creek Above	1.15	**0.82**	12.5	6.33	0.25
N Creek Below	212	**200**	11.1	5.53	0.22
Collected 4/21/2008					
N Creek Above	1.44	**0.82**	13	6.62	0.26
N Creek Below	188	**178**	10	4.91	0.19

* Water quality criteria are for dissolved lead and are based on hardness (WAC 173-201A).

Copper was also analyzed by Ecology in 2008 (Table 2). Copper concentrations exceeded criteria, but not by much. Copper was slightly lower in North Creek below the Club as compared to above the Club, indicating that the Club is not a major source of copper to the creek.

Table 2. Copper Concentrations in North Creek Above and Below the Sportsman's Club.

Station	Copper (ug/L)		Hardness (mg/L)	Water Quality Criteria*	
	Total Recoverable	Dissolved		Acute	Chronic
Collected 4/10/2008					
N Creek Above	2.71	**2.19**	12.5	2.40	1.92
N Creek Below	2.30	**1.96**	11.1	2.14	1.73
Collected 4/21/2008					
N Creek Above	2.68	**2.41**	13	2.49	1.99
N Creek Below	2.6	**2.08**	10	1.94	1.59

* Water quality criteria are for dissolved copper and are based on hardness (WAC 173-201A).

Methods

Study Description

Ecology measured toxicity by exposing bioassay organisms to water samples from North Creek collected above (NCREEK1) and below (NCREEK2) the Sportsman's Club. Sampling locations are shown in Figure 2. Two types of bioassay tests were used to account for responses in different types of organisms:

1. The 48-hour *Daphnia pulex* survival test is considered to be an acute test.
2. The 7-day rainbow trout (*Oncorhynchus mykiss*) survival and growth test meets Environmental Protection Agency's (EPA's) definition of a chronic test due to the growth endpoint, but would usually be considered an acute test due to its duration of only 7 days which is only a fraction of one lifestage.

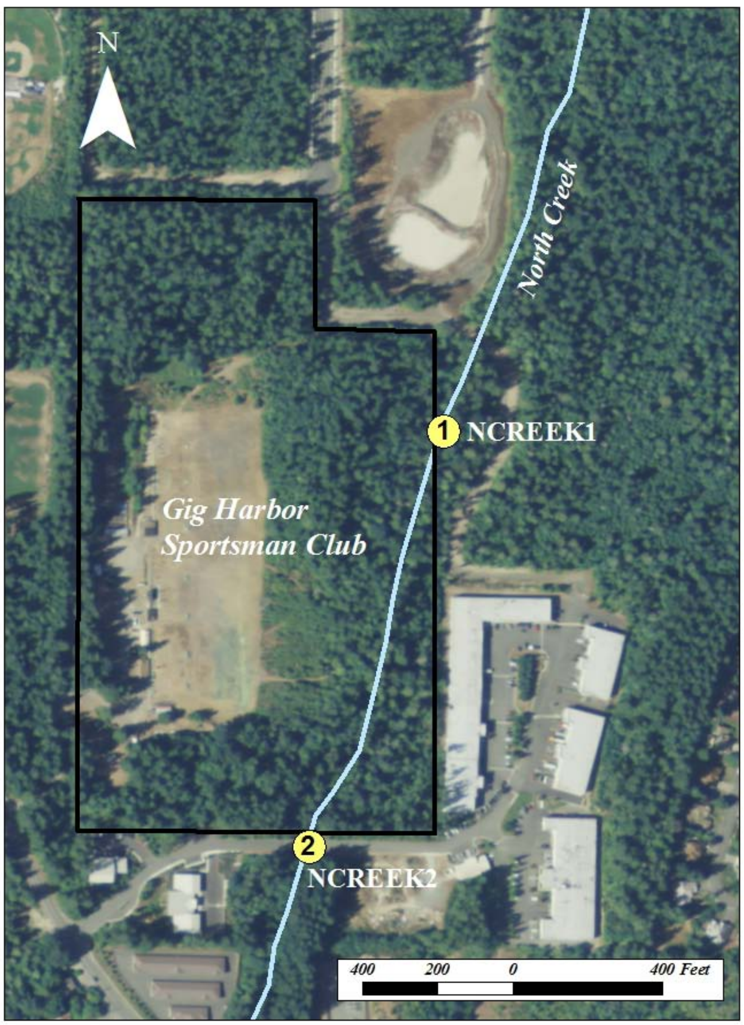

Figure 2. Map Showing the Sportsman Club Boundaries and North Creek Sampling Sites (photo from 2009).

Periphyton was collected upstream (NCREEK1 - control site) and downstream (NCREEK2 - impacted site) of the Club to measure potential adverse biological effects to the periphyton community native to North Creek. Periphyton consists of assemblages of benthic (attached) algae; these algae are made up of various species of diatoms. Selected diatom metrics were used to describe ecological conditions and determine changes in community structure between the impacted and control site. Because North Creek has intermittent flow, diatom metrics were a good choice to measure biological response. Fish and benthic macroinvertebrates are not present in numbers high enough to measure in North Creek.

Diatoms have been proven useful as indicators of water quality because of a number of reasons. They are highly sensitive to physical and chemical factors, are primary producers, and have short reproductive periods. Diatoms also have known environmental requirements and water quality tolerances unique to individual species (Plafkin et al., 1989; Bahls, 1993; Van Dam et al., 1994; Porter et al., 2008). Autecological metrics derived from diatom community data have been used to develop both individual and group metrics for assessing water quality in streams (Lange-Bertalot, 1979; Bahls, 1993; Fore and Grafe, 2002; Delgado et al., 2010).

In support of the biological tests, lead, copper, and general stream parameters were also measured. Hardness and dissolved concentrations of lead and copper were analyzed so they could be compared to Washington State Water Quality Standards.

Samples were collected during the rainy season to ensure adequate flow in North Creek. Water samples were collected on December 22, 2009 and March 4, 2010. Appendix B, Table B-1 shows rainfall amounts measured at the Tacoma-Narrows Airport (located near North Creek) during the study. The week preceding both sampling events had a majority of days with more than 0.10 inches of rain. Periphyton was collected on February 8, 2010.

All sampling was conducted following a published QA Project Plan (Era-Miller, 2009).

Sampling Procedures

Water Samples and Bioassays

Water samples were collected by hand as composites from mid-channel following Ecology's Environmental Assessment Program *Standard Operating Procedure (SOP) for Manually Obtaining Surface Water Samples* (Joy, 2006). Streamflow in North Creek was low and well-mixed so that collecting samples from a single mid-channel point was representative. Individual aliquots were collected with a certified 1-liter, wide-mouth jar and used to proportionally fill the water chemistry and bioassay containers, so each container contained similar water. Field staff wore powder-free nitrile gloves while collecting and handling samples.

Collection of water samples for metals analysis followed the *SOP for the Collection and Field Processing of Metals Samples, Version 1.3* (Ward, 2007). Dissolved metals were filtered in the field using 0.45 micron pre-cleaned Nalgene filters. Samples were then preserved in the field using 1:1 nitric acid from small Teflon® vials. Filtering and preservation was conducted on a clean lab table in the field van using clean methods. Samples were filtered and preserved within an hour of collection.

The testing laboratory, Nautilus Environmental, provided sampling procedures for the collection of water for the bioassay tests. These procedures were carefully followed by field personnel. Thirty liters of stream water were required for the bioassay cubitainers at each site.

After collection, samples were labeled, put on ice in coolers, and kept cool at 4° C. Chain-of-custody was maintained. Bioassay samples were delivered to Nautilus Environmental in Fife, Washington on the same day of collection. Water chemistry samples were delivered to Ecology's Manchester Laboratory in Manchester, Washington the next day. All samples were received and analyzed within holding times.

Field Measurements

A Hydrolab MiniSonde® meter was used to measure water temperature, pH, and conductivity onsite following the *SOP for Hydrolab® DataSonde® and MiniSonde® Multiprobes, Version 1.0* (Swanson, 2007). The MiniSonde® meter was calibrated before and after field sampling following the instruction manual for the meter.

Dissolved oxygen samples were taken and preserved in the field and later analyzed at Ecology's Wet Lab using the Winkler method. This method is described in the *SOP for the Collection and Analysis of Dissolved Oxygen (Winkler Method), Version 1.4* (Ward, 2010).

Flow was measured using a Marsh McBirney flow meter and top-setting rod as described in the *SOP for Estimating Streamflow, Version 1.0* (Sullivan, 2007).

Periphyton

Because North Creek is ephemeral, flowing only during wet months, periphyton was collected outside the normal index period (July 1 through October 15) established for Washington State (Plotnikoff and Wiseman, 2001).

Periphyton (diatom) samples were collected from natural depositional substrates at the upstream and downstream sites on North Creek (Figure 2). Procedures for diatom collection are outlined in the study QA Project Plan (Era-Miller, 2009).

In brief, five sub-samples were collected from depositional areas at each site using a 47-mm diameter Petri dish. The Petri dish was carefully pressed down into the substrate (in cookie-cutter fashion) and then slid tightly onto a spatula to enclose the sample under the water (Figure 3). The five sub-samples were combined into a 500 mL Nalgene® sample bottle using deionized water from a squirt bottle to rinse the sediments into the bottle.

Figure 3. Petri Dish Collection Method for Periphyton.

A sub-sample (~10 mL) was then removed from the composite sample for chlorophyll *a* (chl-*a*) analysis. This subsample was filtered with a 0.7-micron glass microfiber filter. The filters were packaged and labeled and later sent to Manchester Laboratory for chlorophyll *a* analysis. The remaining composite sample was preserved with 5% to 10% Lugol's solution, packaged, labeled, and later sent to Rhithron Associates, Inc. for taxonomic identification.

Diatom Metric Assessments

A suite of diatom-based metrics commonly used for assessing stream health were calculated for each sample (Bahls, 1993; Barbour et al., 1999; Porter, 2008; Van Dam et al., 1994). The metrics of primary interest include those that infer stream health and those associated with metal contamination.

Stream Health Indicator Metrics

1. **Species Richness** is an estimate of the number of diatom species in a sample. High species richness may indicate undisturbed conditions and is predicted to decrease with increasing pollution because many species are stressed (Barbour et al., 1999).

2. **Shannon Diversity Index** is a function of both the number of species in a sample and the distribution of individuals among the taxa present (Weber, 1973). High diversity values occur in diatom communities where no one taxa strongly dominants the community. High diversity values generally indicate healthy, undisturbed streams. For diatom communities under environmental stress, the majority of individuals present belong to a relatively small number of taxa, resulting in lower diversity index values (Bahls, 1993).

3. **Pollution Tolerance Index** is a weighted average of species abundance and tolerance rating within a sample. Many diatom species have assigned tolerance ratings, from 1 to 3, based on knowledge of their tolerance of pollution. Tolerant species get a value of 1 and intolerant a value of 3 (Bahls 1993; Barbour et al., 1999).

4. **Percent Dominant Species** considers the single diatom species having the highest percent relative abundance. In healthy streams, no single species is responsible for a high percentage of the total diatom cells present. The greater the percentage contributed by a single dominant species, the greater the stress (Barbour et al., 1999).

5. **Chlorophyll *a*** is a measure of algal biomass. The response of this metric is variable; however, chlorophyll *a* levels are expected to decrease with decreasing productivity and trophic status (Peterson and Porter, 2002).

Metal Indicator Metrics

1. **Heavy Metals Index** is defined as the total percent abundance of 18 diatom species that tolerate elevated concentrations of heavy metals (Bahls, 1993). Increases in metal-tolerant taxa have been associated with increasing metals concentrations.

2. **Percent *Eunotia* individuals** is defined as the percent of diatom species from the genus *Eunotia*. Species within this genus have been documented to increase with increasing metals concentrations (Verb and Vis, 2000).

3. **Percent *Pinnularia* individuals** is defined as the percent of diatoms species from the genus *Pinnularia*. Species within this genus have been documented to increase with increasing metals concentrations (Potapova and Charles, 2003).

4. **Percent Acidophilous** is the percent of diatoms that mainly occur in water with a pH < 7. Increases in acidophilous taxa have been associated with increasing metals concentrations (Stevenson and Bahls, 1999).

Impacts to the diatom community from the Club were assessed by calculating the deviation between diatom metrics at NCREEK2 (impacted site) and NCREEK1 (control site). To determine if the differences between station metrics were significant, results were compared to metric coefficients of variation (CVs) of replicate samples collected within similar reaches in previous studies (Bahls, 1993; Ecology unpublished data).

Seven water quality attributes for diatom species were applied to each sample, based on those identified by Van Dam et al. (1994). The water quality attributes were used to describe and compare trophic conditions at each sampling station.

Water quality preference values for each diatom species identified were determined using a checklist of autecological guilds provided in Van Dam et al. (1994). Each diatom species was assigned values ranging from 1 through 6 depending on their preferences to following seven water quality attributes: pH, salinity, nutrient enrichment, dissolved oxygen, trophic state, saprobity, and moisture conditions (Appendix C, Table C-1). Preference values were averaged and weighted by species abundance for each sampling station and each attribute to describe trophic conditions.

Laboratory Analysis

Manchester Laboratory analyzed the stream chemistry samples, Nautilus Environmental conducted the bioassay tests, and Rhithron Associates, Inc conducted the periphyton analyses. Analytical methods are shown in Table 3.

Table 3. Analytical Methods and Laboratories.

Analyte	Sample Preparation Method	Analytical Method	Laboratory
Lead - total	Field preserve; laboratory HNO_3/HCl digest	EPA 200.8	Manchester
Copper - total			
Lead - dissolved	Field filter and preserve; laboratory HNO_3/HCl digest		
Copper - dissolved			
Hardness	Field preserve	EPA 200.7	
Total Suspended Solids	N/A	EPA 160.2	
48-hr Daphnid	EPA-821-R-02-012 & Marshall, 2008		Nautilus
7-day Trout	Lazorchak and Smith, 2007 & Marshall, 2008		
Periphyton	WDEQ/WQD, 2005		Rhithron

Data Quality

Stream Chemistry

Manchester Laboratory and the project manager reviewed all of the water chemistry data. The data were found to meet measurement quality objectives as outlined in the QA Project Plan (Era-Miller, 2009).

No significant levels of analytes were detected in method blanks. Laboratory control samples were recovered within laboratory acceptance limits of 85-115%.

Matrix spike (MS) and matrix spike duplicate (MSD) recoveries were within laboratory acceptance limits of 75-125% as shown in Appendix D, Table D-1. Precision of the MS/MSD pairs measured as relative percent difference (RPD) were also within laboratory acceptance limits of less than 20%.

Precision measurements (RPDs) between laboratory duplicates were within acceptance limits for the 3/4/10 sampling (Table D-2). No laboratory duplicates were analyzed for the 12/22/09 sampling; however, precision measurements (RPDs) in the field replicates were excellent for both sampling dates. Table D-3 gives the RPDs for the field replicates. Field replicates are a powerful measurement of precision because they take into account precision in the laboratory analyses as well as field variability.

Field Measurements

The MiniSonde® field meter was calibrated before field measurements were taken and post check measurements were taken upon return from the field. Post check results showed low variability between calibrated results and post check results. For pH, the variability ranged from 1-2%, and for conductivity, variability ranged from 5-12%.

Bioassays

The bioassay analysis methods used by Nautilus Environmental included an adequate amount of QA/quality control (QC) procedures. All QA/QC acceptance limits were met for the bioassay tests as explained in the case narratives provided by Nautilus.

Bioassay results were also reviewed and deemed acceptable by Randall Marshall, Ecology's Whole Effluent Toxicity (WET) Program manager and Ecology's expert on water bioassay testing.

Periphyton

All QA/QC acceptance limits were met for the periphyton testing as explained in the case narratives provided by Rhithron Associates, Inc. QC procedures for taxonomy involved the re-identification of diatoms from a randomly selected sample by independent taxonomists. Taxonomic similarity for identification and enumeration was 85% for the randomly selected periphyton QA sample.

Results

Metals and Stream Chemistry

Results for lead, copper, and general stream chemistry are shown in Table 4.

Table 4. Chemistry Results for North Creek.

Collection Date	12/22/2009		3/4/2010	
Time	11:30	14:45	11:15	13:30
Sample No. Station	0912033-01 NCREEK1	0912033-04 NCREEK2	1003026-01 NCREEK1	1003026-02 NCREEK2
Metals (ug/L)				
Lead - total recoverable	1.06	317	0.96	226
Lead - dissolved	0.997	304	0.825	236
Copper - total recoverable	3.15	2.73	2.69	2.10
Copper - dissolved	2.95	2.52	2.43	2.11
Total Suspended Solids (mg/L)	1 U	1 U	1 U	1 U
Hardness (mg/L)	15.9	14.9	13.1	11.3
Dissolved Organic Carbon (mg/L)	--	--	26	18.8
Dissolved Oxygen (mg/L)	8.5	9.4	6.0	7.3
pH (pH units)	5.36	5.72	5.54	5.75
Conductivity (umhos/cm)	36.4	36.0	32.8	31.4
Temperature (C$^{\circ}$)	5.26	5.86	7.87	7.89
Flow (cubic feet per second)	0.11	0.11	0.01	0.02

-- data not collected.
U - the analyte was not detected at or above the reported sample quantitation limit.

Lead concentrations were extremely elevated at the downstream site NCREEK2 during both sampling events. Lead was several orders of magnitude higher than the upstream site. Copper concentrations were slightly higher at the upstream site when compared to downstream. The dissolved fraction of both lead and copper made up the majority of the total concentrations, ranging from 86% to 104% dissolved.

Lead concentrations in North Creek are the highest concentrations measured in Washington State waters. The next highest concentration, 52.1 ug/L, was measured in SE Thornton Creek in Seattle in 2003 (Ecology's EIM database, accessed 2011).

Dissolved organic carbon (DOC) was high, ranging from 18.8 to 26 mg/L, though it was only measured during the March sampling event. When compared to almost 6,000 DOC measurements from rivers throughout Washington State, 18.8 mg/L was above the 99th percentile (EIM database, accessed 2011). The prevalent tea color of the water also provided visual clues that North Creek contains high amounts of DOC.

Hardness values, ranging from 11.3 – 15.9 mg/L, fell below the 20th percentile for western Washington streams when compared to Ecology's Ambient Freshwater Monitoring Program database (Dave Hallock, personal communication).

Total suspended solids (TSS) were very low in all the samples.

Values for pH ranged from 5.36 – 5.75, indicating that the creek is moderately acidic.

Flows in the creek were so low that they were almost too low to measure accurately, ranging from 0.01 to 0.11 cubic feet per second (cfs).

Bioassays

No toxicity was found in any of the bioassay tests as shown in Table 5. Graphs showing the results for each test on both sampling dates are shown in Appendix E, Figures E-1 through E-8. The endpoint for the daphnid test was survival and the endpoint for the trout test was survival and growth.

Table 5. Bioassay Test Results for North Creek.

<table>
<tr><th>Date</th><th>Bioassay Test</th><th>North Creek 1 Upstream</th><th>North Creek 2 Downstream</th></tr>
<tr><td rowspan="2">12/22/2009</td><td>48-hour Daphnid</td><td rowspan="4">No Toxicity</td><td rowspan="4">No Toxicity</td></tr>
<tr><td>7-day Trout</td></tr>
<tr><td rowspan="2">3/4/2010</td><td>48-hour Daphnid</td></tr>
<tr><td>7-day Trout</td></tr>
</table>

Toxicity was measured as the statistical difference between laboratory controls and the sample results. Since all the organisms survived, except one single daphnid at the upstream site (North Creek 1) from the March 3, 2010 sampling, no statistical difference was measured. No statistical difference could be measured in trout growth. There was no statistical difference between the upstream and downstream sites.

Full test reports provided by Nautilus Environmental are available from the lead Ecology report author upon request.

Periphyton

All diatom metrics associated with stream health indicate a degradation of water quality conditions at NCREEK2 when compared to NCREEK1 (Table 6). Decreases in the number of species and the diversity of species at NCREEK2 are indicative of a stressed diatom community (Barbour et al., 1999; Weber, 1973; Bahls, 1993).

Increases in the percent dominant species and pollution index at NCREEK2 also suggest a stressed diatom community (Barbour et al., 1999). While the differences between pollution indexes were smaller, their respective CV values were still higher than published replicate CV values for respective metrics (Table 6).

Table 6. Values for Selected Diatom Metrics in North Creek.

Metric	NCREEK1 (Control)	NCREEK2 (Impacted)	Response	CV	Replicate CV[1,2]
Stream Health Indicator Metrics					
Species Richness	32	25	Decrease	17.4	5.9[1]
Shannon Diversity Index	3.8	2.7		23.9	4.0[2]
Pollution Index	2.5	2.6	Increase	2.8	2.4[1]
Percent Dominant Species	29.3	41.8		24.9	0.3[2]
Chlorophyll *a* (µg/cm2)	1.8	5.0		65.0	3.31
Metal Indicator Metrics					
Heavy Metal Index	6.7	2.7	Decrease	60.2	48.9[1]
Percent *Eunotia*	28	40	Increase	25.0	-
Percent *Pinnularia*	6.3	9.8		30.7	-
Percent Acidophilous	9.3	13.2		24.5	0.01[1]

CV = coefficient of variation.
[1]CV based on replicate samples collected by Ecology.
[2]Bahls, 1993.

Chlorophyll *a*

Chlorophyll *a* (chl-*a*) concentrations increased from 1.8 ug chl-*a* cm^2 at NCREEK1 to 5.0 ug chl-*a* cm^2 at NCREEK2 (Table 6). This increase would suggest an increase in overall biological productivity or algae biomass at NCREEK2. However, because taxa at both stations indicate similar trophic conditions (see below) the 2.7-fold increase in chl-*a* is unlikely due to increased productivity or change in trophic status.

One explanation for the increase of chl-*a* at NCREEK2 could be that it is correlated with increases in Pb concentrations. Studies measuring the environmental effects of historical mining demonstrated that concentrations of chl-*a* were correlated with increasing zinc, copper, and cadmium concentrations in periphyton (Besser et al., 2007; Calmano et al., 1988).

Another explanation for the increase in chl-*a* could be that live plant material containing chl-*a* was present in the depositional samples collected at NCREEK2. This would elevate chl-*a* concentrations.

Diatom Indicator Metrics

Three of the four metrics that indicate the presence of metals suggest a greater influence of metals at NCREEK2 when compared to NCREEK1 (Table 6). The strongest evidence of metals stress was shown by the increase in the abundance and species of the genus *Eunotia* at NCREEK2. Species in this genus have been documented as dominating streams affected by acid mine drainage (Verb and Vis, 2000; Kingston et al., 1992). Increases in the number of taxa from the genus *Pinnularia,* as well as the number of acidophilous taxa, also suggest metals are influencing the diatom community at NCREEK2 (Hill et al., 2001; Potapova and Charles, 2003).

It should be noted that most reported metal tolerant taxa also have a preference for low pH environments. However, because pH was similar between sampling stations, differences between metals metrics suggest factors other than pH were responsible for the difference.

The heavy metal index suggests there were a greater percentage of metal tolerant taxa at NCREEK1 compared to NCREEK2; however, the overall percentages of each species were relatively small (Appendix C, Tables C-2 and C-3). Also, based on replicate CV values, the heavy metal index had the highest variability of all previously reported CVs for metrics. The diatoms from the genus *Eunotia* and *Pinnularia* are not included as metal tolerant species in the heavy metal index. Combined, these genuses made up 34% of the total taxa at NCREEK1 and 50% of the total taxa at NCREEK2 (Table C-2).

Based on diatom community structure, there were no differences in the trophic state of the North Creek sampling stations (Table 7). Using Van Dam et al. (1994) species preference tables, both stations were categorized as ephemeral, freshwater streams, with a pH <7, low in organic pollution, and high in dissolved oxygen. Although only a limited number and type of water chemistry samples were taken during the study, these results are consistent with chemistry results presented in Table 4.

Table 7. Categories for Selected Trophic Attributes of Diatom Species in North Creek.

Attribute[1]	Water Quality Tendencies	
	NCREEK1 (Control)	NCREEK2 (Impacted)
pH	Acidophilous	Acidophilous
Salinity	Fresh	Fresh
Nitrogen Uptake	Autotroph	Autotroph
Oxygen Metabolism	High	High
Saprobity	Low organic decomposition	Oligosaprobous
Trophic State	Oligo-mesotraphentic	Oligo-mesotraphentic
Moisture	Moist	Moist

[1]Van Dam et al., 1994.

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Discussion

Lead and Copper Results (2008-2010) Compared to Washington State Water Quality Standards

Results for lead and copper from the current 2009-10 study and the 2008 Ecology study are compared to State water quality standards in Table 8 and Table 9. Concentrations of lead and exceedances of the hardness-based standards for lead appear to have slightly increased in the most recent monitoring of North Creek, while trends for copper have remained similar from 2008 to 2010.

Dissolved lead has exceeded (not met) the acute criteria by factors ranging from 36 – 42. Exceedance factors for the chronic criteria range from 910 – 1,072. It is important to note that hardness values in North Creek are relatively low. As hardness decreases, the hardness-based water quality standards also become lower (i.e., more stringent).

Dissolved copper exceeded the acute criteria once (April 21, 2008) by a factor of 1.1. Chronic criteria were exceeded during each of the sampling events, with factors ranging from 1.1 – 1.3.

Table 8. Lead Concentrations in North Creek Compared to State Water Quality Criteria.

Site	Lead		Hardness mg/L	Water Quality Criteria		Dissolved Lead as Factor of	
	Total recoverable (ug/L)	Dissolved (ug/L)		Acute	Chronic	Acute Criteria	Chronic Criteria
Collection Date: 4/10/2008							
NCREEK1	1.15	0.82	12.5	6.33	0.25		3
NCREEK2	212	**200**	11.1	5.53	0.22	36	910
Collection Date: 4/21/2008							
NCREEK1	1.44	0.82	13	6.62	0.26		3
NCREEK2	188	**178**	10	4.91	0.19	36	937
Collection Date: 12/22/2009							
NCREEK1	1.06	0.997	15.9	8.32	0.32		3
NCREEK2	317	**304**	14.9	7.73	0.30	39	1,013
Collection Date: 3/4/2010							
NCREEK1	0.96	0.825	13.1	6.68	0.26		3
NCREEK2	226	**236**	11.3	5.64	0.22	42	1,072

[dashed box] = exceeds chronic criteria only
[solid box] = exceeds both chronic and acute criteria

Table 9. Copper Concentrations in North Creek Compared to State Water Quality Criteria

Site	Copper		Hardness mg/L	Water Quality Criteria		Dissolved Copper as Factor of	
	Total recoverable (ug/L)	Dissolved (ug/L)		Acute	Chronic	Acute Criteria	Chronic Criteria
Collection Date: 4/10/2008							
NCREEK1	2.71	2.19	12.5	2.40	1.92		1.2
NCREEK2	2.30	1.96	11.1	2.14	1.73		1.1
Collection Date: 4/21/2008							
NCREEK1	2.68	2.41	13	2.49	1.99		1.2
NCREEK2	2.60	**2.08**	10	1.94	1.59	1.1	1.3
Collection Date: 12/22/2009							
NCREEK1	3.15	2.95	15.9	3.01	2.36		1.3
NCREEK2	2.73	2.52	14.9	2.83	2.23		1.1
Collection Date: 3/4/2010							
NCREEK1	2.69	2.43	13.1	2.51	2.00		1.2
NCREEK2	2.10	2.11	11.3	2.18	1.76		1.2

[dashed box] = exceeds chronic criteria only
[solid box] = exceeds both chronic and acute criteria

Why No Toxicity to Bioassays?

Dissolved lead concentrations in North Creek below the Sportsman's Club are the highest concentrations ever measured in Washington State, so why was no toxicity observed in the bioassay tests selected for the current study? There are several possible explanations:

1. High amounts of DOC decreased toxicity.
2. Upward pH drift during the bioassay tests lessoned toxicity.
3. The selected bioassay tests were not sensitive enough.

Effects of DOC and Other Parameters on Metals Toxicity

The effect of DOC on the bioavailability of dissolved metals is very complicated. Due to the influence that DOC and other water quality parameters have on the toxicity of metals, current science has moved in the direction of computer modeling to predict toxicity. The Biotic Ligand Model (BLM) for lead is still in development and does not yet have widespread acceptance. A widely accepted BLM has been developed for copper (www.hydroqual.com/wr_blm.html).

DOC includes a mixture of naturally occurring organic compounds, many of which are humic and fulvic acids (Specht, 2005). The primary source of DOC in streams is decomposing plant material (such as the abundance of alder and big leaf maple leaves found in North Creek). Humic and fulvic acids are known to give water a characteristically dark tea color. North Creek had a dark tea color as can be seen in Figure 4.

Figure 4. Sampling Containers at North Creek 2 (Downstream Site).

Both humic and fulvic acids contain many negatively charged binding sites that are capable of binding positively charged dissolved metals such as lead and copper, creating organic-metal complexes, thus reducing toxicity (Specht, 2005).

Positively charged ions such as calcium and magnesium can limit the ability of DOC to reduce metal toxicity, both by competing for binding sites on organic molecules and by reducing the solubility of DOC. On the other hand, calcium and magnesium can also reduce metals toxicity by binding clay particles and DOC into a stable clay-metal-humus complex (Specht, 2005).

Clay particles have a high affinity for binding with metals. Naturally occurring clays or introduced clays from the breakdown of clay pigeons heavily used at the Sportsman's Club could be present in the creek water. Some clay particles are small enough to fit through a 0.45 micron filter. If such small clay particles were present in North Creek surface water, it is conceivable that they could have passed through the filter during field filtering, thus overestimating the true dissolved concentrations of metals.

Calcium and magnesium, measured in this study as hardness, can also compete with lead and copper for fish-gill binding sites. Therefore, lead and copper toxicity are inversely related to water hardness (i.e., toxicity increases as hardness decreases). This is why State water quality standards are based on hardness.

Toxicity is also inversely related to pH, as lead and copper tend to exist more as free ions in acidic conditions. North Creek is slightly acidic.

The bottom line for North Creek is that DOC concentrations are likely overriding the effects of the naturally occurring low hardness and low pH in the creek. DOC will bind with lead and copper and reduce the amount of bioavailable lead and copper.

pH Drift during Bioassay Tests

pH drifted up in the samples, becoming less acidic, during the course of the laboratory bioassay tests. For the 7-day trout survival and growth bioassay test, the 100% concentration sample (no dilution) from the December 22, 2009 downstream site drifted from 5.27 to 6.00 (pH units). The March 4, 2010 sample from the same location (downstream) drifted from 5.43 to 6.25. For the 48-hour daphnid survival test, the 100% concentration sample at the downstream site drifted from 4.86 – 5.03 in December and from 5.74 – 6.44 in March.

It is common for pH to drift up or vary during bioassay tests. Nitric acid is sometimes added during bioassay tests to maintain consistent pH values. Though pH drifted during the bioassay tests, pH concentrations met the bioassay method acceptance criteria.

Because metals tend to be more toxic at lower pH concentrations, the increase in pH during the course of the North Creek bioassay tests could have effectively lowered toxicity to the testing organisms.

Sensitivity of Selected Bioassay Tests

It's likely that the 7-day trout survival and growth tests and the 48-hour daphnid survival test are simply not sensitive enough to pick up chronic lead toxicity in North Creek. Acute toxicity threshold concentrations are generally much higher than chronic toxicity threshold concentrations. Chronic toxicity bioassay tests are typically longer in duration and encompass other lifestage endpoints such as development and reproduction.

Though technically considered to be a chronic toxicity test because of the growth endpoint, the 7-day trout growth and survival test is actually more of an acute test.

A 2008 study developed acute-to-chronic ratios (ACRs) for trout response to lead (Mebane et al., 2008). They found that chronic lead toxicity thresholds in a 69-day early life stage (ELS) rainbow trout survival test are typically 2 – 5 times lower than acute survival toxicity thresholds for rainbow trout. The acute LC50 (lethal concentration 50%), based on a 4-day swim-up stage survival test, was 120 ug/L lead, and the chronic LC50 for the 69-day test was 55 ug/L lead.

The EPA EcoTox database was searched for lead toxicity bioassay results. Results are shown in Table 10 (see Appendix F, Table F-1 for references to these EcoTox studies). The tests are a mix of acute and chronic tests and they cover variable concentrations of hardness and DOC. The last two tests in Table 10 have DOC concentrations similar to North Creek, and the LC50 values are very high at 2,913 (fathead minnow 2-day test) and 1,090 (mayfly 4-day test) ug/L total lead.

Table 10. EPA EcoTox Database Lead Toxicity Results of Potential Relevance to North Creek.

Organism	Endpoint	Effect	Days	Type	Lead (µg/L)	Hardness (mg/L)	DOC (mg/L)	Year
rainbow trout (egg)	LC50	mortality	69	diss	55	19.7	≤ 2	2007
	MATC	growth	69		36 - 88	19.7	≤ 2	
daphnid (neonate)		mortality	1	total	150	20	n/a	1995
mayfly (larval)		molt	10	diss	130	20.7	≤ 2	2007
rainbow trout (egg)	LC50	mortality	62		120	29.4	≤ 2	
	MATC	growth	62		12 - 57	29.4	≤ 2	
rainbow trout	LC50	mortality	14	total	140	30	n/a	1971
daphnid (neonate)	EC50	reproduction	21		100	45.3	n/a	1972
daphnid (neonate)*	LC50	mortality	2		29.4	80	5.7	1997
fathead minnow (larval)*			2		2913	n/a	28	
mayfly (nymph)			4		1090	n/a	21.6	1994

*nitric acid lead salt added during test.
n/a = data not available.
diss = dissolved concentration.
LC50 = lethal concentration 50%.
EC50 = effective concentration 50%.
MATC = maximum acceptable toxicant concentration.

Conclusions

Results of this 2009-2010 study support the following conclusions:

- Dissolved lead concentrations in North Creek downstream of the Sportsman's Club continue to be extremely elevated, exceeding (not meeting) Washington State aquatic life chronic criteria by a factor of over 1,000. Dissolved lead concentrations upstream of the Sportsman's Club are much lower, exceeding the aquatic life chronic criteria by a factor of only 3. The Sportsman's Club continues to be the obvious source of lead to North Creek.

- The periphyton community in North Creek is more impacted downstream of the Sportsman's Club. Several periphyton community metrics point to the possibility that lead may be impacting periphyton community health.

- No acute toxicity to bioassay organisms exposed to North Creek water was measured using Daphnid and Trout. Further research into chronic effects would be needed to rule out chronic toxicity to bioassay organisms exposed to North Creek water.

- High dissolved organic carbon concentrations from decaying leaves and plant matter in North Creek are likely buffering lead and copper toxicity in North Creek.

- Dissolved copper concentrations continue to be moderate, exceeding the chronic criteria by a factor of approximately 1.2, both above and below the Sportsman's Club. The Sportsman's Club is not the likely source of copper to North Creek.

Recommendations

Recommendations for future options to address lead contamination in North Creek include:

1. Ecology could accept the current bioassay toxicity results as indicative of toxicity levels in North Creek under the current environmental conditions. The high concentration of dissolved organic carbon (DOC) in North Creek is likely mitigating acute lead toxicity. As long as lead does not increase and DOC does not decrease, acute toxicity will be unlikely.

2. Development in the North Creek watershed, along with any lead clean-up actions on the Sportsman's Club property, could impact lead and DOC concentrations in the future. It would be useful to monitor lead and DOC in North Creek if either of these watershed activities were to occur.

3. Further study of toxicity in North Creek would help define toxic thresholds in North Creek. Further research to understand toxicity thresholds could be done in the following ways:

    a. Have a laboratory repeat the same toxicity tests to (1) reduce the uncertainties with the existing results and (2) provide some predictability of the consequences of development around North Creek. The laboratory would need to employ measures to keep pH and hardness near levels in North Creek in all bioassay test chambers throughout the duration of toxicity tests. Dissolved lead would need to be measured at the beginning, middle, and end of the tests to confirm concentrations.

       Lead chloride could be added to a series of concentrations of both the upstream and downstream water to determine how much additional lead is necessary to reach toxic thresholds. DOC in stream samples could be reduced by dilution with clean water in the laboratory, and the ambient dissolved lead concentration could be restored by addition of lead chloride to produce another series for evaluation of lead toxicity. This would require much work, but would confirm (or not confirm) the existing toxicity results and provide information on which to base cleanup decisions in a changing environment where lead concentrations might increase or DOC concentrations might decrease.

    b. Chronic bioassay tests could be run to supplement the existing test results and fill gaps in organism lifestages and biological endpoints. Lead tends to be more of a chronic toxicant than an acute toxicant and has been known to cause developmental effects. Rainbow trout embryo viability testing and *Daphnia magna* 21-day or *Ceriodaphnia* 7-day reproduction testing would be appropriate tests.

    c. The lead Biotic Ligand Model (BLM) has reached a level of developmental maturity which may allow it to be useful for understanding lead toxicity in North Creek. The current lead BLM available from HydroQual could be used to evaluate what-if scenarios involving changing lead, DOC, hardness, and temperature levels. Assessment of competition between lead and copper, zinc, and arsenic may also be possible by a qualified contractor. BLM testing could be more convenient and affordable than additional laboratory toxicity testing. If successful, the lead BLM could be applied to other sites with metals contamination.

d. In 2008 Ecology found that, due to the effects of dilution, concentrations of lead and copper in Donkey Creek downstream of the North Creek confluence were low and did not exceed (met) Washington State water quality standards (Golding, 2008). Donkey Creek is a salmon-bearing perennial stream that drains directly to Puget Sound. It is possible that in other years, with different precipitation conditions, North Creek could have a greater impact on the biology of Donkey Creek.

   If concentrations of dissolved lead in Donkey Creek were measured at levels exceeding State water quality standards in the future, then toxicological impacts to Donkey Creek could be assessed with bioassay testing upstream and downstream of North Creek's confluence with Donkey Creek.

   The rainbow trout embryo-to-fry, in-situ (instream) toxicity test is recommended. Ecology successfully conducted a pilot in the city of Olympia's Indian Creek in spring 2010 and got an excellent upstream (no adverse effects to trout) to downstream (severe mortalities and some developmental effects to trout) comparison. The embryo-to-fry, in-situ test is a true chronic test which runs through three lifestages (embryo, alevin, and fry), lasts for a month (more or less depending on water temperature), and uses a species of salmonid.

e. Another way to measure toxicity in both North Creek and Donkey Creek would be through stream sediment bioassays, along with measuring lead and copper concentrations in the stream sediments. Lead and copper can bind to particulates and settle out into the substrate. It is recommended that both an acute and chronic test be used.

4. Impacts to groundwater from the Sportsman's Club should be a consideration. Lead from lead shot and polycyclic aromatic hydrocarbons (PAHs) from the breakdown of pitch-based clay pigeons on the upland portion of the Club could potentially leach down in to groundwater if the hydrological conditions dictated it. Groundwater wells in the vicinity could be monitored for the contaminants of concern.

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Appendices

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Appendix A. Glossary, Acronyms, and Abbreviations

Glossary

Acidophilous: An organism that tolerates acidic conditions.

ACR: The ratio between chemical concentrations exerting a lethal versus sublethal toxic effect. It's often used to predict chronic effects to bioassay organisms.

Autecological: The natural habitat conditions and preferences of a species.

Bioassay: Standard biological test. Usually a laboratory test which exposes organisms to the medium of interest (e.g., amphipod exposure to sediment). Results indicate the toxicity of the medium to that particular organism.

Biomass: A combined survival and growth toxicological endpoint calculated by dividing the total mass of surviving fish at the end of the test by the number of fish introduced into test chambers at the beginning of the test.

Clean Water Act: A federal act passed in 1972 that contains provisions to restore and maintain the quality of the nation's waters. Section 303(d) of the Clean Water Act establishes the TMDL program.

Conductivity: A measure of water's ability to conduct an electrical current. Conductivity is related to the concentration and charge of dissolved ions in water.

Diatom: A major group of algae that possess a rigid siliceous cell wall.

Dissolved oxygen (DO): A measure of the amount of oxygen dissolved in water.

EC50: Effective Concentration 50. The concentration of a substance that has a specified non-lethal effect on half of the test organisms within a specified period of time. Effects measured are often number of young produced, time to reproduction, etc. Definition used in bioassay testing.

Endpoint: For bioassay tests, the endpoint is the end of the test by which the anticipated effect is measured. For example, the endpoint for a survival bioassay is either the death or survival of an organism.

Eutrophic: Waters or systems with high primary productivity of new organic matter.

LC50: Lethal Concentration 50. The estimated concentration of a substance required to cause death in 50% of the test organisms in a specified time period. Definition used in bioassay testing.

MATC: Maximum Acceptable Toxicant Concentration. The maximum acceptable toxicant concentration defined by the highest tested value leading to no observed harmful effect. It is an approximation of the toxic threshold. Definition used in bioassay testing.

Metric: A system of related measures that facilitates the quantification of some characteristic.

Oligotrophic: Waters or systems with low primary productivity of new organic matter.

Oligo-mesotraphentic: Waterbodies with an intermediate level of productivity of new organic matter.

Parameter: Water quality constituent being measured (analyte). A physical, chemical, or biological property whose values determine environmental characteristics or behavior.

Periphyton: A complex mixture of algae, cyanobacteria, heterotrophic microbes, and detritus that is attached to submerged surfaces.

pH: A measure of the acidity or alkalinity of water. A low pH value (0 to 7) indicates that an acidic condition is present, while a high pH (7 to 14) indicates a basic or alkaline condition. A pH of 7 is considered to be neutral. Since the pH scale is logarithmic, a water sample with a pH of 8 is ten times more basic than one with a pH of 7.

Salmon: Any fish that belong to the family *Salmonidae*. Basically, any species of salmon, trout, or char. www.fws.gov/le/ImpExp/FactSheetSalmonids.htm

Saprobity: the physiological and biochemical characteristics of an organism that permit it to live in water with some amount of organic matter, that is, with some degree of pollution.

Surface waters of the state: Lakes, rivers, ponds, streams, inland waters, salt waters, wetlands and all other surface waters and water courses within the jurisdiction of Washington State.

Trophic status: The relative productivity of a waterbody (e.g., eutrophic).

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a central collector such as a stream, river, or lake at a lower elevation.

303(d) list: Section 303(d) of the federal Clean Water Act requires Washington State to periodically prepare a list of all surface waters in the state for which beneficial uses of the water – such as for drinking, recreation, aquatic habitat, and industrial use – are impaired by pollutants. These are water quality-limited estuaries, lakes, and streams that fall short of state surface water quality standards and are not expected to improve within the next two years.

Acronyms and Abbreviations

BOD	Biochemical oxygen demand
Club	Gig Harbor Sportsman's Club
DOC	Dissolved organic carbon
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
QA	Quality Assurance
QC	Quality Control
RPD	Relative percent difference
RSD	Relative standard deviation
SOP	Standard operating procedure
WRIA	Water Resource Inventory Area

Units of Measurement

°C	degrees centigrade
cfs	cubic feet per second
mg/L	milligrams per liter (parts per million)

mL	milliliters
mm	millimeters
µg/cm2	micrograms per centimeter squared
µg/L	micrograms per liter (parts per billion)
umhos/cm	micromhos per centimeter

Appendix B. Rainfall at Tacoma-Narrows Airport

Table B-1. Rainfall (inches) at the Tacoma Narrows Airport during the 2009-2010 Sampling Season.

2009				2010					
November		December		January		February		March	
1	0.00	1	0.07	1	0.48	1	0.01	1	0.01
2	0.00	2	0.00	2	0.43	2	0.14	2	0.00
3	0.00	3	0.00	3	0.30	3	0.00	3	0.12
4	0.00	4	0.00	4	0.06	4	0.25	4	0.01
5	0.00	5	0.01	5	1.13	5	0.17	5	0.00
6	0.00	6	0.00	6	0.10	6	0.03	6	0.00
7	0.00	7	0.00	7	0.00	7	0.12	7	0.00
8	0.00	8	0.00	8	0.17	8	0.07	8	0.10
9	0.00	9	0.00	9	0.94	9	0.01	9	0.17
10	0.23	10	0.00	10	0.00	10	0.00	10	0.12
11	0.00	11	0.00	11	0.19	11	0.32	11	0.01
12	0.00	12	0.00	12	0.88	12	0.33	12	0.74
13	0.00	13	0.00	13	0.24	13	0.45	13	0.33
14	0.07	14	0.02	14	0.35	14	0.46	14	0.04
15	0.00	15	0.60	15	0.33	15	0.13	15	0.02
16	0.12	16	0.22	16	0.55	16	0.25	16	0.00
17	0.00	17	0.58	17	0.10	17	0.05	17	0.20
18	0.00	18	0.01	18	0.17	18	0.00	18	0.00
19	0.00	19	0.20	19	0.00	19	0.00	19	0.00
20	0.71	20	0.26	20	0.00	20	0.00	20	0.00
21	0.2	21	0.42	21	0.02	21	0.00	21	0.00
22	0.00	22	0.12	22	0.00	22	0.00	22	0.06
23	0.00	23	0.05	23	0.00	23	0.00	23	0.00
24	0.26	24	0.00	24	0.00	24	0.46	24	0.00
25	0.09	25	0.00	25	0.45	25	0.40	25	0.06
26	0.63	26	0.01	26	0.01	26	0.24	26	0.48
27	0.67	27	0.01	27	0.00	27	0.36	27	0.00
28	0.00	28	0.01	28	0.00	28	0.08	28	0.14
29	0.00	29	0.01	29	0.01			29	0.56
30	0.00	30	0.19	30	0.07			30	0.97
		31	0.02	31	0.24			31	0.16

0.10 inches of rainfall or more in 24 hours.

0.01 - 0.09 inches of rainfall in 24 hours.

Monitoring events.

Appendix C. Periphyton Information

Table C-1. Classification of ecological indicator values from Van Dam et al. (1994).

(R) pH				
1	acidobiontic	optimal occurrence at pH < 5.5		
2	acidophilous	mainly occurring at pH < 7		
3	circumneutral	mainly occurring at pH about 7		
4	alkaliphilous	mainly occurring at pH > 7		
5	alkalibiontic	exclusively occurring at pH > 7		
6	indifferent	no apparent optimum		
(H) Salinity				
		Chloride (mg/l)	*Salinity*	
1	fresh	< 100	< 0.2	
2	fresh-brackish	< 500	< 0.9	
3	brackish-fresh	500 - 1000	0.9 - 1.8	
4	brackish	1000 - 5000	1.8 - 9.0	
(N) Nitrogen uptake metabolism				
1	nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen			
2	nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen			
3	facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen			
4	obligately nitrogen-heterotrophic taxa, needing continuously elevated concentrations of organically bound nitrogen			
(O) Oxygen metabolism				
1	continuously high (about 100% saturation)			
2	fairly high (above 75% saturation)			
3	moderate (above 50% saturation)			
4	low (above 30% saturation)			
5	very low (about 10% saturation)			
(S) Saprobity				
		Water quality class	*Oxygen saturation (%)*	*BOD (mg/l)*
1	oligosaprobous	I, I-II	> 85	< 2
2	beta-mesosaprobous	II	70 - 85	2 - 4
3	alpha-mesosaprobous	III	25 - 70	4 - 13
4	alpha-meso-/polysaprobous	III-IV	10 - 25	13 - 22
5	polysaprobous	IV	< 10	> 22

(T) Trophic state	
1	oligotrophic
2	oligo-mesotrophic
3	mesotrophic
4	meso-eutrophic
5	eutrophic
6	hypereutrophic
7	oligo- to eutrophic (hypereutrophic)
(M) Moisture	
1	never, or only very rarely, occurring outside waterbodies
2	mainly occurring in waterbodies, sometimes on wet places
3	mainly occurring in waterbodies, also rather regularly on wet and moist places
4	mainly occurring on wet and moist or temporarily dry places
5	nearly exclusively occurring outside waterbodies

Table C-2. North Creek 1 Diatom Species List and Species Preference Values.

North Creek 1 (Upstream /Control)			Species preference values[1]						
Species	Count	PRA	R	H	N	O	S	T	M
Achnanthes[2]	6	0.010	3	2	1	1	1	3	3
Achnanthes rupestoides	4	0.007	4	2	1	1	1	1	4
Achnanthes subhudsonis[2]	8	0.013	3	2	1	1	1	3	3
Achnanthidium minutissimum[2]	10	0.017	3	2	1	1	1	3	3
Aulacoseira crenulata	20	0.033	3	1	1	1	1	1	2
Encyonema perminutum	2	0.003	-	-	-	-	-	-	-
Eolimna minima	12	0.020	-	-	-	-	-	-	-
Eunotia minor	34	0.057	2	1	-	-	1	-	4
Eunotia muscicola v. tridentula[2]	122	0.203	2	1	1	1	1	2	3
Eunotia soleirolii	10	0.017	3	1	2	1	2	1	3
Fragilaria neoproducta	20	0.033	-	1	1	1	1	-	-
Fragilaria virescens	176	0.293	2	1	1	1	1	2	3
Frustulia vulgaris[2]	6	0.010	2	1	1	1	1	2	3
Gomphonema micropus	12	0.020	4	2	2	2	2	5	3
Gomphonema parvulius[2]	6	0.010	3	2	1	1	2	3	3
Gomphonema parvulum	12	0.020	3	2	3	4	4	5	3
Meridion circulare	18	0.030	4	2	2	2	2	-	1
Meridion circulare v. constrictum	8	0.013	4	2	2	2	2	-	2
Nitzschia[2]	4	0.007	-	-	-	-	-	-	-
Nitzschia lacunarum[2]	24	0.040	4	3	2	2	2	5	
Nitzschia palea	4	0.007	3	2	4	4	5	6	3
Pinnularia acuminata[2]	18	0.030	2	1	1	2	1	2	3
Pinnularia kuetzingii[2]	2	0.003	2	1	1	2	1	2	3
Pinnularia microstauron	10	0.017	3	2	2	3	2	-	3
Pinnularia subcapitata	8	0.013	2	2	2	3	2	2	3
Planothidium	2	0.003	-	-	-	-	-	-	-
Planothidium lanceolatum	2	0.003	-	-	-	-	-	-	-
Psammothidium subatomoides	12	0.020	-	-	-	-	-	-	-
Stauroneis	4	0.007	3	2	2	2	2	4	3
Stauroneis kriegeri[2]	14	0.023	3	2	2	2	2	4	3
Surirella angusta	6	0.010	4	2	2	2	2	5	3
Synedra ulna	6	0.007	-	2	-	-	-	-	-
Weighted average			2	1	1	1	1	2	3

[1]Refer to Appendix C-3, Classification of ecological indicator values (Van Dam et al., 1994).
[2] Average for the entire genus from Van Dam et al.'s master list (Fore, 2010; Lane, 2007; Van Dam et al., 1994).
-No information.
PRA = percent relative area.
R = pH; H = salinity; N = nitrogen uptake metabolism; O = oxygen metabolism; S = saprobity;
T = trophic state; M = moisture.

Table C-3. North Creek 2 Diatom Species List and Species Preference Values.

North Creek 2 (Downstream/Impacted)			Species preference values[1]						
Species	Count	PRA	R	H	N	O	S	T	M
Aulacoseira crenulata	1	0.002	3	1	1	1	1	1	2
Cymbella naviculiformis	1	0.00	3	2	2	2	2	5	2
Eolimna minima	6	0.01	-	-	-	-	-	-	-
Eucocconeis depressa	3	0.01	-	-	-	-	-	-	-
Eunotia bilunaris	3	0.01	-	2	2	2	2	-	3
Eunotia bilunaris v. mucophila	31	0.05	2	2	2	2	1	2	4
Eunotia minor	35	0.06	2	1	-	-	1	-	4
Eunotia muscicola v. tridentula	169	0.28	2	1	1	1	1	2	3
Eunotia paludosa	3	0.01	1	1	1	1	1	1	4
Fragilaria capucina	3	0.01	3	2	-	-	2	3	-
Fragilaria virescens	251	0.42	3	1	1	1	1	2	3
Gomphonema clavatum	6	0.01	3	1	1	1	1	4	2
Gomphonema parvulum	7	0.01	3	2	3	4	4	5	3
Meridion circulare v. constrictum	13	0.02	4	2	2	2	2	-	1
Navicula	1	0.00	-	-	-	-	-	-	-
Navicula gerloffii	1	0.00	2	-	-	-	-	-	-
Pinnularia	9	0.02	-	-	-	-	-	-	-
Pinnularia divergens	4	0.01	3	1	-	-	1	1	3
Pinnularia kuetzingii	3	0.01	2	1	1	2	1	2	3
Pinnularia microstauron	6	0.01	3	2	2	3	2	-	3
Pinnularia nodosa	15	0.03	2	1	1	1	1	1	3
Pinnularia sinistra	13	0.02	2	1	1	2	1	2	3
Pinnularia subcapitata	9	0.02	2	2	2	3	2	2	3
Psammothidium daonense	3	0.01	-	-	-	-	-	-	-
Stauroneis kriegeri	4	0.01	3	2	2	2	2	4	3
Weighted average			2	1	1	1	1	2	3

[1]Refer to Appendix C-3, Classification of ecological indicator values (Van Dam et al. 1994)
[2] Average for the entire genus from Van Dam et al.'s master list (Fore, 2010; Lane, 2007; Van Dam et al., 1994)
-No information
PRA = percent relative area.
R = pH; H = salinity; N = nitrogen uptake metabolism; O = oxygen metabolism; S = saprobity;
T = trophic state; M = moisture.

Appendix D. Data Quality

Table D-1. Matrix Spike Recoveries (%).

Sample Date:	12/22/2009			3/4/2010		
Parameter	MS	MSD	RPD	MS	MSD	RPD
Hardness (mg/L)	97	96	1%	98	97	1%
DOC (mg/L)	na	na	nc	102	na	nc
Metals (ug/L):						
Lead - TR	103	102	1%	75	108	3%
Lead - dissolved	102	103	1%	100	76	2%
Copper - TR	107	105	1%	96	104	5%
Copper - dissolved	96	96	1%	102	101	1%

RPD = relative percent difference reported by Manchester Laboratory as the difference between the concentrations in the matrix spike samples.
MS = matrix spike
MSD = matrix spike duplicate
na = not analyzed
nc = not calculated
TR = total recoverable

Table D-2. Precision of Laboratory Duplicate Samples.

Sample Date:	12/22/2009			3/4/2010		
Parameter	Result	Duplicate	RPD	Result	Duplicate	RPD
TSS (mg/L)	2 U	3 U	nc	15	16	6%
Hardness (mg/L)	na	na	nc	na	na	nc
DOC (mg/L)	na	na	nc	26.0	26.4	2%
Metals (ug/L):						
Lead - TR	na	na	nc	7.87	8.02	2%
Lead - dissolved	na	na	nc	0.164	0.148	10%
Copper - TR	na	na	nc	5.95	6.09	2%
Copper - dissolved	na	na	nc	0.60	0.574	4%

na = not analyzed
nc = not calculated
RPD = relative percent difference
TR = total recoverable
U = not detected

Table D-3. Precision of Field Replicate Samples.

Sample Date:	12/22/2009			3/4/2010		
Sample No:	0912033-01	0912033-02		1003026-02	1003026-03	
Parameter	Result	Result	RPD	Result	Result	RPD
TSS (mg/L)	1 U	3 U	nc	1 U	1 U	nc
Hardness (mg/L)	15.9	15.7	1%	11.3	11.4	1%
DOC (mg/L)	na	na	nc	18.8	19.0	1%
Metals (ug/L):						
Lead - TR	1.06	1.05	1%	226	229	1%
Lead - dissolved	0.997	0.973	2%	236	230	3%
Copper - TR	3.15	3.35	6%	2.10	2.10	0%
Copper - dissolved	2.95	2.95	0%	2.11	2.31	9%

na = not analyzed
nc = not calculated
RPD = relative percent difference
TR = total recoverable
U = not detected

Appendix E. Bioassay Results

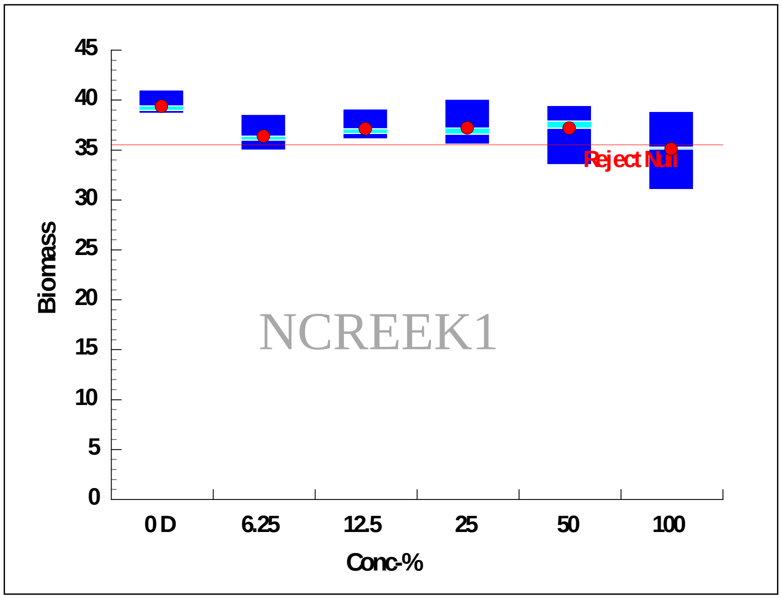

Figure E-1. Trout 7-day Survival and Growth Test Results from North Creek 1 (Upstream Site) for Samples Collected on December 22, 2009.

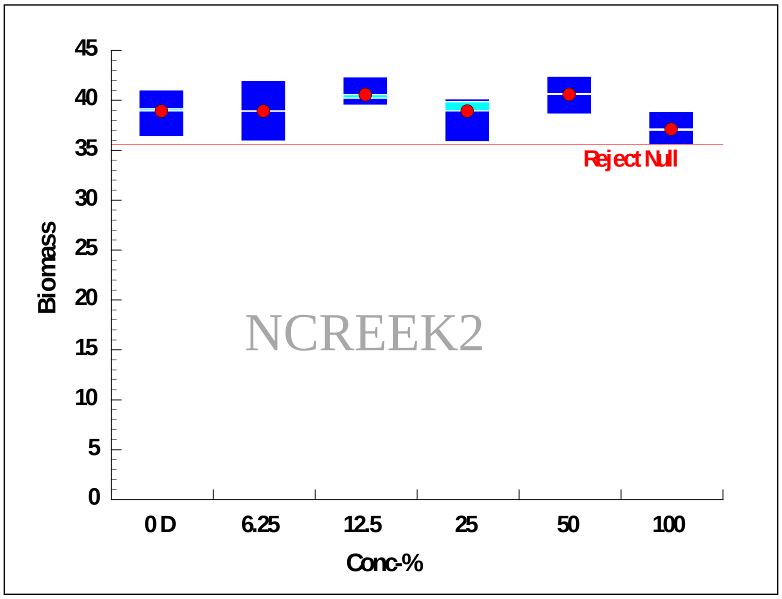

Figure E-2. Trout 7-day Survival and Growth Test Results from North Creek 2 (Downstream Site) for Samples Collected on December 22, 2009.

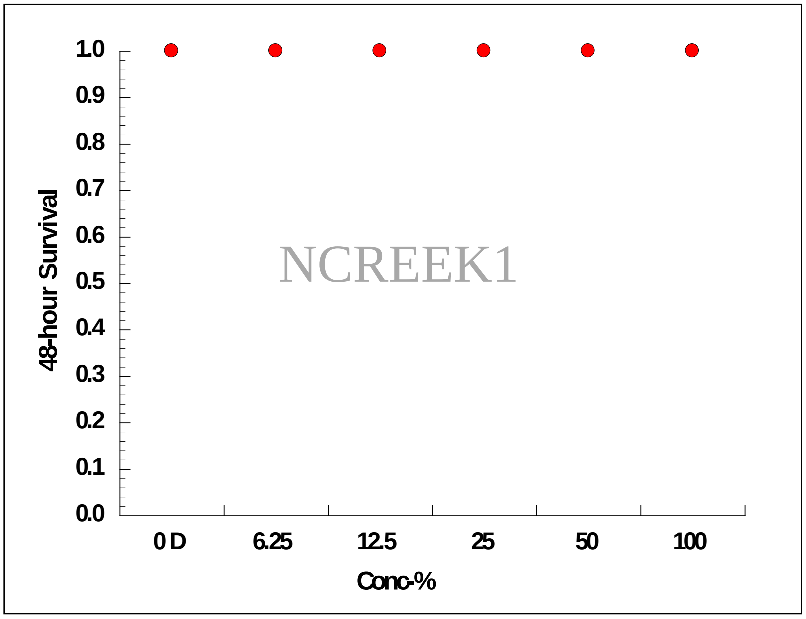

Figure E-3. Daphnid 48-hour Survival Test Results from North Creek 1 (Upstream Site) for Samples Collected on December 22, 2009.

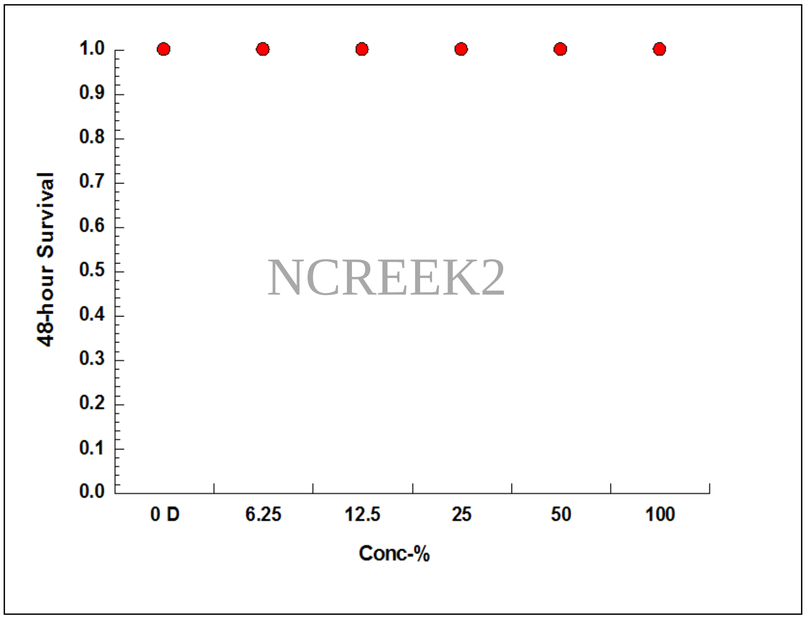

Figure E-4. Daphnid 48-hour Survival Test Results from North Creek 2 (Downstream Site) for Samples Collected on December 22, 2009.

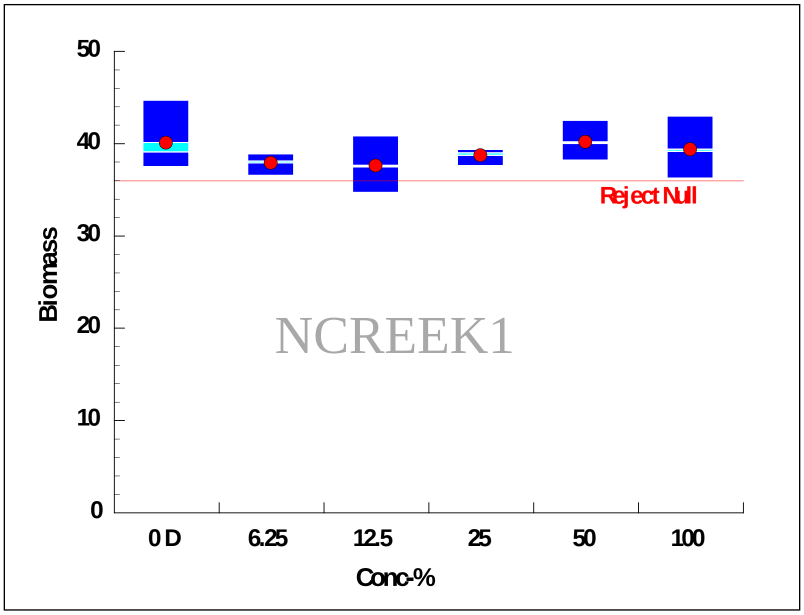

Figure E-5. Trout 7-day Survival and Growth Test Results from North Creek 1 (Upstream Site) for Samples Collected on March 4, 2010.

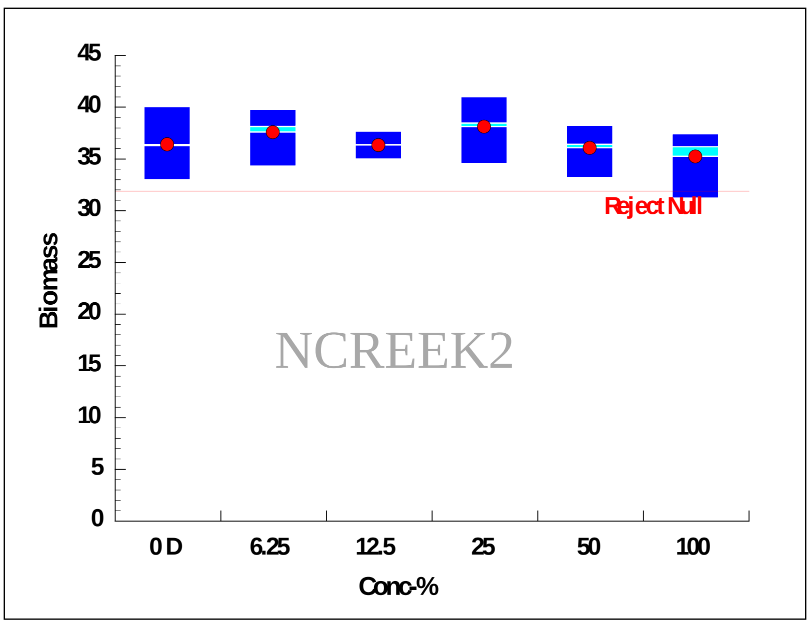

Figure E-6. Trout 7-day Survival and Growth Test Results from North Creek 2 (Downstream Site) for Samples Collected on March 4, 2010.

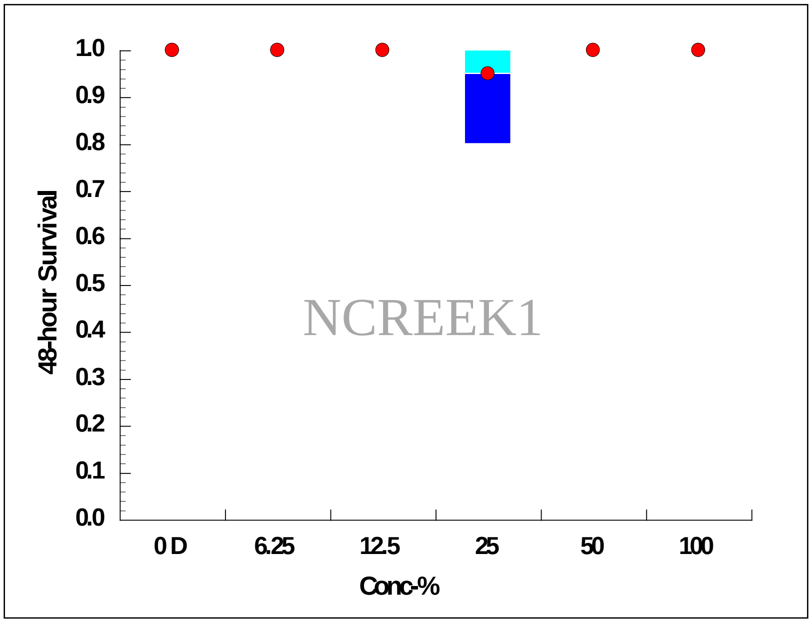

Figure E-7. Daphnid 48-hour Survival Test Results from North Creek 1 (Upstream Site) for Samples Collected on March 4, 2010.

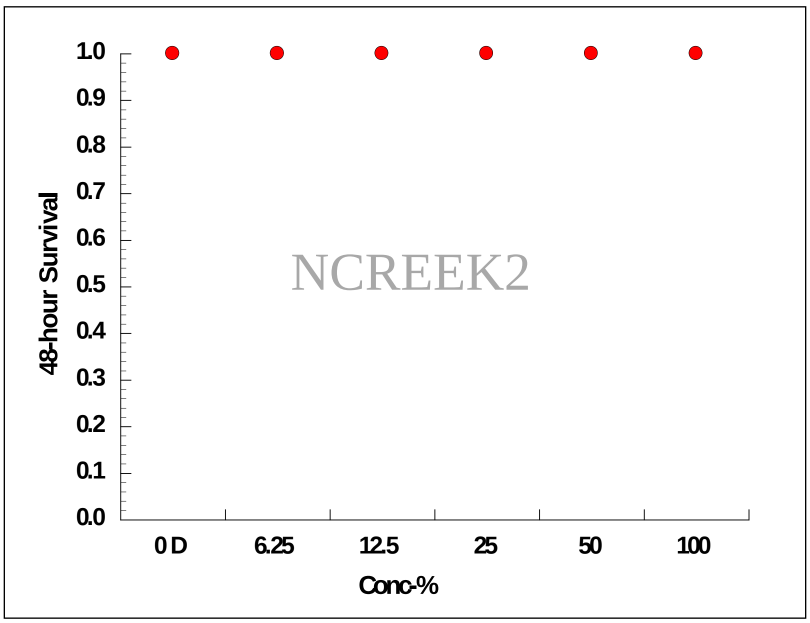

Figure E-8. Daphnid 48-hour Survival Test Results from North Creek 2 (Downstream Site) for Samples Collected on March 4, 2010.

Appendix F. EPA EcoTox Study References

Organism	Endpoint	Effect	Days	Toxicity Measured?	type	Lead (µg/L)	Hardness (mg/L)	DOC (mg/L)	Author	Title	Source	Year
rainbow trout (egg)	LC50	mortality	69	yes	dissolved	55	19.7	≤ 2	Mebane, C.A., D.P. Hennessy, and F.S. Dillon	Developing Acute-to-Chronic Toxicity Ratios for Lead, Cadmium, and Zinc Using Rainbow Trout, a Mayfly, and a Midge	Water Air Soil Pollut. :21p. (DOI - 10.1007/s11270-007-9524-8)	2007
	MATC	growth				36 - 88						
daphnid (neonate)	MATC	mortality	1	yes	total	150	20	--	Jop, K.M., A.M. Askew, and R.B. Foster	Development of a Water-Effect Ratio for Copper, Cadmium, and Lead for the Great Works River in Maine Using Ceriodaphnia dubia and Salvelinus fontinalis	Bull. Environ. Contam. Toxicol. 54(1):29-35	1995
mayfly (larval)	MATC	molt	10	yes	dissolved	130	20.7	≤ 2	Mebane, C.A., D.P. Hennessy, and F.S. Dillon	Developing Acute-to-Chronic Toxicity Ratios for Lead, Cadmium, and Zinc Using Rainbow Trout, a Mayfly, and a Midge	Water Air Soil Pollut. :21p. (DOI - 10.1007/s11270-007-9524-8)	2007
rainbow trout (egg)	LC50	mortality	62			120	29.4					
	MATC	growth				12 - 57	29.4					
rainbow trout	LC50	mortality	14	no	total	140	30	--	Goettl, J.P.	Water Pollution Studies	Fed. Aid Proj. No.F-33-R-6, Colorado Game, Fish and Parks Div., Div. Wildl., Ft. Collins, CO :130 p.	1971
daphnid (neonate)	EC50	reproduction	21	no	total	100	45.3	--	Biesinger, K.E., and G.M. Christensen	Effects of Various Metals on Survival, Growth, Reproduction and Metabolism of Daphnia magna	J.Fish Res.Board Can. 29(12):1691-1700	1972
daphnid (neonate)*	LC50	mortality	2	no	total	29.4	80	5.7	Diamond, J.M., D.E. Koplish, J. McMahon III, and R. Rost	Evaluation of the Water-Effect Ratio Procedure for Metals in a Riverine System	Environ. Toxicol. Chem. 16(3):509-520	1997
fathead minnow (larval)*						2913	--	28				

Organism	Endpoint	Effect	Days	Toxicity Measured?	type	Lead (µg/L)	Hardness (mg/L)	DOC (mg/L)	Author	Title	Source	Year
mayfly (nymph)	LC50	mortality	4	yes	total	1090	--	21.6	Gerhardt, A.	Short Term Toxicity of Iron (Fe) and Lead (Pb) to the Mayfly Leptophlebia marginata (L.) (Insecta) in Relation to Freshwater Acidification	Hydrobiologia 284(2):157-168	1994

*nitric acid lead salt added during test
diss = dissolved concentration
LC50 = lethal concentration 50%
EC50 = effective concentration 50%
MATC = maximum acceptable toxicant concentration