Development and Implementation of Integrative Bioassessment Techniques to Delineate Small Order Acid Mine Drainage Impacted Streams of the North Fork Powell River, Southwestern Virginia.

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(Abstract)

Acid mine drainage (AMD) results from the oxidation of pyretic mineralogy, exposed by mining operations to oxygen and water. This reaction produces sulfuric acid and liberates heavy metals from the surrounding mineralogy and impairs water quality and freshwater communities. The U.S. Army Corps of Engineers has begun an ecosystem restoration project to remediate the abandoned mine land (AML) impacts to the North Fork Powell River (NFP) and is utilizing the ecotoxicological rating (ETR) system to delineate these affects to focus restoration efforts. The ETR was developed to summarize the integrative data into a single number ranging from 0 to 100, which is descriptive of the environmental integrity of a sampling station. The ETR is conceptualized to work as an academic grading scale (0 through 100), rating reference stations with A's (90-100) and B's (80-89) and impacted stations with C's (70-80), D's (60-70) and failures (F ≤ 60). Two rounds of ETR investigations have evaluated seven headwater tributaries to the NFP including investigations of Ely and Puckett's Creek from 1997 and 1998. This thesis contains the results of the second series of ETR investigations at 41 stations in Cox Creek, Jone's Creek, Reed's Creek, Summers Fork, Straight Creek, and areas in the NFP. Eight stations were recommended for reclamation; CC 03, JCRF2 02, JCRF2 01, RCPS 09B, RCPS 11B, SULF 01, SU 02, and SU 01. Summers Fork was the most severely impacted watershed of the second round of ETR investigations. An effort to streamline the ETR to the most ecologically predictive parameters was successful in creating a system more time and cost efficient then the initial ETRs and exclusive of benthic macroinvertebrate surveys. The Modified ETR streamlined the ETR to just 5 parameters including; mean conductivity, mean Asian clam survival, mean aluminum (Al) and manganese (Mn) in the water column, and mean habitat score to describe the AMD impacts to small headwater streams. Also, an investigation was conducted to determine the mode of toxicity, (i.e., exposures to metal contaminated surface waters or sediments) by which Al and iron (Fe) dominated AMD impairs benthic macroinvertebrate communities. It was found that water column exposures both within and beyond the zone of pH depression are the most likely mode by which AMD impairs the benthic macroinvertebrate communities of the NFP.

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Preface

Acid mine drainage (AMD) is created from pyretic materials (FeS_2) which have been oxidized upon exposure to water and oxygen, a process often brought about through mining activities. The resultant reaction produces sulfuric acid, ferric hydroxides, and mobilizes other trace metals, depending on the surrounding mineralogy. These toxic acids and metals flow to surface waters, precipitate, and coat streambeds with metal oxides destroying habitat and adversely affecting water quality in over 13,000 miles of U.S. rivers [1]. In fact, AMD has been singled out as the primary cause of water quality impairment in the Appalachian Region [2].

The Cumberland Plateau of southwestern Virginia and the Tennessee River drainage once supported over 73 native unionid mussels [3,4]. The Powell River alone supported 42 different species [5], but after a century of mining impacts, a 33 % decline in biodiversity has occurred. Currently, only 28 surviving species remain in the river, 6 are listed as federally endangered, and all mussel fauna in the upper Powell River have been eliminated above Dryden, Virginia, at ~mile 165 [6,7]. Wolcott and Neves et al attributed this decline in mussel populations in the Powell River to coal waste and metal loading associated with active mining and/or abandoned mined lands (AML) [6,7].

In 1994, the U.S. Army Corps of Engineers, the Virginia Department of Mines, Minerals, and Energy, Division of Mined Land Reclamation (VA DMLR), and Virginia Tech began the ecosystem restoration to remediate the AMD impacts to the North Fork Powell River [8]. As part of this project an integrative bioassessment technique known as the Ecotoxicological rating (ETR), was developed and modeled after the Sediment-Quality Triad [9,10]. The Sediment-Quality Triad uses bulk sediment chemistry, sediment bioassays, and in situ studies to derive three separate values grading the environmental state of the station [11]. In contrast, the ETR results in a single value, summarizing several parameters: water and sediment chemistry, benthic macroinvertebrate indices, laboratory acute water column survivorship and sediment chronic toxicity test impairment of standard test organisms, and in situ toxicity testing with the Asian clam, *Corbicula fluminea*. Multiple Linear Regression Analysis (MLRA) determined a subset of 10 parameters that best describe the macroinvertebrate indices. A single number, summarizing this subset, was produced with a rating scale from 0-100, 0 being the most environmentally impacted and 100 as the most pristine. This rating allows officials, responsible for allocating ecological remediation funds, to prioritize spending relative to the environmental condition of a particular tributary or watershed.

Initially, Ely and Puckett's Creeks were evaluated with the ETR. Both of these creeks were found to have acute water column toxicity, chronic sediment toxicity and a paucity of benthic macroinvertebrate assemblages at most of the sampling stations studied [9, 10]. Ten of the 20 sampling stations in Ely Creek had significantly reduced survival of test organisms in sediment toxicity tests and in Puckett's Creek; one station had a 48-hour LC50 value of <2.5 % effluent. The AMD impacts from these two tributaries were found to significantly impact the receiving systems of Stone/Straight Creek and even in the NFP [12]. In June of 2000, the ETR evaluation of the NFP was extended to include five more tributaries.

This thesis evaluated Cox Creek, Ely Creek, Jone's Creek, Puckett's Creek, Reed's Creek, Straight Creek, Summers Fork, and associated area in the NFP utilizing integrative bioassessment techniques (Figure 1). The first chapter is the extended ETR evaluation of 41 sampling stations in Cox Creek, Jone's Creek, Reed's Creek, Straight Creek, Summers Fork, and the associated areas in the NFP, recommending specific areas for reclamation as part of the ecosystem restoration by the U.S. Army Corps of Engineers. Chapter two is an attempt to streamline the ETR process by reducing the number of techniques necessary to complete an ETR evaluation of the AMD impacts to the NFP by utilizing both historical data in Ely and Puckett's Creeks as well as new data from Reed's Creek. Chapter three also investigates these three tributaries in an attempt to determine the mode of toxicity by which AMD impairs benthic communities in small order streams both within and beyond the zone of pH depression.

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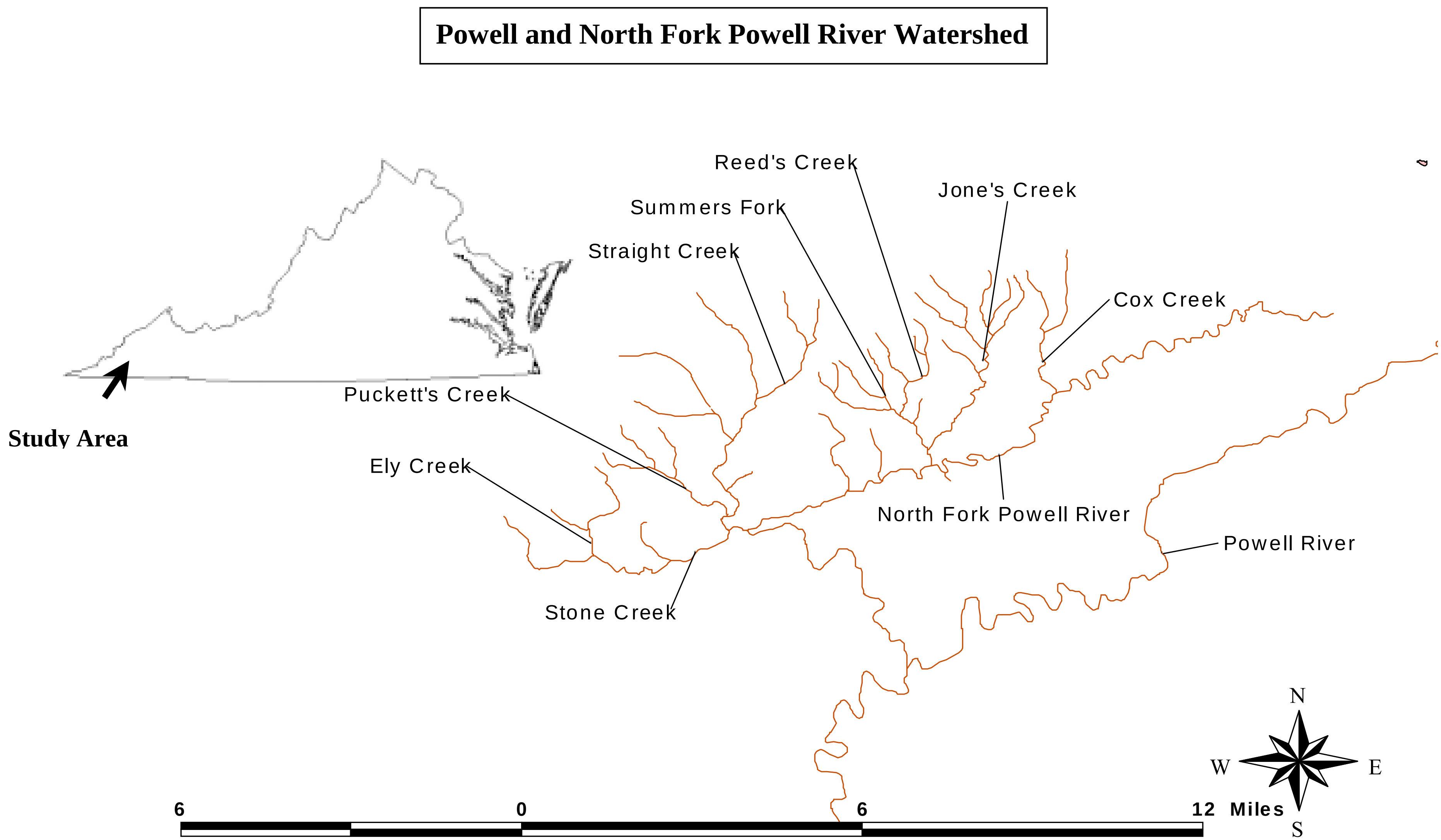

Figure 1. Map of the Sub-watersheds of the North Fork Powell River investigated in these studies.

Chapter 1.

Delineating Acid Mine Drainage Impacted Tributaries of the North Fork Powell River.
Revision of Technical Report Submitted to David Millar and Associates

Executive Summary - A comprehensive study utilizing physical, chemical, ecological and toxicological testing procedures was carried out in five sub-basins (Cox Creek, Jones Creek, Reeds Creek, Summers Fork, Straight Creek, and parts of the North Fork Powell River [NFPR]) watershed to evaluate the ecotoxicological rating of 41 sampling stations over a two-year period. The purpose of this study was to evaluate the input of abandoned mined land (AML) and acid mine drainage (AMD) impacts in these sub-basins and in several sites in the North Fork Powell River in order to develop an ecotoxicological restoration plan of the AML/AMD sources for the US Army Corps of Engineers. A similar study plan has been in place for Ely Creek and Puckett's Creek, two sub-basins that were studied for restoration purposes two to three years earlier in the lower portion of the NFPR watershed.

The ecotoxicological restoration potential was processed around an ecotoxicological rating (ETR) of each sampling site to determine the degree of impairment from AML/AMD input. The ETR contained 10 parameters, two of which were benthic macroinvertebrate parameters (taxa richness, percent Ephemeroptera) and a third was pH. These three parameters carried the highest weight because of their ability to more clearly indicate environmentally stressed conditions. For the middle-weight ETR weighted scale, three toxicity tests were used, an acute water column test with *Ceriodaphnia dubia*, a 10-day *Daphnia magna* sediment chronic testing, and an *in situ* clam survival test extended for 35 days, plus a fourth parameter of habitat assessment. Three parameters, conductivity, aluminum and iron concentrations in the water column carried the least weight. The total weighting of the 10 parameters amounted to 100 points, and ETRs of "A, B, C, D and F" were developed relative to an academic score or grade on a high school or college test. For example: "A" grades were 90-100, "B" from 80-89, "C" from 70-79, "D" were 60-69, while "F" were ≤59. The dismal "D" and failing "F" ETR grades for any sampling station required close evaluation for ecotoxicological restoration, especially all "F" graded sites.

The sub-basin with the lowest ETR scores (41.4, 44.6 and 55.2), which were "F" grades, was Summers Fork with three sites impacted by AML/AMD inputs. Summers Fork also had a downstream site with a "D" grade of 68.5. Reeds Creek appeared to rank second in overall low ETR scores with two "F" (52.8 and 58.1) and two "D" (64.3 and 65.4) grades. Cox Creek was rated third in environmental impact with an F at one AMD site (score of 55.9) and three others with "D" grades (64.5-64.9) due to agricultural and active mining inputs. Jones Creek was ranked fourth in the sub-basins from one site with AMD input (ETR=64.7), and the up-stream site was not much better at 66.7. Straight Creek was ranked least with no ETRs having F grades but three had "D's" from 67.2-69.8. It appeared that urban influence more adversely impacted Straight Creek than did AMD.

There were seven sites in four of the five sub-basins that were recommended for restoration of AML/AMD inputs. They included one in Cox Creek (CC 03 with an ETR=55.9) and two in Jones Creek (JCRF2 01 [ETR=66.7] and JCRF2 02 [ETR=64.2]). Straight Creek had no sites recommended for AML/AMD restoration. Two other sub-basins with two sites each recommended for AML/AMD restoration were Reeds Creek (RCPS 09B [ETR=52.8] and RCPS 11B [ETR=58.1]) and Summers Fork [ETR=55.2], SULF 01 [ETR=41.4] and SU 02). Although Summers Fork had three sites with failing ETRs of 41.4-55.2, repairing the upper two sites on two separate forks (SULF 01 and SU 02) because they converged into the downtown mainstem site (SU 01A with an ETR-44.6), would allow the down-stream mainstem site to recover by natural ecological processes.

Keywords— Ecotoxicological rating, integrative bioassessment, acid mine drainage.

Introduction

The upper Tennessee River system, including the Clinch, Holston and Powell Rivers in southwestern Virginia, contains aquatic habitat richer in unionid mussel species than any other area of North America. Thirty-four endangered freshwater mussel species reside in the tributaries of the upper Tennessee River drainage [1]. The lower Powell River system provides habitat for a number of mussel species including the endangered *Quadrula intermedia*, *Elliptio crassidens*, *Fusconaia cor*, and others [1-2]. However, all mussel fauna in the upper Powell River have been eliminated above Dryden, Virginia, at ~mile 165 [3]. Wolcott attributed the decline in mussel populations in the Powell to metal loading associated with acid mine drainage [4].

Acid mine drainage (AMD) occurs when pyrite (FeS_2) is oxidized upon exposure to water and oxygen through mining activities. The process produces sulfuric acid, ferric hydroxides, and mobilizes other trace metals, depending on the surrounding mineralogy. These toxic metals flow to surface waters and precipitate, coating streambeds with metal oxides, destroying habitat, and adversely affecting water quality in over 13,000 miles of U.S. rivers [5]. Recently, AMD was singled out by the US EPA as the primary water quality problem in the Appalachian region [6].

The Virginia Department of Mines, Minerals and Energy, Division of Mine Land Reclamation and the Army Corps of Engineers has begun to remediate the AMD impacts of the Powell River watershed. Reclaiming an entire watershed is a costly and time-consuming process requiring an approach capable of quantitatively evaluating segments/tributaries relative to a particular segments environmental condition. The resulting evaluation was to allow officials responsible for allocating ecological remediation funds to prioritize spending relative to the environmental condition of a sampling station or watershed.

The Ecotoxicological Rating (ETR) was modeled after the Sediment-Quality Triad (SQT) and uses integrative bioassessment to quantify environmental contamination.

such as AMD. The SQT uses bulk sediment chemistry, sediment bioassays, and *In situ* studies to derive three separate values grading the environmental state of each site studied [7]. Like the SQT, the ETR uses chemical, toxicological, and ecological data, but synthesizes these data into one number summarizing several parameters. The ETR is conceptualized to work as an academic grading scale (0 through 100), rating reference stations with A's (90-100) and B's (80-89) and impacted stations with C's (70-80), D's (60-70) and failures ($F \leq 60$). This system synthesizes data in such away that a diversity of scientists, natural resource managers, and engineers can easily and efficiently prioritize sampling stations for remediation.

Two sub-watersheds within the NFP River drainage, Ely and Puckett's Creek, have been assessed using the ETR approach, effectively synthesizing 6 to 10 parameters into ETR scores which differentiated between multiple levels of AMD impacts (i.e. acidic vs. circum-neutral AMD) [8-10]. These watersheds are rural, mostly forested drainages primarily impacted by AMD. The next phase of the Powell River restoration project included five AMD impacted sub-watersheds; Cox Creek, Reed's Creek, Jone's Creek, Straight Creek, and Summers Fork which were also impacted by agriculture, active mining, and urban development. The purpose of the present study was to conduct an ETR evaluation of 41 sampling stations in the aforementioned streams, to prioritize these stations for remediation from AMD impacts.

Materials and Methods

Sampling stations/sampling schedule

Water column and *In situ* toxicity as well as benthic macroinvertebrate sampling were conducted simultaneously in six systems; Cox Creek (6 stations), Jones Creek (7 stations), Reed's Creek (10 stations), Straight Creek (5 stations), Summer's Fork (6 stations), and at all sub-systems confluences with the North Fork (7 stations) of the Powell River. Sampling was conducted under both high and low flow conditions. The first round of sampling occurred May 2nd, 2000 during a spring high flow event in which we sampled 73 potential AMD seeps for water column toxicity. On a follow up trip, May 19th, 2000, only those sampling points found to be toxic on the previous trip and 41 newly established in-stream sampling stations were tested for water column toxicity. These 41 in-stream stations were again tested for water column acute toxicity on June 21st, December 3rd, 2000, and March 17th of the following year 2001. A 33-day *In situ* Asian clam (*Corbicula fluminea*) chronic toxicity test was started on May 19th, 2000 because water temperatures were above 15°C, which is the threshold for the predictability of clam growth and survival (McMahon et al., 1986). Benthic samples were collected on June 5, 2000. All stations in a given system were sampled for sediment toxicity on the same given day (November to January), except the NFP stations, which were sampled along with the local sub-system sampling.

Water column and sediment chemistry

Water column chemistry was measured both in the field and the laboratory. Field samples were stored at 4 °C and were analyzed within 24 hours of collection. The pH was measured using either a Markson Field® pH meter with combination electrode or an Accumet® (Fisher Scientific, Pittsburgh, PA,USA) pH meter equipped with an Accumet gel-filed combination electrode. Conductivity was measured with a Hach® (Hach, Loveland, CO,USA) conductivity/TDS meter. Titration was used to measure alkalinity and hardness as described in APHA 1992 [12]. Metals analyzed were total aluminum (Al), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Filtered water (0.45 μm pore size) samples were analyzed by inductively coupled plasma (ICP) spectrometry either by Spectrum Laboratories, Coeburn Virginia or at the ICP laboratory at Virginia Tech.

Sediments were digested in 50% (*v*/*v*) nitric, 20% (*v*/*v*) hydrochloric acid (Fisher Scientific, Pittsburgh, PA, USA.) with metals analysis following U.S. EPA protocol (1991) [13]. Total Al, Cu, Fe, Mn, and Zn were measured using ICP spectrometry. Sediments were collected from various points within each sampling station, placed in a zip lock bag, and stored at 4 °C. Each sample was homogenized and two replicate, one-gram samples were dried at 280 °C for 24 hours and weighed again to determine mean percent water content.

Benthic macroinvertebrate sampling

Benthic macroinvertebrate surveys followed U.S. EPA Rapid Bioassessment Protocols (RBP) [14]. Riffle, run, pool and shoreline rooted areas were sampled and replicated once, using dip nets of 800 μm mesh. Two composite samples were collected simultaneously for five-minute periods at each sampling station. Organisms were identified to lowest practical taxaomic level and community indices determined using the RBP tier III approach and standard taxaomic keys [15-16].

In situ clam toxicity testing

Asian clams (*C. fluminea*) used for testing were collected from the New River near Ripplemead, Virginia, using clam rakes. Clams were maintained in Living Streams® (Toledo, OH) at the Ecosystems Simulation Laboratory, Virginia Tech, Blacksburg, VA. until used for *In situ* toxicity testing. Five clams were given a distinctive mark from a file, measured for width, and placed into 18 cm by 36 cm bags with mesh ~0.5 cm^2. At each sampling station 5 bags were tied to a stake and left in the field for 33 days. After this period clams were collected and transported to Virginia Tech where mean survival and growth were determined for each bag and station. Clams were considered dead if found gapping or easily opened, or failed to close when the visceral mass was touched.

Water column toxicity testing

Acute 48-hour toxicity tests were preformed following U.S. EPA standard procedures [17]. *Ceriodaphnia dubia* test organisms were cultured at Virginia Tech, with mean survival being used for statistical analysis. Filtered culture/diluent water came from Sinking Creek, Newport, Virginia. Toxicity testing followed the Standard Operating Procedure as outlined in the Virginia Tech laboratories. Organisms were fed 18ml/30ml of a 1:1 (*v*/*v*) mixture containing *Selenastrum capricornutum* and Yeast-

Cereal-Trout Chow (YCT) prior to testing. Five organisms were place into one of two replicate 50 ml beakers containing site water. Tests were 48 hours long and temperature was maintained at 25 °C. Sinking Creek water was used as a control. Mean survival was determined for each of four test periods.

Sediment toxicity testing

Ten-day sediment toxicity tests were preformed using *Daphnia magna*. Sediment was collected in the field with a polyurethane dipper and maintained in plastic bags at 4 °C until testing, which was conducted within 14 days of sample collection. Testing followed the standard protocols as outlined in [12, 18-19], with little modification. Fifty-ml beakers were filled with 15ml of site sediment and overlaid with 35ml of Sinking Creek water and one *D. magna*. *Daphnia* were fed 10 ml *Selenastrum*/YCT daily. Temperature during the experiment was maintained at 25 °C, with the overlaying water changed daily. Each sampling station sediment sample was replicated eight times. Both water and sediment controls were run with each test and all met U.S. EPA and ASTM standards. Mean survival and reproduction, as percent of mean control, determined by averaging each sediment controls reproduction data, was determined for statistical analysis.

Habitat assessment

Habitat assessments were conducted at all stations using U.S. EPA RBPs (1999) [21]. Ten parameters were measured: (1) epifaunal substrate/ available cover, (2) embeddedness, (3) velocity/depth regime, (4) sediment deposition, (5) channel flow status, (6) channel alteration, (7) frequency of riffles (or bends), (8) bank stability, (9) vegetative protection, and (10) riparian vegetation zone width. Two scores for each station were made by independent observers with the mean score calculated for statistical purposes.

Ecotoxicological Rating (ETR) development

Parameter selection

In past studies parameter selection was based on Multiple Linear Regression Analysis and bivariate correlations of chemical, ecological, toxicological, and physical parameters, selecting those most sensitive to aquatic impairment resulting from different levels of AMD input into the watershed (i.e., acidic, intermittently acidic, and neutralized AMD). However, the paucity of sampling stations in Cox Creek, Jones, Reeds, Straight Creeks, Summers Fork and the North Fork Powell River limited the statistical development of ETR parameters for each of these individual sub-watersheds. As a result parameters were selected based on best professional judgment from past research in the North Fork Powell watershed, Ely, Puckett's, and Reed's Creek [8-10].

Specific parameters selected

Ecological- EPT richness and % Ephemeroptera abundance were found to be highly sensitive to various levels of AMD inputs (i.e., reference, intermittently acidic AMD, acidic AMD, neutralized AMD) in Ely and Puckett's Creek and are used throughout the literature as sensitive indicators of heavy metal contamination.

Toxicological-Three toxicological parameters were highly correlated with benthic macroinvertebrate community indices in Ely and Puckett's Creek and were included as ETR parameters. *Ceriodaphnia dubia* acute (48-hour) water column survival provided responses to water column toxicity; while *D. magna* sediment test survival represented short-term chronic responses to sediment toxicity at sites where water column toxicity may not be present but sediment toxicity was. *In situ* clam survival represented long-term (i.e., 33 days) responses to both media.

Chemical-The four chemical parameters that were selected as ETR parameters were water column iron and aluminum, conductivity and pH because they were found to be highly correlated with benthic macroinvertebrate community biodiversity in Ely and

Puckett's Creek and were negatively correlated with AMD seeps. As the four parameters increased (or decreased for pH), AMD toxicity increased and limited the benthic macroinvertebrate assemblages at those AMD influenced sampling stations.

Physical-One physical parameter was selected as an ETR parameter based on new research approaches taken with the Ely and Puckett's Creek data and new data collected in Reed's Creek. Habitat degradation, as a result of metals flocculation and sediment accumulation from mining and AML activities, was found to be the parameter with the highest mean correlation with four benthic community indices selected for their sensitivities to heavy metal pollution.

Rating of stations

Once ETR parameters were selected, each station was given a relative rating for each parameter (Table 1). For example, when total taxa richness was selected, the station with the highest value for taxa richness would receive a 1.0 for that parameter while all others received values equal to their proportion (0 to 1.0) of the highest value. This process was used for the ecological parameters.

For toxicological endpoints, either proportion surviving would be used, or proportion of the highest value in the case of the sediment test, daphnid reproduction (Table 1). Stations would be rated for chemical values such as conductivity based on 1 minus the percent of the highest value at any station. For pH, stations with values that were acutely toxic (i.e., <4.0 standard units (SU)) received a score of 0, and values of 4.0 to 4.5 SU, which may cause chronic if not acute toxicity, received a score of 0.25. Stations with pH values that may not be toxic themselves but may cause increased toxicity of metals present (4.5 to 6.0 SU) received scores of 0.5 (4.5 to 5.0 SU), 0.75 (5.0 to 6.0 SU) or 1.0 (>6 SU).

Table 1. Ecotoxicological Rating (ETR) procedure where parameters were selected based on best professional judgment.

Parameters	Station rating procedure (0 to 10 Points)											Weighting[A]
	0			2.5		5			7.5		10	
% Taxa richness (% of Ref.)	0			25		50			75		100	1.25
Ephem. Abund. (% of ref.)	0			25		50			75		100	1.25
pH	≤3.9			4.0-4.4		4.5-5.0			5.1-6.0		>6.0	1.25
% C. *dubia* survival	0			25		50			75		100	1.0
% D. *magna* survival	0			25		50			75		100	1.0
% In situ clam survival	0			25		50			75		100	1.0
Habitat score (% of Ref.)	0			50		100			150		200	1.0
Conductivity 1-(% of Ref.)	1187[B]			900		570			285		48[B]	0.75
Al in H_2O (mg/L)	>0.75	0.75-0.68	0.68-0.62	0.62-0.55	0.55-0.49	0.49-0.42	0.42-0.35	0.35-0.29	0.29-0.22	0.22-0.15	<0.15	0.75
Fe in H_2O (mg/L)	>1.0	1.0-0.9	0.9-0.8	0.8-0.7	0.7-0.6	0.6-0.5	0.5-0.4	0.4-0.3	0.3-0.2	0.2-0.1	<0.1	0.75

[A] Weightings of 1.25, 1.0 and 0.75 are multiplied by values of 0 to 10 points each with a perfect score for a pristine site = 100 points and those with ≤69 points were failing sites. [B] Example values taken from Cox Creek. Each sub-watershed was evaluated utilizing the highest value in that sub-watershed.

Finally, for water column metals parameters, stations would receive a value ranging from 0 to 1.0 based on the concentration found at a station relative to a scale ranging from the U.S. EPA ambient Water Quality Criterion (WQC) for that metal which received a 0, to concentrations found at the pristine Boggs Hollow reference site, which received a 1.0 (Table 1) [22]. Ten levels of concentrations were established between these values and were given scores increasing from the WQC to the Boggs Hollow levels at 0.1 point value per level. The ETR values of the six sampling stations scored from 97.4 at Boggs Hollow to 55.9 at station CC 03 (Table 2). The Boggs Hollow reference station had a combination of the most unique setting of undisturbed habitat, low water quality values, high survivorship of test organisms, and maximum benthic macroinvertebrate assemblages. Taxa richness of 23.5 was usually more than double that of the other stations which ranged from 8.5 to 12.0. Percent mayfly abundance was 25.8% at the reference station compared to 0 to 0.9% at the other stations. Station CC 03 had the lowest ETR score of 55.9 due to high Al and Fe concentrations in the water column, high conductivity (1,155 umhos/cm), lowest taxa richness and no mayflies, and the lowest Asian clam and D. magna survival (Table 2). The habitat assessment score was 58.5, much lower than the other stations.

Results

Cox Creek

Three stations had similar scores all depressed compared to Boggs Hollow, scoring as follows; 64.5 for CCLF 01, 64.5 for CC 04, and 64.9 for CC 02 (Table 2). These stations had high conductivities of 833, 1187, and 964 umhos/cm due to upstream active mining and agricultural practices, although pH was neutral to slightly alkaline. Total Fe in the water column was high, ranging from 0.36 to 0.73 mg/L compared to Boggs Hollow at 0.15 mg/L. Taxa richness at these three stations ranged from 10.0 to 12.5 with habitat scores of 71.5 to 90.0. The station (CC 01) furthest downstream in the watershed near the North Fork of the Powell River had an ETR of 75.4. This ETR indicated some recovery of water quality, benthic macroinvertebrates, and improved habitat, scoring 137.5, with no toxicity (100% survival) to *C. dubia*, Asian clam, and *D. magna*.

In summary for Cox Creek, there was one reference station in the lower part of the sub-watershed, which was Boggs Hollow (Table 2). There is no other reference tributary to rank any higher in the entire North Fork of the Powell River. The low score of the upper four stations indicates some influence of AMD whereby three stations (CCLF 01, CC 04, and CC 02) received a "D" or dismal score while station CC 03 was the only one that failed at 55.9. None of the four upper stations received AMD inputs resulting in acute or chronic toxicity to test organisms, as demonstrated by the high pH and high test organism percent survival. However, high water column metals and conductivity had some effect in the tributary. A more likely cause of these depressed scores is the active mining and agricultural activity in the upper portion of the sub-watershed and perhaps the active mining input in the headwaters of the two branches that form Cox Creek. Active mining, agricultural (i.e. cattle land), and urban (i.e. roads and houses) influences contribute to the sedimentation of the habitat at these stations, causing low habitat assessment scores, depressed taxa richness and percent mayfly benthic macroinvertebrate community indices because of little available habitat, resulting in dismal "D" ETR scores. However, station CC 03 just downstream from all the AMD seep inputs received a failing grade.

For these reasons, any potential AMD reclamation activities proposed for Cox Creek would mitigate water quality without greatly improving upon observed toxicity or streambed habitat, having little effect on the environmental integrity of these stations, excluding CC 03. AMD/AML are not the primary impacts to this sub-watershed making any reclamation efforts in the system as a whole to be focused on land use and habitat improvements. Reclamation to mitigate the mine drainage contributing to CC 03 would seemingly only improve this station from an "F" to a "D" rating.

Table 2. Cox Creek with ETR parameters and final scores and grades.												
Stations	*Aluminum, total (mg/l)*	*Iron, total (mg/l)*	*Conductivity uS*	*pH*	*Taxa richness*	*%Mayfly*	*C. dubia survival*	*Clam survival*	*D. magna survival*	*Habitat score*	*ETR scores*	*ETR grades*
CCLF1 01	0.15	0.55	833	7.84	12.5	0.0	100	88	87.5	71.5	64.5	D
CC 04	0.10	0.36	1187	7.81	10.0	0.5	95	96	93.8	73.5	64.5	D
CC 03	0.34	0.52	1155	7.86	8.5	0.0	100	80	75.0	58.5	55.9	F
CC 02	0.21	0.73	964	7.71	10.5	0.0	100	100	100.0	90.0	64.9	D
Boggs Hollow	0.15	0.15	48	6.61	23.5	25.8	100	92	100.0	140.0	97.4	A
CC 01	0.16	0.27	768	7.93	12.0	0.9	100	100	100.0	137.5	74.6	C

Jones Creek

In Jones Creek, the seven sampling stations scored from a high of 89.2 at the furthest station downstream (JC 01) to 64.2 at the uppermost station in the sub-watershed, located in the Robins Chapel drainage (JCRF2 02) (Table 3). Along with JCRF2 02, JCRF2 01 had a marginal ETR score of 66.7. Reasons for these dismal ETR scores were due to low percent mayfly, reduced *Ceriodaphnia* survival (75%), and low Asian clam and *Daphnia* survival of 24 and 50% at JCRF2 02. Station JCRF2 01 had a low ETR score due to high Fe (2.5 mg/L), 0 percent mayfly, and the lowest taxa richness of 11.5 in the sub-watershed. The next four sampling stations down the sub-watershed (JC 04A, JC 04B, JC 03, and JC 02) had "C" or average type ETR scores ranging from 72.8 to 76.8. The JC 01 station received a "B+" with an ETR score of 89.2 due to low Al and Fe concentrations in the water column, with highest taxa richness (22.0) and percent mayfly of 30.2%, and relatively high test organism survivorship from 100% for *Ceriodaphnia* to 75% for *Daphnia*. Habitat score was also the highest at JC 01 scoring 168.5.

Overall, Jones Creek had a range of the more mediocre ETR grades and scores in the five sub-watersheds (Table 3). The lowest (D) and highest (B+) ETR grades were found at the top (JCRF2 02) and bottom (JC 01) stations respectively, of the sub-watershed. The five other stations received ETR grades in the mid C's and mid D's with scores generally declining from JC 01 to JCRF2 02. Previous reclamation in the Robins Chapel area above JCRF2 01 may have improved water quality; however, high levels of Fe in the water column (2.5 mg/L) and associated flocculent suppressed the benthic macroinvertebrate community at this station (0 percent mayfly abundance).

Table 2. Jones Creek with ETR parameters and final scores and grades.

Stations	*Aluminum, total (mg/l)*	*Iron, total (mg/l)*	*Conductivity uS*	*pH*	*Taxa richness*	*%Mayfly*	*C. dubia survival*	*Clam survival*	*D. magna survival*	*Habitat score*	*ETR scores*	*ETR grades*
JCRF2 02	20	0.15	58	6.18	16	0.5	78	24	50	126.0	64.2	D
JCRF2 01	0.19	2.5	167	7.17	11.5	0.0	87.5	96	100	133.5	66.7	D
JC 04A (JC05)	0.28	0.34	523	7.67	17.5	5.5	96.5	92	100	142.0	72.8	C
JC 04B	0.16	0.46	453	7.70	17	7.3	100.0	92	75	178.0	76.4	C
JC 03	0.21	0.29	499	7.76	17.5	5.2	100.0	100	100	162.5	76.8	C
JC 02	0.23	0.40	429	7.88	18.5	8.7	100.0	100	100	119.5	75.9	C
JC 01	0.17	0.14	379	7.73	22.0	30.2	100.0	92	75	168.5	89.2	B+

Reeds Creek

In Reeds Creek, the ten sampling stations had ETR scores of 90.7 (RC 06) to a low of 52.8 (RCPS 09B) and 58.1 at RCPS 11B (Table 4). The "A" at RC 06 was due to the lowest concentration of Al (0.12 mg/L), highest taxon richness (18.0) and percent mayfly

(8.2), 100% survival of the three test organisms in toxicity tests, and high habitat score. For the two stations previously mentioned as having failing grades, Al (highest RCPS09B, 2.37 mg/L) and Fe (highest at RCPS 11B, 1.92 mg/L) concentrations were the highest. Both failing stations were characterized by AMD influences that flowed through natural wetlands. Taxa richness was the lowest at these stations relative to the rest and toxicity fell to 45.0 and 62.5 % survival in two of the three tests. Station RCPS 09B had the most acidic pH (5.51) in this sub-watershed.

Five stations (RC 14, RC 8A, RCPS 08, RCPS 02B, and RC 01) had ETR scores in the "C" range, varying from 70.5 (C – at RCPS 08) to 79.8 (C + at RC 01) (Table 4). There were two stations with D grades, RCPS 12 and RC 03. The Fe concentrations at these stations were somewhat high, ranging from 0.54 to 0.67 mg/L, and the benthic macroinvertebrate community was depressed by low taxa richness (ranging 10-12) and very low percent mayfly (0 to 1 percent). Although test organism survivorship was high (87.5 to 96.0 %) for three tests at RCPS 12, the habitat score of 98.0 was second lowest in the sub-watershed. The lowest habitat score was 75.5 at RCPS 11B which had a failing ETR grade of 58.1.

There were two stations (RCPS 09B and RCPS 11B) that had failing ETR grades and two others (RCPS 12 and RC 03) with ETR grades of D (Table 4). Sites RCPS 09B and RCPS 12 were the sampling stations immediately below the Mcpherson coal bond forfeiture and below the confluence with another tributary. It is recommended that remediation efforts be focused in this area. Drainage at RCPS 09B was acutely toxic to *Ceriodaphnia* (mean survival 45%) and flocculation of heavy metals from this drainage impacted the habitat at RCPS 12. Stations RC 08A and RCPS 08 received C grades and contributed to drainage below the Mcpherson mine.

Drainage from the Bee mine area (RCPS 11B) had both high Al and Fe concentrations combined with the lowest habitat assessment score resultant from urban development (Table 4). These adverse environmental impacts caused this station to fail.   Reclamation of the Bee mine area would greatly improve water quality; however, improvement of habitat availability would also be necessary to significantly enhance the environmental integrity of this area.

Table 4, Reeds Creek. ETR Parameters and final ratings.												
Stations	*Aluminum, total (mg/l)*	*Iron, total (mg/l)*	*Conductivity uS*	*pH*	*Taxa richness*	*%Mayfly*	*C. dubia survival*	*Clam survival*	*D. magna survival*	*Habitat score*	*ETR scores*	*ETR grades*
RC 14	0.12	0.11	237	7.16	12.0	0.0	100.0	96.0	75.0	132.5	74.2	C
RCPS 09B (RC12)	2.37	0.30	721	5.51	9.0	0.0	45.0	92.0	100.0	130.0	52.8	F
RC 8A (RC 11)	0.18	0.94	160	6.99	17.5	4.7	97.5	32.0	87.5	120.0	73.0	C
RCPS 08	0.19	0.99	209	6.66	13.5	0.0	100.0	96.0	87.5	139.0	70.5	C-
RCPS12 (RC10)	0.18	0.59	485	6.99	10.0	0.0	90.0	96.0	87.5	98.0	64.3	D
RC 06	0.12	0.46	301	6.80	18.0	8.2	100.0	100.0	100.0	130.0	90.7	A
RCPS 11B (RCLF 01)	0.31	1.92	296	6.57	6.5	2.5	96.5	88.0	62.5	75.5	58.1	F
RC03 (RC04)	0.18	0.67	323	6.75	12.0	1.0	100.0	68.0	62.5	120.0	65.4	D
RCPS 02B (RC03)	0.28	0.54	321	6.37	11.0	6.2	97.5	88.0	50.0	130.5	74.8	C
RC 01	0.23	0.17	246	7.12	17.5	4.9	97.5	96.0	37.5	130.5	79.8	C+

Although having a C score of 74.8, station RCPS 02B received mine drainage that caused the stream to occasionally become acidic (low pH of 4.10) and elevated stream Al concentration as high as 1.49 mg/L. This was the lowest pH and the third highest Al concentration measured in the Reeds Creek sub-watershed. Although the influences of the Mcpherson and Bee mine drainages are more constant, RCPS 02B does periodically receive toxic mine drainage, and the overall median pH was high and metals concentrations (Fe, Al) were intermediate (Table 4). It should be mentioned that there was an increase in sediment toxicity observed in Reeds Creek from RCPS 11B to RC 01 (Table 4). This sediment toxicity is not corroborated by increases of total sediment metals concentrations or of a significant increase of a single metal in the sediment. However, urban development increased significantly in this area, suggesting urbanization may be influencing sediment toxicity in this section of the sub-watershed.

Summers Fork

In Summers Fork, the six sampling stations had ETR scores ranging from 89.6 or B+ at SULF 03 to F at three stations, SU 02 (55.2), SULF 01 (41.4) and SU 01A (44.6) (Table 5). At SULF 03, the lowest Al (0.21 mg/L) and Fe (0.26 mg/L) concentrations were found. Taxa richness was high (22.0) there while percent mayfly (30.7) was highest among all stations. Test organism survivorship in the three tests ranged from 96.0 to 100% while habitat score was the second highest at 138.5.

The three stations with failing ETR scores of 41.4 (SUFL 01) 44.6 (SU 01A) and 55.2 (SU 02) had the lowest ETR scores in all five sub-watersheds (Table 5). At SULF 01, the worst failing station, the highest median Al concentration (1.74 mg/L) was measured, the lowest median pH (5.74), the third lowest percent mayfly abundance and lowest survivorship scores of 0% for Asian clams and 25.5% for *Ceriodaphnia*. The other failing station, SU 01A, influenced by drainage from SU 02 and SULF 01, had such a low rating due to the lowest taxa richness and percent mayfly abundance values (5.5 and 0%), low test organism survivorship (16.0 and 66.7%) for Asian clams and *Ceriodaphnia*, and the second lowest habitat score of 100.5. Site SU 02 failed due to the second highest median Fe concentration (2.80), the third highest median Al (0.53 mg/L) concentration, second lowest median pH (6.48), 0 percent mayfly abundance and the lowest habitat score (84.0) in the sub-watershed.

Overall, there were two stations directly influenced by AMD in each of the left (SULF 01) and right (SU 02) forks of Summers Fork (Table 5). These two AMD influenced forks merged contributing high concentrations of Al, Fe, and acidic pH’s to the next two downstream stations, SU 01A and MF 01. Reclamation of the areas associated with SU 02 and SULF 01 would greatly improve the environmental integrity of Summers Fork and the receiving streams of Meadow Fork and Reeds Creek.

Table 5. Summers Fork. ETR Parameters and final ratings.												
Stations	*Aluminum, total (mg/l)*	*Iron, total (mg/l)*	*Conductivity uS*	*pH*	*Taxa richness*	*%Mayfly*	*C. dubia survival*	*Clam survival*	*D. magna survival*	*Habitat score*	*ETR scores*	*ETR grades*
SU 04	0.22	0.36	171	6.89	26.5	23.3	100.0	96.0	100.0	110.0	84.8	B
SU 02	0.53	2.80	246	6.48	14.0	0.0	75.0	100.0	100.0	84.0	55.2	F
SULF 03 (SULF 02)	0.21	0.26	146	6.74	22.0	30.7	100.0	96.0	100.0	138.5	89.6	B+
SULF 01	1.74	0.49	260	5.74	15.0	2.1	25.0	0.0	87.5	129.0	41.4	F
SU 01A (SU 01)	0.58	0.76	243	7.06	5.5	0.0	66.7	16.0	100.0	100.5	44.6	F
MF 01	0.36	0.52	173	7.16	16.0	2.6	97.5	100.0	75.0	161.0	68.5	D

Straight Creek

In Straight Creek, ETR grades were mediocre, ranging from B (84.7) to three stations with C (72.0 – 75.3) and three having D grades with ETR scores of 67.2 - 69.8 (Table 6). The highest scoring station was SW 18 with a score of 84.7 or B. It had high taxa richness (16.5) and the highest percent mayfly abundance (21.0) and the highest habitat score (148) in the sub-watershed. The toxicity test response ranged from 50 to 100% survivorship for the three test species. Station SW 19, the lowest station located in Straight Creek, had a C grade due to the lowest habitat score in the sub-watershed, sub-marginal survivorship in two toxicity tests (62.5 – 80.0%), and fairly high to mediocre values in taxa richness (16.0) and percent mayfly abundance (12.7).

The three stations with D grades were SC 6B, SC 5A, and SC 5B with ETR scores ranging from 67.2 to 69.8 (Table 6). Station SC 5A scored low with high Al (1.67 mg/L) and Fe (3.11 mg/L) concentrations, mediocre percent mayfly abundance (11.8) and the second lowest habitat score (101.0) in the sub-watershed. Station SC 6B had moderate Al (0.33 mg/L) and Fe (0.59 mg/L) concentrations, high conductivity (742 *u*S), low percent mayfly abundance (3.2), and low *Daphnia* survivorship (37.5%).

For AML/AMD reclamation in Straight Creek, there are no particular stations that stand out above the rest other than the Lone Mountain blow out area at SC 6A and SC 6B, and the Slick Lizard area, which contributes minimal mine drainage into Straight Creek between sites SC 5A and SC 5B.

Table 6. Straight Creek with ETR parameters and final scores and grades.

Stations	*Aluminum, total (mg/l)*	*Iron, total (mg/l)*	*Conductivity uS*	*pH*	*Taxa richness*	*%Mayfly*	*C. dubia survival*	*Clam survival*	*D. magna survival*	*Habitat score*	*ETR scores*	*ETR grades*
SC 2	0.20	0.07	485	8.10	13.0	0.3	100.0	76.0	62.5	139.5	72.0	C
SC 6A (SC6C)	0.16	0.03	516	8.34	15.5	6.2	100.0	92.0	50.0	110.0	75.3	C
SC 6B (SC6)	0.33	0.59	742	8.13	17.0	3.2	97.5	92.0	37.5	133.5	67.2	D
SC 5A (SC6)	1.67	3.11	580	8.16	14.5	11.8	100.0	92.0	100.0	101.0	67.5	D
SC 5B (SC5)	2.04	4.00	600	8.05	18.0	18.0	100.0	100.0	37.5	127.5	69.8	D+
SW 18	0.28	0.68	312	7.33	16.5	21.0	97.5	100.0	50.0	148.0	84.7	B
SW 19	0.26	0.17	774	8.08	16.0	12.7	100.0	80.0	62.5	79.0	73.5	C

North Fork Powell River

In the North Fork Powell River stations, ETR grades ranged in the mid- B to C range (Table 7). The three stations with B grades had ETR scores from 86.5 to 87.3. The two other stations had solid C grades with ETR scores of 77.3 to 78.5. The median Al and Fe concentrations between these five stations were low, ranging from 0.14 to 0.25 mg/L (Al) and 0.12 to 0.24 mg/L (Fe). These median concentrations were an order of magnitude lower than high median Al and Fe found in some of the other sub-watershed sampling stations. Median specific conductance was low from 297 to 384 (*u*S), whereas values in some sub-watershed stations reached median highs of 774 (*u*S) in Straight Creek to 1,187 (*u*S) in Cox Creek. The pH was consistently above neutral (7.73 to 7.84), whereas it declined to 5.51 in Reeds Creek and 5.74 in Summers Fork.

Table 7. North Fork Powell River stations with ETR parameters and final scores and grades.

Stations	*Aluminum, Total (mg/l)*	*Iron, Total (mg/l)*	*Specific Conductance uS*	*pH*	*Taxa Richness*	*%Mayfly*	*C. dubia Survival*	*Clam Survival*	*D. magna survival*	*Habitat Score*	*ETR Scores*	*ETR Grade*
NFP 07 A	0.25	0.24	297	7.84	25.5	14.5	95.0	96	58.3	171.5	77.3	C
NFP 07 B	0.15	0.15	370	7.75	23.5	22.5	100.0	92	100.0	166.5	86.0	B
NFP 6A	0.14	0.12	298	7.79	24.0	27.5	100.0	92	62.5	158.5	87.3	B
NFP 05B	0.17	0.12	333	7.73	22.5	29.5	97.5	76	87.5	157.0	86.3	B
SW 02	0.22	0.23	384	7.83	22.5	22.0	97.5	80	75.0	152.0	78.5	C

Taxa richness was high, ranging from 22.5 to 25.5, while percent mayfly abundance was also evaluated from 14.5 to 29.5 (Table 7). Taxa richness values in some sub-watersheds were quite low, down to 5.5 in Summers Fork, 6.5 in Reeds Creek, and 8.5 in Cox Creek. The percent mayfly abundance values were high in the North Fork Powell River stations as well, ranging from 14.5 to 29.5% compared to low values of 0% in Cox Creek, Reeds Creek, Summers Fork, and 0.3% in Straight Creek. The *Daphnia* survivorship values varied the most, between stations (58.7 to 100%) and were least for *Ceriodaphnia* (95.0 to 100%). The habitat scores for all five stations were high (152.0 to 171.5) when compared to the low scores of 71.3 in Cox Creek, 75.5 in Reeds Creek, 84.0 in Summers Fork, and 79.0 in Straight Creek.

Although no reclamation should be considered in the North Fork Powell River, there appears to be some impact upon the river depressing the ETR score (77.3, or C) at the uppermost station above Cox Creek (NFP 7A) and below the North Fork Powell River confluence with Straight/Stone Creek (SW 02 where the ETR score was 78.8 or C). Previous studies have determined that urban impacts from the township of Saint Charles are contributing to the degraded environmental state of SW 02 as well as mine drainage from Ely, Puckett's, and Straight Creek. However, little work has investigated the inputs contributing to the highest median Al (0.25 mg/L) and Fe (0.24 mg/L) concentrations, the lowest measured percent mayfly abundance (14.5) and lowest percent survivorship of *Daphnia magna* (58.3%) found in the North Fork Powell River at NFP 7A.

Discussion/Recommendations

There were six sampling stations that had failing ETR scores that ranged from 41.4 to 55.2 in Summers Fork, 52.8 to 58.1 in Reeds Creek, and 55.9 in Cox Creek (Table 8). In addition, there were 11 stations with "D" ratings from 64.5 to 64.9 in Cox Creek, 64.3 to 65.4 in Reeds Creek, 64.7 to 66.7 in Jones Creek, 68.5 in Summers Fork, and 67.2 to 69.8 in Straight Creek. We assume that no station with a "C" or better ETR grade (≥70 ETR score) deserves reclamation activity, so the stations to be considered are those 17 stations with ETR scores less then 69.8, or ETR grades of "D" and "F".

In Cox Creek, Station CC 03 received drainage from AMD seeps that had been reclaimed but still impact the receiving system as evidenced by the ETR score of 55.9 (Table 8). The CC 03 site needs to be reclaimed again. This sub-watershed is capable of supporting ideal stream habitat as seen in the high ETR score (97.4) of one of its tributaries (Boggs Hollow).

In Jones Creek, no station had an "F" and the lowest rated station, JCRF2 02, had an ETR score of 64.7 (Table 8). However, JCRF2 02 was an upper reference station in this sub-watershed, located upstream of AML/AMD inputs. Reasons for this station scoring so low include its ephemeral state and low flow through most of the year. Also, Al and Fe concentrations in the water column are periodically as high as stations below AML/AMD inputs. For these reasons it is believed JCRF2 02 may receive seasonal seep water from naturally occurring pyretic substrates. However, station JCRF2 01 also received a "D" scoring 66.7. This sampling station is influenced by AML/AMD from a previously reclaimed mine area that requires additional reclamation.

In Reeds Creek, two stations had failing scores of 52.8 (RCPS 09B) and 58.1 (RCPS 11B) (Table 8). Both stations are influenced by AML/AMD impacts and are recommended for reclamation. Of the two stations with "D" ratings, RCPS 12 had a 64.3 score but would recover on its own when the upper station (RCPS 09B) is restored. Therefore, RCPS 12 is not recommended for reclamation. Station RC 03 had a rating of 65.4 and may be impacted by station RCPS 11B. Because RCPS 11B is upstream of RC 04, reclamation of RC 03 may not be necessary as it may also improve with the remediation of the impacts to the sub-watershed from RCPS 11B.

In Summers Fork, three stations had "F" ratings, SULF 01 (41.4) in the left branch of Summers Fork, SU 02 (55.2) in the right branch of Summers Fork, and station SU 01A (44.6) that was below these two AMD impacted sites, and adversely influenced by the upper two branches of the sub-watershed (Table 8). Stations SULF 01 and SU 02 have been adversely impacted by AML/AMD impacts and are recommended for reclamation while station SU 01 A is not. If station SULF 01 and SU 02 are reclaimed, then station SU 01A located below would recover by natural ecological processes. Station MF 01 is further downstream of the AML/AMD influences in Summers Fork, and it is influenced by neutralized AMD as evidenced by its rating of 68.5. This station will also recover through natural ecological processes once SULF 01 and SU 02 are reclaimed.

In Straight Creek, no station had a failing ETR score while three stations had "D" to "D+" score of 67.2 to 69.8 (Table 8). The reference station (SW 18) had an ETR score of 84.7, which was the lowest score for a reference station in the five sub-watersheds where scores ranged from 89.2 to 97.4. None of these stations were recommended for reclamation because their degraded "D" status was more influenced by urban runoff and development than by AML/AMD influences.

Table 8. Summary of failing and marginal stations in the five sub-watersheds.			
Sub-watersheds	*ETR Score Range*		
	Highest	*Lowest*	*Other Low ETR Scores*
Cox Creek	97.4, Boggs Hollow	55.9, CC 03*	64.5, CCLF1 01 *** 64.5, CC04 *** 64.9, CC02 ***
Jones Creek	89.2, JC 01	64.2, JCRF2 02* 66.7, JCRF2 01*	--
Reeds Creek	90.7, RC 06	52.8, RCPS 09B* 58.1, RCPS 11B*	64.3, RCPS 12 ** 65.4, RC 03 **
Summers Fork	89.6, SULF 03	41.4, SULF 01* 55.2, SU 02* 44.6, SU 01A**	68.5, MF 01**
Straight Creek	84.7, SW 18	--	67.2, SC 6B **** 67.5 SC 5A **** 69.8, SC 5B ****

* Recommended for restoration.
** Station will recover on its own after the AMD impacted sites above it are reclaimed.
*** Low ETR scores due to high conductivity, moderate metals, low percent mayfly due to active mining.
**** Low ETR scores due to non-point and other urban source inputs that cannot be reclaimed under this study.

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Chapter 2.

Modification of an Ecotoxicological Rating to Bioassess Small Acid Mine Drainage Impacted Watersheds Exclusive of Benthic Macroinvertebrate Analysis.

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Abstract—The Modified Ecotoxicological Rating (METR) approach to synthesizing integrative bioassessment data into a single number ranging from 0 to 100 was found to differentiate between multiple levels of impacts from Acid Mined Drainage (AMD). Our objective was to develop a more cost effective and time efficient bioassessment technique than previously used in other large scale ecotoxicological assessments by minimizing the number of parameters required to rank stations to only those most descriptive of the benthic macroinvertebrate community responses to AMD. Nineteen physical, chemical, toxicological and ecological measurements were made at 38 stations in two adjacent watersheds. The most descriptive parameters were selected through Multiple Linear Regression Analysis (MLRA), bivariate correlation analysis and one way analysis of variance (ANOVA). We found that habitat assessment, 30-d in situ Asian clam survival, mean conductivity, and mean total water column concentration of aluminum and manganese were the most descriptive parameters. The METR constructed from these parameters was equally effective at differentiating stations as were two previous published ecotoxicological that incorporated up to 10 parameters, including benthic macroinvertebrate indices. When the METR was applied to a new watershed the scores were significantly correlated with benthic macroinvertebrate indices for those stations.

Keywords—Acid mine drainage, Rapid integrative bioassessments, Ecotoxicological rating system.

Introduction

Large-scale restoration efforts utilizing integrative bioassessment techniques to locate and rank contaminated areas of watersheds require intense sampling regimes [1-4]. For example, in the Leading Creek Ecological Enhancement Plan, over 150 different bioassessment parameters were measured on multiple occasions at 29 different stations for five years [5]. During an initial assessment of the (AMD) impacts upon the Powell River, Lee County southwest Virginia, 44 different parameters were measured at 38 different stations [6]. Recently, the US Army Corps of Engineers (US ACE) in conjunction with the Virginia Department of Mines, Minerals and Energy (VA DMME), Division of Mine Land Reclamation, has begun a large-scale reconnaissance of the AMD impacts upon the Powell River watershed. Because of the scale of the Powell River Ecosystem Restoration Project, projected to remediate numerous sites throughout the watershed including 11 sub-watersheds of the North Fork of the Powell River (NFP River), it is in the interest of those involved to utilize only the most descriptive and efficient bioassessment techniques available.

Integrative bioassessment approaches incorporating both field and laboratory techniques such as the Sediment Quality Triad have become increasingly popular [7-12]; a number of researchers have conducted assessments of heavy metal impacted watersheds by comparing chemical, physical, and biological data to describe the environmental condition of the associated aquatic ecosystem [1,13-17]. Many of these studies used a Sediment Quality Triad and weight-of-evidence approach to determine which stations were impacted and to provide insight toward causality of the observed impacts [18-20]. Often, these types of studies summarize integrative data with multivariate statistics and utilize a tabular decision matrix or triangular plot to categorize station groups relative to their environmental integrity in a visual manor easily understood by non-scientists [1,8,18,21-23]. However, summarizing data in this way limits the ability to statistically distinguish different levels of impact (i.e. heavily vs. slightly impaired).

The ETR system was designed to summarize abiotic and biotic parameters into a single value and to allow both rank ordering of individual stations according to relative impact and statistical comparisons between station groups. The ETR is conceptualized to work as an academic grading scale (0 through 100), rating reference stations with A's (90-100) and B's (80-89) and impacted stations with C's (70-80), D's (60-70) and failures ($F \leq 60$). Two sub-watersheds within the NFP River drainage, Ely and Puckett's Creek, have been assessed using the ETR approach, effectively differentiating between multiple levels of AMD impacts (i.e. acidic vs. circum-neutral AMD) [13,16]. These investigations employed over 20 different bioassessment parameters, including water and sediment chemistry/metals, habitat assessments, laboratory sediment and water column toxicity testing, in situ toxicity testing, and benthic macroinvertebrate collection and analysis including percent Ephemeroptera abundance and Ephemeroptera – Plecoptera – Tricoptera (EPT) and taxon richness indices. Because the ETR is precise enough to differentiate between multiple levels of impact, has a quantitative basis for ranking stations environmental integrity, and is easily understood, the US ACE has adopted the ETR approach for use in further AMD remediation efforts within the Powell River watershed.

A common attribute of the previous ETR assessments, and most other ecological impact investigations, is the collection and analysis of the resident benthic macroinvertebrate communities. Valuable information can be derived from analyzing these benthic communities as they act as a continuous biological monitoring system, describing impacts in time frames far beyond a snapshot-sampling event of water chemistry or acute toxicity. However, benthic macroinvertebrate collection and analysis is a time consuming and costly process [23]. Therefore, the objective of this study was to create an ETR approach specific to the NFP River related AMD impacts, with only a few highly descriptive parameters to increase sampling efficiency and cost effectiveness, and to potentially create an assessment technique exclusive of benthic macroinvertebrate surveys without losing the ability to predict their community structure.

Materials and Methods

Watershed characterization

Throughout the NFP River watershed the same geological formation, the Upper Mason coal seam, has been heavily mined and is the primary source of AMD. Discharge from the Upper Mason coal seam empties into two adjacent tributaries of the NFP River, Ely and Puckett's Creek. These two tributaries share other similarities such as area (6.0 to 7.4 km^2, respectively), mean pH range (2.9-7.7 and 2.9-8.0, respectively), and number of AMD seeps (5 and 4, respectively) (Table 1). Acid mine drainage seeps contribute most of the water draining from the predominately rural, reclaimed lands that encompass the Ely and Puckett's Creek watersheds. These similarities make the Ely and Puckett's Creek systems (hereafter referred to as the paired system) ideal for investigating which bioassessment techniques best describe benthic macroinvertebrate communities responses to AMD impacts from the Upper Mason coal seam, while accounting for the variability in community responses to other environmental differences between watersheds.

Table 1. Comparison of watershed characteristics between Ely, Puckett’s, and Reed’s Creek.

Parameter	Ely Creek	Puckett's Creek	Reed's Creek
Watershed area (km^2)	6.0	7.4	11.8
Mean flow range (million gallons/day)	85-3110	72-1588	0.29-1343
Mean pH range	2.9-7.7	2.9-8.0	5.5-7.2
Mean conductivity range (μmhos/cm)	50-2420	65-1600	148-710
# AMD seeps[a]	5	4	19
Mean in situ Asian clam survival [b]	44	59	82
Mean *Ceriodaphnia dubia* survival [b]	53	62	87

[a] Acid mined drainage

[b] Describes the average survival of the test organisms at all stations within the watershed.

To validate the new METR that was developed using data from the paired system, analysis of a third tributary impacted by AMD from the Upper Mason Coal Seam and within the NFP River watershed was necessary. Reed's Creek is a relatively larger system as compared to Ely Creek or Puckett's Creek (11.8 vs. 6.0 - 7.4 km^2) but with somewhat lower total discharge characteristics (Table 1). Although Reed's Creek is impacted by more AMD seeps (19 areas of concentrated seepage) these seeps appear to cause less average toxicity (82 % in situ Asian Clam survival and 87% *C. dubia* survival, n = 16 stations) at stations through out the watershed as compared to the seeps impacting Ely (n = 20) and Puckett's Creek (n= 21) (44 and 53 % in situ Asian Clam survival, 59 and 62 % *Ceriodaphnia dubia* survival). The lesser degree of toxicity is thought to be a product of the diffuse and relatively neutral pH (mean pH's ranging from 5.5 to 7.2) of the seepage impacting Reed's Creek. The watershed characteristics of Reed's Creek provide an opportunity to test the effectiveness of the METR in a watershed with a different level of AMD impact.

Sample stations and ETR groups

Samples were collected in Ely Creek (January 1997 to March 1997), Puckett's Creek (October 1997 to July 1998) and Reed's Creek (December 1999 to November 2000) over a four-year period. A total of 53 sampling stations were selected, 16 stations in Reed's Creek, 20 in the Ely Creek study [13], and 21 in the Puckett's Creek study [16], with 4 overlapping stations between the latter two studies. Mean values were determined for all parameters that were measured at overlapping stations.

Each station was categorized according to relative level of AMD input as determined by location within the watershed and mean pH to facilitate statistical comparisons between the mean ETR scores of differentially impacted stations by ANOVA [16]. Group one (group 1) sampling stations were categorized as being upstream of all known AMD inputs within the sub-watersheds or in the NFP River. A second category (group 2) consisted of stations subjected to intermittent AMD input, which was designated as such based on the finding of wide pH ranges over time (e.g., 3.17-7.79), or being downstream of such a station but upstream of any continuous AMD input. Criteria for group three stations (group 3) consisted of stations continuously subjected to AMD input and having acidic mean pH values ≤ 4.5 (no stations in Reed's Creek met this criterion). Stations continuously subjected to AMD input but that had mean pH values >4.5, were categorized as group four stations (group 4). These stations generally occurred in third-order streams and are referred to as being neutralized AMD stations rather than neutral mine drainage stations. Group five stations (group 5) occurred in all three watersheds, generally found in fourth order streams, but fit the criterion that they had one additional level of dilution downstream of group four stations.

Sample collection

Detailed descriptions of the sample collection and analysis methods for the Ely and Puckett's Creek studies can be found in Cherry et al. [13], and Soucek et al. [16]. All data collected in Reed's Creek followed the procedures outlined in the two previous studies with the following exceptions. The lower detection limits for aluminum (Al) and manganese (Mn) in water column samples from Reed's Creek were 0.06 and 0.024 mg/L, respectively. Habitat assessments using the United States Environmental Protection Agency Rapid Bioassessment Protocols [24] were conducted at all stations as part of the original Ely and Puckett's creeks studies; however, habitat assessments were not utilized as part of the Ely or Puckett's ETRs. Habitat assessments conducted in Reed's Creek followed the United States Environmental Protection Agency Rapid Bioassessment Protocols, Second Edition [25]. Data transformations included a log (X+1) transformation of all toxicological data.

Ecotoxicological parameter selection procedures

Three different approaches were used in the parameter selection that produced the ranking systems resulting in the original Ely and Puckett's Creek ETRs, and the new METR scores for Ely Creek and Puckett's Creek. Best professional judgment was used for parameter selection to develop the original Ely Creek ETR as described in Cherry et al. [13]. The ETR parameter selection used in the original Puckett's Creek assessment utilized the chemical, ecological, and toxicological parameters that produced the largest statistical differences between station groups [16]. To produce the METR, multiple linear regression analysis (MLRA) was used to select parameters that best described benthic macroinvertebrate community structure as it changed relative to AMD impact, using only chemical, physical, and toxicological parameters to develop ETR scores.

Statistical analyses performed to develop the METR were conducted using JMP IN® [26] software to select the chemical, physical, and or toxicological parameters (independent variables) that best correlated with the four selected benthic macroinvertebrate indices (total taxon richness, percent Ephemeroptera (E), Ephemeroptera - Plecoptera - Tricoptera (EPT) richness, and EPT abundance. Step-wise MLRA procedures were used to select an independent variable or variables (i.e. pH, sedimentary Al) that described each of the four dependent variables (i.e. benthic macroinvertebrate indices) with model significance determined at the ($\alpha \leq 0.05$) level. Those independent variables selected in the step-wise procedures were then used in bi-variate analyses to calculate correlation coefficients (r) with the dependant variables. Then, means of the absolute values of correlation coefficients were calculated between selected chemical, physical, and toxicological parameters and benthic macroinvertebrate indices, for the Ely and Puckett's Creek data sets. To determine differences in the mean correlations an ANOVA and post hoc Tukey's t-test with model significance at the $\alpha \leq 0.05$ levels were performed. Those independent variables found to have significantly larger average correlation coefficients than the others were selected to construct the METR scores for the stations in Ely and Puckett's Creeks.

Construction of EcoToxicological scores

Transformation of integrative data into ETR values for the METR followed Soucek et al. [16], except for water column metals. All chemical parameters were transformed into a percentage of the highest value measured in the sub-watershed, and then subtracted from one, (1-(value measured at a station/highest value measured in sub-watershed)). This procedure creates values ranging from 0 to 1, giving stations with high chemical concentrations (e.g. sedimentary Al) values closer to 0 than stations with relatively low levels of contamination. A similar procedure often used with Sediment Quality Triad studies, commonly referred to as ratio-to-reference (RTR) is described in Chapman [27] and Del Valls et al. [21]. To create actual ETR scores each integrative parameter selected for use in the METR was transformed to a numeric unit ranging from 0-1, averaged together and then multiplied by 100. This procedure assumes each parameter contributes equal weight to the final score and results in a value ranging from 1 to 100. Higher scores are indicative of environmental conditions that are less impacted as compared to stations with lower scores. For example, an unimpacted reference station might have an ETR score of 95, as compared to an impacted station with an ETR score of 52.

Comparison of ETR parameter selection procedures

Comparisons between the original Ely and Puckett's Creek ETRs and the METR were used to test how parameter selection procedures affect ETR performance in differentiating between station group categories (groups 1 through 5). Two separate ETR scores were developed for all stations in Ely Creek using the original Ely Creek ETR and the METR. Two separate ETR scores were also developed for each station in Puckett's Creek, by using the original Puckett's Creek ETR and the METR. Mean station group ETR scores were compared between the original Ely Creek and the METR, and between the original Puckett's Creek and the METR using an ANOVA with model significance at the $\alpha \leq 0.05$ level and a post hoc Tukey's *t*-test ($\alpha \leq 0.05$) (Table 5).

Statistical analysis of METR validation

To validate that the effectiveness of the METR at differentiating between station groups and capability of rank ordering stations in any AMD impacted sub-watersheds of the NFP River, the same ETR approach used in the Ely and Puckett's Creek sub-watersheds was applied to data collected in Reed's Creek. In addition, to validate that the METR was predictive of the benthic macroinvertebrate communities, correlations coefficients were developed between the same four benthic macroinvertebrate indices used to develop the METR, and the METR score for stations in Reed's Creek.

Results

ETR parameter selection

Step-wise MLRA-produced models explained 70% to 95% of the variation in the dependent variables from station to station in Ely Creek and 55% to 68% of the variation in dependent variables between stations in Puckett's Creek (Table 2). Twelve of the 18 chemical, toxicological, and physical parameters were selected by one of the eight regression models at least once: (1) *Chironomus tentans* survival, (2) *Chironomus tentans* weight, (3) *Daphnia magna*

Table 2. Prediction equations for benthic macroinvertebrate community indices based on multiple linear regression analysis for Ely [a] and Puckett's Creeks [b], [c]

Taxa richness	[(a)] Y = -23.54 + 0.38 (Habitat).	(R^2 = 0.74, total *df* = 19, $p < 0.0001$).
	[(b)] Y= 24.14 + -0.01(Conductivity) - 0.129(Sedimentary Zn).	(R^2 =0.55, total *df* = 20, $p = 0.0007$).
%E. abundance	[(a)] Y = -85.74 + 0.001(Sedimentary Fe) - 0.675(Sedimentary Zn) + 1.38(Habitat).	(R^2 = 0.70, total *df* = 19, $p = 0.0002$).
	[(b)] Y =60.14 - 0.0337(Conductivity) - 1.76(Sedimentary Ni).	(R^2 =0.68, total *df* = 20, $p < 0.0001$).
EPT richness	[(a)] Y = -14.89 + 0.24(Habitat).	(R^2 =0.72, total *df* = 19, $p < 0.0001$).
	[(b)] Y = 22.51 - 0.01(Conductivity) – 4.66(*Daphnia magna* reproduction [d]).	(R^2 =0.63, total *df* = 20, $p = 0.0001$).
EPT abundance	[(a)] Y = -189.68 + 14.04(Asian clam survival) + 50.20(Chironomus tentans survival) - 137.50(*Chironomus tentans* weight) + 11.80(Sedimentary Ni) - 3.86(Sedimentary Zn) + 7.80(Al in H_2O) - 18.32(Mn in H_2O) + 2.08(Fe in H_2O) + 2.54(Habitat).	(R^2 =0.95, total *df* = 19, $p < 0.0001$).
	[(b)] Y = 61.53 - 148.93(*Daphnia magna* reproduction [d]) - 6.19(Sedimentary Ni) - 8.49(Fe in H_2O) + 4.63(Habitat).	(R^2 =0.65, total *df* = 20, $p = 0.0015$).

[a] Regression equation for Ely Creek.

[b] Regression equation for Puckett's Creek.

[c] The best model for each index as determined by a step-wise selection procedure is shown. For each model, all variables contribute significantly to the overall model, ($\alpha \leq 0.05$).

[d] *Daphnia magna* reproduction (sediment toxicity end-point) is shown as number of neonates (% of control).

reproduction, (4) water column iron (Fe), (5) habitat assessment, (6) in situ Asian clam survival, (7) water column Al, (8) water column conductivity, (9) water column Mn, (10) sedimentary Fe, (11) sedimentary nickel (Ni), and (12) sedimentary zinc (Zn). Habitat assessment appeared in five of the eight MLRA models, while water column conductivity, and sedimentary Ni and Zn were in three models. *Daphnia magna* reproduction and water column Fe occurred in two MLRA models, with the remaining five parameters contributing to only one model.

Table 3. Mean (± SD) absolute value of bivariate correlation coefficients (r) for comparisons of the twelve parameters selected in multiple linear regression analysis with four-benthic macroinvertebrate indices in Ely and Puckett's Creek, Southwestern, VA. [a]

Ecotoxicological Rating parameter	Mean correlation coefficients
Habitat	0.61 ± 0.24 A
Asian clam survival	0.52 ± 0.10 AB
Conductivity	0.48 ± 0.14 AB
Mn in H_2O	0.47 ± 0.16 AB
Al in H_2O	0.45 ± 0.11 ABC
Fe in H_2O	0.38 ± 0.10 BCD
Chironomus tentans weight	0.25 ± 0.06 CDE
Chironomus tentans survival	0.24 ± 0.03 CDE
Sedimentary Zn	0.24 ± 0.13 CDE
Sedimentary Ni	0.23 ± 0.15 DE
Sedimentary Fe	0.19 ± 0.14 DE
Daphnia magna reproduction [b]	0.15 ± 0.08 E

[a] Means followed by the same uppercase letter are not significantly different, Tukey's *t*-test results ($p < 0.0001$).

[b] *Daphnia magna* reproduction (sediment toxicity end-point) is shown as number of neonates (% of control).

These twelve selected parameters were then subjected to bivariate correlation analysis with the four ecological parameters, and mean correlation coefficients were calculated for each parameter and analyzed with an ANOVA. This analysis indicated that habitat assessment was significantly more descriptive of the four ecological indices than the other parameters, with a mean r-value of 0.61 ($p < 0.0001$) (Table 3). The other eleven parameters produced mean r-values ranging from 0.15 to 0.52. In situ Asian clam survival (mean r-value = 0.52), water column conductivity (0.48), Mn (0.47), and Al (0.45), were all found not to be statistically lower than habitat assessment. The r-values of the other seven parameters ranged from 0.15 to 0.38 but were found to be statistically different from habitat assessment. All parameters found to be similar to habitat assessment were used to construct the METR (Table 4).

Table 4. Parameters selected in respective ecotoxicologic rating (ETR) systems. [a]

Original Ely Creek ETR	Original Puckett's Creek ETR	METR	METR Ranking Procedure
Mean conductivity	Mean conductivity	Mean conductivity	1 - % of highest value
Asian clam in situ survival		Asian Clam in situ survival	% of highest value
Al in H_2O		Al in H_2O	1 - % of highest value
Ceriodaphnia dubia survival	*Ceriodaphnia dubia* survival		
Daphnia magna survival	*Daphnia magna* survival		
% Ephemeroptera	% Ephemeroptera		
Taxa richness	EPT richness	Mn in H_2O	1 - % of highest value
Mean pH	Fe in H_2O	Habitat	% of highest value
Sedimentary Fe			
Chironomus tentans survival			

[a] Parameters selected for the original Ely Creek ETR were based on best professional judgment. Parameters selected for the original Puckett's Creek ETR were based on sensitivity, multiple linear regression analysis, and correlation analysis. Parameter selected for the METR were based on multiple linear regression analysis and correlation analysis with benthic macroinvertebrate indices, selecting parameters most descriptive of those indices. A station's rankings were then averaged, and multiplied by 100 to result in the final ETR score.

Two ETR scores were constructed for each station within Ely and Puckett's Creek using the original ETR designed for the respective sub-watersheds and the METR as described above. Mean ETR score for each station group were then calculated for each method and compared by ANOVA (Table 5). Significant differences ($p < 0.0001$) were observed among groups in all four

ETRs. The original ETR constructed for Ely Creek had the same significant differences between station groups as the METR for Ely Creek and the same was true for the Puckett's Creek ETRs. In all cases, reference stations (group 1) had the highest average ETR score, with the two METR scores averaging higher ETR scores then the original ETR. In all cases again, group 3 stations had the lowest average ETR scores, with the original ETRs having the lower two average values. The original ETRs constructed for Ely and Puckett's Creeks had the same significant differences between station groups as the METRs.

Table 5. Mean (± SD) ecotoxicologic ratings (ETR score) created for station group in Ely and Puckett's Creek, as created by the modified ETR and the original ETR for that watershed. [a]

Ely Creek ETRs			Puckett's Creek ETRs		
Station group [b]	METR scores	Original ETR scores	Station group [b]	METR scores	Original ETR scores
1 (n = 5)	94.5 ± 7.2 A	79.5 ± 11.3 A	1 (n = 7)	93.8 ± 2.3 A	80.1 ± 11.1 A
2 (n = 3)	59.0 ± 12.3 BC	40.8 ± 7.2 BC	2 (n = 3)	73.5 ± 11.8 B	54.9 ± 4.7 B
3 (n = 3)	37.8 ± 12.7 C	20.6 ± 14.4 C	3 (n = 4)	30.1 ± 16.4 C	15.9 ± 14.7 C
4 (n = 5)	65.9 ± 11.2 B	49.5 ± 11.6 B	4 (n = 3)	73.6 ± 9.1 B	53.8 ± 4.3 B
5 (n = 4)	82.2 ± 8.2 AB	63.8 ± 11.6 AB	5 (n = 4)	86.6 ± 3.6 AB	69.8 ± 6.0 AB

[a] Means followed by the same uppercase letter are not significantly different, Tukey's *t*-test $p < 0.0001$.
[b] 1 = No AMD impact, 2 = Intermittent AMD impact, 3 = Acidic AMD stations, 4 = Neutral AMD impact, 5 = Receiving system stations. AMD = acid mine drainage.

METR validation

In the validation sub-watershed, Reed's Creek, group 1 (reference) stations, the average METR score was 87.6 (Table 6). These stations were not found to be statistically different from group 5 stations (highest average METR score of 88.7) which are located one level of dilution downstream of group 4 stations. Intermittently acidic stations (group 2) averaged the lowest METR score, while group 4 stations (neutralized AMD stations averaged 70.2), were found not to be different from groups 1, 2, and 5 stations. The range of the average METR scores for

Table 6. Mean (± SD) modified ecotoxicologic rating of each station groups in Reed's Creek. [a]	
Station group [b]	METR scores
1 (n = 3)	87.6 ± 2.7 A
2 (n = 3)	55.4 ± 21.5 B
3 (n = 0)	N/A
4 (n = 7)	70.2 ± 9.7 AB
5 (n = 2)	88.7 ± 2.5 A

[a] Means followed by the same uppercase letter are not significantly different, Tukey's *t*-test $p < 0.0001$.

[b] 1 = No AMD impact, 2 = Intermittent AMD impact, 3 = Acidic AMD stations, 4 = Neutral AMD impact, 5 = Receiving system stations. AMD = acid mine drainage.

stations within the Reed's Creek sub-watershed was 33.3. When group 3 stations are excluded from the Ely and Puckett's Creek ETRs for comparison (Ely Creek ETRs, Original = 38.7, Reed's Creek METR = 35.5; Puckett's Creek, Original = 26.3, Reed's Creek METR = 20.3) the Reed's Creek Modified ETRs average range of score is consistent with those found in the paired watershed system (Table 5). All correlation coefficients between METR score and the four benthic macroinvertebrate indices were significant with p-values ≤ 0.01 and r-values ranging from 0.62 for EPT abundance to 0.70 for percent Ephemeroptera (Table 7).

Table 7. Correlation coefficients (r) for comparisons of mean modified ecotoxicologic ratings with benthic macroinvertebrate indices of Reed's Creek.

Benthic macroinvertebrate indices	Correlation coefficients
Taxa richness	0.67 (p = 0.006)
% Ephemeroptera abundance	0.70 (p = 0.004)
EPT richness [a]	0.67 (p = 0.007)
EPT abundance	0.62 (p = 0.010)

[a] EPT = Ephemeroptera – Plecoptera – Trichoptera

Discussion

The results of the present study suggest that the METR approach synthesizes integrative data from AMD impacted sampling stations into a single numerical index sensitive enough to statistically differentiate multiple levels of impact (i.e. upstream acidic vs. neutral mine drainage). By utilizing benthic macroinvertebrate community indices to select parameters for the purpose of ranking stations, data from other sub-watersheds (i.e. Ely and Puckett's Creeks) can be used to develop a system that can rank stations in other sub-watersheds relative to AMD impacts and retain the ability to describe the benthic community at those stations (i.e. Reed's Creek). Because the METR utilizes fewer, more descriptive parameters then past ETRs, and can be applied to new sub-watersheds of the NFP River without benthic macroinvertebrate analysis, it is a more time and cost-efficient ranking system than previous ranking approaches used in the NFP River watershed (i.e. original ETRs for Ely and Puckett's Creek).

Chapman [18,28] and Chapman et al. [29] established that summary indices should be avoided when using Sediment Quality Triad studies intended to rank impaired stations because they do not effectively distinguish intermediate impacts [1,10]. Both the original and METRs constructed for Ely and Puckett's Creek differentiated reference (group 1) and recovery stations (group 5) from stations receiving both acutely toxic AMD inputs (group 3) and intermittently impacted stations (group 2 and 4) (Table 5). To further assess the resolution of the METR in systems of intermittent and intermediate impacts a third more diffusely impacted sub-watershed of the NFP River, Reed's Creek, was investigated with the METR. Reed's Creek is a system without acutely toxic AMD impairments as demonstrated by the lack of group 3 stations (stations with mean acid pH) and greater mean percent survival of two test organisms (Asian clam and *Ceriodaphnia dubia*) at all stations than in the paired watersheds (Table 1). However, when group 3 stations were removed for comparison, the mean range METR scores between station groups in Reed's Creek (mean score range = 33.3) is similar or larger then the mean score ranges found in Ely (35.5) or Puckett's Creek (20.3). These data suggest that the METR has similar or improved resolution in ranking stations impacted by intermittent or intermediate AMD impacts as compared to the past ETR studies.

The METR takes a novel approach to utilizing integrative data often not associated with triad based ranking studies. Historically, information regarding the environmental integrity of impacted stations is pooled together from the ecological, toxicological, and chemical aspects of the Sediment Quality Triad, assuming that utilizing all three data types will establish a chain of evidence of contamination or biological impairment by a stressor. The result is a triad of information including data from multiple levels of biological organization, both field and laboratory validated, that establish a chain of evidence demonstrating environmental contamination and biological impairment. Benthic macroinvertebrate analyses alone contribute the largest extent of this information and by design the METR approach recognizes this utility and uses these ecological indices as the focus of parameter selection. Correlations between the chemical/toxicological parameters and the ecological parameters (benthic indices) can establish a chain of evidence between those parameters most correlated with changes in the resident benthic communities. For example, habitat assessment and Al in the water column, as measured at each station, explained an average of 78% and 69%, respectively, of the variation in the four benthic indices analyzed; however, sedimentary Ni and Fe only explained 48% and 44% on average (Table 3). This might suggest that habitat degradation and high levels of Al in the water column significantly contribute to the observed resident benthic infaunal impairment relative to reference areas. With this approach resource managers might focus efforts on remediation techniques that improve in stream habitat and lower Al levels in the water column in Ely and Puckett's Creeks to more efficiently remediate these systems.

In the development of a summary index such as the ETR, the parameters selected for use in the ranking procedures must be evaluated according to the weight or descriptive power each parameter contributes to the ranking. The METR approach also provides logic to how much weight each parameter is given and how much it contributes to the ranking system. By utilizing those parameters that have statistically similar descriptive power of the resident community structure, equal distribution of the descriptive nature of each parameter is pre-determined and all selected parameters are treated equally.

The use of a sensitive 30-d in situ test with Asian clams contributes significantly to capabilities of the METR (Table 3). The significance of the contribution of this parameter to the

METR is ultimately found in the bridge it builds between changes in the resident benthic macroinvertebrate community structure as impacted by AMD and the laboratory measured contaminant levels and toxicity [29,30]. Through the conduction of benthic macroinvertebrate surveys during or immediately following the 30-d in situ Asian clam test, differences in the community structure found at stations can be associated with the survival of Asian clams at those stations during that time period. By co-joining water column and sediment chemical analysis with these field measurements a reasonable argument can be made for causality if necessary.

This study demonstrates how the METR approach can be used to develop a numerical summary index synthesizing integrative data and ranking stations relative to how the resident benthic macroinvertebrate community structure is impacted by AMD. The METR is a summary index sensitive enough to differentiate between multiple levels of environmental impacts from AMD and is an approach that results in a time and cost efficient bioassessment easily understood by non-scientists. ETR approaches are currently being utilized by US Army Corps of Engineers to prioritize AMD impacted stations in sub-watersheds of the North Fork of the Powell River, Southwest Virginia USA, for ecological restoration.

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Chapter 3.

Integrative Assessment of Benthic Macroinvertebrate Community Impairment From Metal-Contaminated Waters in Tributaries of the Upper Powell River, Virginia.

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Abstract— Benthic macroinvertebrate communities of the North Fork Powell River, southwest Virginia, appear to be impacted by aluminum (Al) and iron (Fe) from acid mine drainage (AMD) beyond the zone of pH depression. As part of a watershed restoration project, we used integrative techniques including water column, sediment, and in situ toxicity tests, sediment and water column chemistry, and habitat assessments to detect AMD impacts. Analysis of variance (ANOVA), least significant difference (LSD), and spearman correlations were used to test the sensitivity of these integrative techniques to detect various (i.e., acidic or neutralized) levels of AMD input and to determine the mode of impairment (metal contaminated sediments or water) to the benthic macroinvertebrate communities. Benthic macroinvertebrate indices were the most sensitive endpoint to AMD inputs and were significantly correlated ($\alpha \leq 0.05$) with water column metal concentrations, in situ and water column toxicity tests. Sediment chemistry and toxicity did not detect AMD impacts and were not significantly correlated with benthic macroinvertebrate indices. These results suggest that the primary mode of impairment to the benthic macroinvertebrate communities were water column concentrations of Al and Fe.

Keywords— Benthic macroinvertebrates, integrative bioassessment, mine drainage, aluminum, iron.

Introduction

Acid mine drainage (AMD) results from the reduction of pyretic materials, which have been oxidized upon exposure to water and oxygen, a process often brought about through mining activities. The pyretic acidification reactions produce sulfuric acid, ferric hydroxides, and mobilize other trace metals, depending on the surrounding mineralogy. These toxic acids and metals flow to surface waters, where the acid is eventually neutralized, causing metals to precipitate and coat streambeds with metal oxides, impairing habitat and adversely affecting water quality in over 13,000 miles of U.S. rivers [1]. The biotic effects associated with AMD impacted surface waters include acute impairment of benthic and fish communities as a result of low pH and elevated levels of dissolved heavy metals [2-5]. A decrease in benthic macroinvertebrate diversity and increase of tolerant organisms is also associated with heavy metal pollution in streams.

In the North Fork Powell River watershed (NFP), southwest Virginia, stream communities have experienced decades of impairment from drainage and sedimentation associated with mining activities and abandoned mine lands. In the main-stem of the NFP, populations of unionid mussels have been extirpated, and reductions in populations of the common stonefly *Acroneuria* in one particular reach have been attributed to the chronic toxic affects of neutralized mine drainage [6-7]. Headwater streams such as Ely and Puckett's Creek are direct recipients of AMD, rendering surface and pore waters of these tributaries acutely toxic to cladocerans and transplanted Asian clams [8-11]. Benthic macroinvertebrate community indices were correlated with the acute toxicity testing endpoints in Ely and Puckett's Creek.

Reconnaissance of other tributaries draining mined areas in the NFP watershed revealed impaired benthic communities in streams without acidic pH values and only slightly elevated water column metals concentrations near or below U.S. EPA Water Quality Criteria [12]. Recent investigations of heavy metal laden stream sediments in the NFP and other watersheds have suggested that those metals may be bioavailable, cause acute or chronic toxicity to standard test organisms, and may smother or cause physical abrasion of the resident infauna [10, 13-15]. Many of these studies attributed sediment toxicity to sediment copper (Cu), cadmium (Cd), and lead (Pb) or, pore-water and water column concentrations of these metals. Because Cu, Cd, and Pb are found in relatively low concentrations in the NFP river sediments, the potential for sediment toxicity may be less in these streams. The objective of the present study was to investigate the sensitivity of various assessment techniques to different levels of AMD input, and further to determine the likely mode of impairment (exposure to metal-contaminated sediments or water) to the benthic macroinvertebrate communities at both acidic and cirum-neutral AMD impacted tributaries of the NFP.

Materials and Methods

Sampling regime and station categorization

Samples were collected over a four-year period in Ely Creek (January 1997 to March 1997), Puckett's Creek (October 1997 to July 1998) and Reed's Creek (December 1999 to November 2000). A total of 36 sampling stations were selected, 12 in each of the three sub-watersheds. All stations are found in 1^{st} to 3^{rd} order streams. To facilitate statistical comparisons between different levels of AMD impact, each station was categorized according to the relative level of AMD input, mean pH, and position within the watershed. The reference station category (upstream) included stations categorized as upstream of all known AMD inputs. Stations continuously subjected to AMD input, but that had median pH values > 4.5, were categorized as neutralized AMD impacted stations. A third station category (acidic AMD) consisted of stations continuously subjected to AMD input and having median pH values ≤ 4.5. No stations in Reed's Creek met the acidic station criterion.

Water column and sediment chemistry

Water column chemistry was measured both in the field and laboratory. Field samples were stored at 4 °C and analyzed within 24 hours of collection. The pH was measured using either a Markson Field® pH meter with combination electrode or an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) meter equipped with a gel-filed combination electrode. Conductivity was measured with a Hach® (Hach, Loveland, CO, USA) conductivity/TDS meter. Alkalinity and hardness were measured by titration [16]. Metals analyzed included total Al, Fe, and manganese

(Mn). Copper, Cd, Zinc (Zn), and Pb were measured in earlier studies but were not found above detection limits. Filtered water (0.45 μm pore size) samples were analyzed by inductively coupled plasma (ICP) spectrometry either by Spectrum Laboratories, Coeburn Virginia, or at the Virginia Tech Soil Testing Laboratory. The lower detection limits for Al, Fe and Mn were 0.06, 0.027, and 0.024 mg/L, respectively. When concentrations were below detection limits, one half of that limit was used as the measured value for statistical analysis. Water quality parameters for each station were reported as median values.

Sediments were digested in 50% (*v*/*v*) nitric acid, 20% (*v*/*v*) hydrochloric acid (Fisher Scientific, Pittsburgh, PA, USA.) with metals analysis following U.S. EPA protocol [17]. Total Al, Cu, Fe, Mn, and Zn were measured using ICP spectrometry. Sediments were collected with a polyurethane scoop from various points within a sampling station, placed in a freezer lock bag, and stored at 4 °C. Each sample was homogenized and one-gram samples were dried at 80 °C for 24 hours and weighed again to determine mean percent water content. Mean values of replicate samples were used for statistical analysis.

In situ clam toxicity testing

Asian clams (*Corbicula fluminea*) [Müller] were collected from the New River near Ripplemead, Virginia, using clam rakes. Clams were maintained in Living Streams® (Toledo, OH) at the Ecosystem Simulation Laboratory (ESL), Virginia Tech, Blacksburg, VA, until needed for in situ toxicity testing. Five clams were placed into 18 cm by 36 cm mesh (~0.5 cm^2) bags. At each sampling station 5 bags were tied to a stake and left in the field for 30 days. After 30 days, clams were retrieved and transported to Virginia Tech where mean survival was determined for each station. Clams were considered dead if found gaping, were easily opened, or failed to close when the visceral mass was touched with a blunt object.

Water column toxicity testing

Acute toxicity tests were preformed using *Ceriodaphnia dubia* cultured at Virginia Tech. Filtered culture/diluent water came from Sinking Creek, Newport, Virginia. Organisms were fed 0.18ml/30ml test solution of a 1:1 (*v/v*) mixture containing *Selenastrum capricornutum* and

Yeast-Cereal-Trout Chow (YCT) prior to testing. For toxicity tests, five organisms were place into replicate 50 ml beakers (two replicates for Reed's Creek, four for Puckett's Creek, five replicates for Ely Creek) containing site water. Tests were 48 hours long and temperature was maintained at 25 ± 1 °C. Sinking Creek water was used as a control. Ely Creek stations were tested on once occasion for water column toxicity, Puckett's Creek three times, while Reed's Creek was tested on four occasions. For the purpose of statistical comparisons, mean survival for each test period was determined.

Sediment toxicity testing

Ten-day sediment toxicity tests were conducted within 14 days of sample collection using procedures outlined in Ingersoll et al. [18] Nebeker et al. [19], U.S. EPA, [20], and ASTM, [21], with modifications. Similarities in test procedures included the use of five to six day old *Daphnia magna,* an ambient temperature of 25 ± 1ºC, overlying reference water collected from Sinking Creek, and all controls met U.S. EPA and ASTM standards. Control sediments for Ely and Reed's Creek were also collected from Sinking Creek; however, the control sediments for the Puckett's Creek investigation were formulated using a mixture of sand and potting soil (4:1, *w/w*). Overlying water was changed daily and test organisms were fed 0.18ml/30ml of a 1:1 (*v/v*) mixture containing *S. capricornutum* and YCT daily. To minimize the effect of the different sediment test techniques used in these studies mean survival and reproduction for each station were reported as percent of mean control.

Sediment toxicity tests performed for the Ely and Puckett's Creek investigations utilized five replicated 1-L beakers filled with 200 ml of sediment and 800 ml of overlying water per station. Five *D. magna* were placed in each beaker. The sediment test chambers in the Reed's Creek investigation were 50 ml beakers each filled with 15 ml of site sediment, overlaid with 35 ml of water, and containing one test organism each. Eight replicates were used per station.

Benthic macroinvertebrate sampling

Benthic macroinvertebrate surveys followed U.S. EPA Rapid Bioassessment Protocols (RBP) [22]. Two composite samples were collected at each station from riffle, run, pool and shoreline rooted areas using dip nets of 800 μm mesh. Organisms were identified to lowest practical taxonomic level (usually genus) from standard taxonomic keys [23]. The community indices calculated included total taxon richness, Ephemeroptera-Plecoptera-Tricoptera (EPT) richness, Ephemeroptera richness, Plecoptera richness, Tricoptera richness, EPT minus (Hydropsychidae), EPT minus (Leuctridae), and EPT minus (Hydropsychidae + Leuctridae). Community indices were calculated for each composite sample, and were combined to obtain a mean for each station.

Habitat assessment

Habitat assessments in Ely and Puckett's Creek were performed using U.S. EPA RBP [22]. Nine parameters were measured including: (1) bottom substrate/available cover; (2) embeddedness; (3) velocity/depth; (4) channel alteration; (5) bottom scouring and deposition; (6) pool/rifle-run/bend ratio; (7) bank stability; (8) bank vegetative stability; and (9) streamside cover. In Reed's Creek, habitat assessments were performed using the revised U.S. EPA RBP [24]. Ten parameters were measured: (1) epifaunal substrate/ available cover, (2) embeddedness, (3) velocity/depth regime, (4) sediment deposition, (5) channel flow status, (6) channel alteration, (7) frequency of riffles (or bends), (8) bank stability, (9) vegetative protection, and (10) riparian vegetation zone width. Ratings ranging from 0 - 10 or 0 - 20 (depending on the parameter) were used to distinguish physical integrity of the sampling station and its availability of niches for aquatic life. Two independent researchers conducted habitat assessments at each station simultaneously.   In all cases, habitat assessment scores were reported as percent of reference and were reported as means.

Statistical analysis

To analyze the differences between station categories (i.e. upstream of AMD impacts, acidic AMD impacts, and neutralized AMD impacts), means and medians for each data type (i.e., pH or sediment Fe (mg/kg)) from each station were pooled and averaged for all stations within a category for a given sub-watershed. As these pooled means did not meet the primary assumptions of normality and homogeneity of variance, all data were rank transformed, and mean station category ranks were compared by ANOVA and Least Significant Difference (LSD) post hoc tests, using SAS® software [25]. For example, median pH was reported for each station (n = 36). The median pH values were then rank transformed and pooled into the three station categories (i.e. upstream of AMD impacts, acidic or neutralized AMD impacted stations). These three pooled mean pH values for upstream of impact, acidic AMD impacted, and neutralized AMD impacted stations were then compared by ANOVA and LSD.

To characterize the relationships between the integrative data at different pH regimes, stations were segregated into two subsets; acidic AMD impacted stations and neutralized AMD impacted station. Integrative data from the upstream station group were added to both subsets creating data sets of upstream and acidic AMD impacted stations as well as an upstream with neutralized AMD impacted stations data set. Because these two data sets did not meet the assumptions of normality and homogeneity of variance, spearman correlation analysis was used to compare the different types of assessment endpoints using SAS® software.

Results

Chemical and physical parameters

Mean water column metals concentrations and conductivity at upstream stations were significantly lower than neutralized or acidic AMD impacted stations in Ely and Puckett's Creeks (Table 1). In Reed's Creek, only mean conductivity was found to distinguish upstream from neutralized AMD impacted stations. In general, acidic stations in Ely and Puckett's Creeks averaged higher water column metals concentrations and conductivity than neutralized stations.

Table 1. Mean ± (SD) of water chemical parameters at each station category in Ely, Puckett's and Reed's Creeks.[a]				
Water chemistry	Station category	Ely Creek (n = 12)	Puckett's Creek (n = 12)	Reed's Creek (n = 12)
Al in H_2O (mg/L)	Upstream	0.09 ± 0.01 B	0.18 ± 0.14 C	0.18 ± 0.05 A
	Neutralized AMD	2.28 ± 1.16 A	2.44 ± 0.49 B	0.31 ± 0.17 A
	Acidic AMD	5.22 ± 4.48 A	29.34 ± 21.95 A	N/A
Fe in H_2O (mg/L)	Upstream	0.26 ± 0.07 B	0.34 ± 0.22 C	0.42 ± 0.36 A
	Neutralized AMD	6.80 ± 5.29 A	2.46 ± 0.77 B	1.09 ± 0.83 A
	Acidic AMD	7.29 ± 6.34 A	18.54 ± 18.42 A	N/A
Mn in H_2O (mg/L)	Upstream	0.02 ± 0.00 B	0.5 ± 0.5 B	0.15 ± 0.19 A
	Neutralized AMD	0.94 ± 0.22 A	1.11 ± 1.38 A	0.35 ± 0.22 A
	Acidic AMD	1.68 ± 1.72 A	2.98 ± 1.42 A	N/A
Conductivity (µS)	Upstream	107 ± 47 B	195 ± 113 B	179 ± 40 B
	Neutralized AMD	373 ± 53 A	541 ± 123 AB	284 ± 95 A
	Acidic AMD	418 ± 127 A	980 ± 654 A	N/A
PH	Upstream	7.27 ± 0.11 A	7.39 ± 0.73 A	6.95 ± 0.18 A
	Neutralized AMD	5.81 ± 0.42 B	7.21 ± 0.25 AB	6.81 ± 0.24 A
	Acidic AMD	3.62 ± 0.57 C	3.61 ± 0.68 B	N/A
[a] Means followed by the same uppercase letter are not significantly different, LSD $p < 0.05$.				

However, in Puckett's Creek, significant differences were observed between all three stations categories for mean concentrations of Al and Fe. Similarly, in Ely Creek, significant differences were observed between all three station categories for mean pH (7.27, upstream; 5.81, neutralized; and 3.62 acidic AMD impacted stations), while in Puckett's Creel, only upstream stations (7.39) and acidic stations (3.61) mean pH were significantly different, and no differences found in Reed's Creek. Few significant differences in sediment metals concentrations (mg/kg) were observed between stations categories (Table 2). Mean habitat score at upstream stations were generally higher than at either acidic or neutralized AMD stations. The only significant differences observed for mean habitat score were in Ely Creek.

Table 2. Mean ± (SD) of sediment chemical and physical parameters at each station category in Ely, Puckett's and Reed's Creek.[a]

Sediment chemistry	Station category	Ely Creek (n = 12)	Puckett's Creek (n = 12)	Reed's Creek (n = 12)
Sediment Al (mg/kg)	Upstream	1,623 ± 533 A	5,428 ± 954 A	3,802 ± 836 A
	Neutralized AMD	1,480 ± 354 A	5,300 ± 826 A	4,592 ± 757 A
	Acidic AMD	1,664 ± 638 A	4,686 ± 2,772 A	N/A
Sediment Cu (mg/kg)	Upstream	3.04 ± 1.96 A	8.56 ± 3.33 A	9.98 ± 12.17 A
	Neutralized AMD	2.78 ± 1.24 A	8.03 ± 1.80 A	7.84 ± 2.15 A
	Acidic AMD	1.38 ± 1.6 A	8.97 ± 5.01 A	N/A
Sediment Fe (mg/kg)	Upstream	4,398 ± 1,744 A	26,860 ± 12,318 B	18,240 ± 1,798 A
	Neutralized AMD	5,071 ± 3,726 A	24,400 ± 1,609 B	26,655 ± 9,709 A
	Acidic AMD	1,0134 ± 7,162 A	86,050 ± 58,897 A	N/A
Sediment Mn (mg/kg)	Upstream	107.0 ± 43.9 A	980.6 ± 679.1 A	1,004 ± 675 A
	Neutralized AMD	126.9 ± 163.4 A	779.7 ± 119.9 A	1,235 ± 1,317 A
	Acidic AMD	33.7 ± 24.1 A	111.3 ± 97.5 B	N/A
Sediment Zn (mg/kg)	Upstream	14.12 ± 3.72 A	45.20 ± 11.84 A	49.87 ± 16.29 B
	Neutralized AMD	16.35 ± 5.22 A	80.57 ± 7.87 A	79.79 ± 19.13 A
	Acidic AMD	7.67 ± 3.79 A	45.03 ± 31.86 A	N/A
Habitat Score[b]	Upstream	96.9 ± 2.2 A	91.9 ± 10.2 A	77.8 ± 7.9 A
	Neutralized AMD	71.1 ± 7.3 B	85.7 ± 7.9 A	70.5 ± 18.1 A
	Acidic AMD	62.3 ± 11.5 B	66.3 ± 26.2 A	N/A

[a] Means followed by the same uppercase letter are not significantly different, LSD $p < 0.05$.

[b] Percent of Reference.

Toxicological parameters

In Ely Creek, 48-hour *C. dubia* and 30-day in situ Asian clam toxicity tests had significantly greater survival at upstream stations than at acidic AMD impacted stations (Table 3). *Ceriodaphnia* and Asian clam survival at neutralized AMD impacted stations were not different from upstream stations or acidic AMD impacted stations.

In Puckett's Creek, *C. dubia* survival was significantly greater at upstream stations as compared to neutralized AMD impacted or acidic AMD impacted stations (Table 3). However, at neutralized AMD impacted stations, *C. dubia* survival was significantly greater than that at acidic AMD impacted stations. Asian clam survival was significantly greater at upstream stations than at either neutralized or acidic AMD impacted stations. No differences in Asian clam survival were observed between neutralized or acidic AMD impacted stations.

In Reed's Creek, no significant differences in *C. dubia, D. magna* and Asian clam survival or *D. magna* reproduction were observed (Table 3). Survivorship for each station category was high ranging from 80 to 100 percent survival among the three test organisms.

Table 3. Mean ± (SD) for toxicological parameters at each station category in Ely, Puckett's and Reed's Creek[a].

Toxicological parameters	Station category	Ely Creek (n = 12)	Puckett's Creek (n = 12)	Reed's Creek (n = 12)
Daphnia magna reproduction[b]	Upstream	75.2 ± 92.0 A	67.8 ± 14.8 A	87.8 ± 25.8 A
	Neutralized AMD	292.8 ± 170.2 A	92.7 ± 21.6 A	59.5 ± 16.8 A
	Acidic AMD	77.0 ± 133.4 A	49.0 ± 58.1 A	N/A
Daphnia magna survival[b]	Upstream	35.0 ± 52.0 A	67.0 ± 12.2 A	103.6 ± 13.7 A
	Neutralized AMD	80.0 ± 24.5 A	84.7 ± 2.1 A	96.4 ± 18.3 A
	Acidic AMD	46.7 ± 50.3 A	53.0 ± 61.2 A	N/A
Clam survival	Upstream	80.0 ± 40.0 A	92.8 ± 5.22 A	80.0 ± 32.0 A
	Neutralized AMD	45.0 ± 44.7 AB	36.0 ± 34.2 B	83.0 ± 29.1 A
	Acidic AMD	0 ± 0 B	0 ± 0 B	N/A
Ceriodaphnia dubia survival	Upstream	98.8 ± 2.5 A	86.2 ± 11.1 A	99.4 ± 1.3 A
	Neutralized AMD	40.0 ± 54.8 AB	45.3 ± 32.7 B	90.7 ± 12.9 A
	Acidic AMD	0 ± 0 B	0 ± 0 C	N/A

[a] Means followed by the same uppercase letter are not significantly different, LSD $p < 0.05$.
[b] Percent of Reference.

Table 4. Mean ± (SD) for ecological parameters at station categories in Ely, Puckett's, and Reed's Creeks.[a]

Ecological Parameters	Station Type	Ely Creek (n = 12)	Puckett's Creek (n = 12)	Reed's Creek (n = 12)
Taxon rich.	Upstream	13.0 ± 2.9 A	18.5 ± 5.6 A	19.5 ± 6.2 A
	Neutralized AMD	1.2 ± 1.6 B	9.3 ± 2.5 B	11.9 ± 4.4 A
	Acidic AMD	0.7 ± 0.6 B	4.6 ± 1.5 C	N/A
EPT rich.	Upstream	2.8 ± 1.5 A	4.8 ± 1.8 A	2.0 ± 1.9 A
	Neutralized AMD	0.2 ± 0.4 B	0.3 ± 0.6 B	0.7 ± 1.0 A
	Acidic AMD	0 ± 0 B	0.4 ± 0.5 B	N/A
Ephemeroptera rich.	Upstream	2.8 ± 0.5 A	5.3 ± 0.8 A	3.1 ± 1.0 A
	Neutralized AMD	0.2 ± 0.4 B	1.5 ± 0.5 B	1.4 ± 0.6 B
	Acidic AMD	0 ± 0 B	0.5 ± 0.7 B	N/A
Plecoptera rich.	Upstream	2.8± 0.5 A	2.7 ± 1.7 A	2.4 ± 0.3 A
	Neutralized AMD	0.0 ± 0.0 B	2.5 ± 1.0 A	1.4 ± 1.0 B
	Acidic AMD	0.3 ± 0.6 B	0.1 ± 0.3 B	N/A
Tricoptera rich.	Upstream	8.3 ± 1.3 A	12.8 ± 3.8 A	7.5 ± 2.5 A
	Neutralized AMD	0.4 ± 0.9 B	4.3 ± 1.0 B	3.5 ± 2.2 B
	Acidic AMD	0.3 ± 0.6 B	1.0 ± 0.7 C	N/A
EPT rich. – Hydrop. rich.	Upstream	7.5 ± 1.0 A	11.6 ± 3.1 A	6.6 ± 2.3 A
	Neutralized AMD	0.4 ± 0.9 B	3.0 ± 0.5 B	2.9 ± 1.9 B
	Acidic AMD	0.3 ± 0.6 B	0.9 ± 0.8 C	N/A
EPT rich. – Leuctr. rich.	Upstream	8.3 ± 1.3 A	11.8 ± 3.8 A	6.5 ± 2.5 A
	Neutralized AMD	0.4 ± 0.9 B	3.7 ± 1.3 B	2.6 ± 2.1 B
	Acidic AMD	0.3 ± 0.6 B	0.8 ± 0.5 C	N/A
EPT rich. – (Hydrop. rich. + Leuctr. rich.)	Upstream	7.5 ± 1.0 A	10.6 ± 3.1 A	5.6 ± 2.3 A
	Neutralized AMD	0.4 ± 0.9 B	2.3 ± 0.8 B	2.1 ± 1.8 B
	Acidic AMD	0.3 ± 0.6 B	0.3 ± 0.6 C	N/A

rich- richness, Hydrop.- Hydropsychidae, Leuctr- Leuctridae, [a] Means followed by the same uppercase letter are not significantly different, LSD $p < 0.05$.

Benthic macroinvertebrate parameters

In Ely Creek, all eight benthic macroinvertebrate indices had significantly lower values at acidic and neutralized AMD impacted stations than upstream stations (Table 4). However, no differences were found in any benthic macroinvertebrate indices between acidic and neutralized AMD impacted stations. Again in Puckett's Creek, all eight benthic macroinvertebrate indices statistically differentiated between upstream and AMD impacted stations (Table 4). In Reed's Creek, only taxon richness and Ephemeroptera richness were not sensitive to AMD impacted station categories (Table 4).

Upstream and acidic AMD impacted stations correlation analysis

Correlations between water column Al, Fe, Mn, conductivity, pH and all eight benthic macroinvertebrate indices were significant, ranging from – 0.39 (conductivity vs. Tricoptera richness) to – 0.82 (water column Mn vs. EPT richness minus Hydropsychidae, and EPT richness minus (Hydropsychidae + Leuctridae)) (Table 5). However, there were few significant correlations between sediment chemistry and the benthic macroinvertebrate indices except for sediment Mn and Zn. Habitat assessment score was significantly correlated with many benthic macroinvertebrate indices, ranging from 0.48 for taxon richness and EPT richness minus Leuctridae; to 0.59 for Plecoptera richness.

Table 5. Correlation coefficients between ecological parameters, chemical and physical data at upstream and acidic AMD impacted stations (n = 16).

Chemical and physical vs. ecological parameters	Al in H_2O	Fe in H_2O	Mn in H_2O	Conductivity	pH	Sediment Al	Sediment Cu	Sediment Fe	Sediment Mn	Sediment Zn	Habitat[a]
Taxon rich.	-0.64*	-0.72*	-0.64*	-0.54*	0.88*	0.22	0.18	-0.03	0.71*	0.41	0.48*
EPT rich.	-0.66*	-0.74*	-0.69*	-0.62*	0.89*	0.29	0.21	-0.10	0.76*	0.40	0.51*
Ephemeroptera rich.	-0.61*	-0.70*	-0.66*	-0.65*	0.83*	0.27	0.19	-0.04	0.74*	0.39	0.39
Plecoptera rich.	-0.63*	-0.74*	-0.73*	-0.64*	0.81*	0.42	0.29	-0.10	0.85*	0.50*	0.59*
Tricoptera rich.	-0.76*	-0.78*	-0.69*	-0.54*	0.82*	-0.15	-0.16	-0.35	0.38	0.06	0.36
EPT rich. – Hydrop. rich.	-0.66*	-0.74*	-0.68*	-0.63*	0.87*	0.30	0.20	-0.12	0.75*	0.39	0.50*
EPT rich. – Leuctridae rich.	-0.65*	-0.74*	-0.68*	-0.60*	0.88*	0.23	0.15	-0.12	0.72*	0.35	0.48*
EPT rich. - (Hydrop. rich. + Leuctr. rich.)	-0.66*	-0.75*	-0.68*	-0.63*	0.87*	0.28	0.18	-0.15	0.74*	0.36	0.50*

rich- richness, Hydrop.- Hydropsychidae, Leuctr- Leuctridae, Sed.- Sediment, * Significant correlation at the $p<0.05$ level. [a]Percent of reference.

Significant correlations occurred between all water chemistry parameters and both *C. dubia* (ranging from -0.72 to –0.90) and Asian clam survival (-0.69 to –0.89) at upstream and acidic AMD impacted stations (Table 6). Daphnia survival and reproduction were not correlated

Table 6. Correlation coefficients between select toxicological parameters, chemical and physical data at acidic AMD impacted stations (n = 16).

Toxicological vs. chemical and physical parameters	*Ceriodaphnia dubia* survival	Asian Clam survival	*Daphnia magna* reproduction[a]	*Daphnia magna* survival[a]
Al in H_2O	-0.90*	-0.85*	-0.22	-0.03
Fe in H_2O	-0.78*	-0.89*	-0.21	-0.2
Mn in H_2O	-0.74*	-0.77*	-0.20	-0.1
Conductivity	-0.72*	-0.69*	-0.31	-0.25
PH	0.84*	0.84*	0.31	0.19
Sediment Al	-0.10	0.03	0.37	0.47
Sediment Cu	-0.17	-0.00	0.17	0.30
Sediment Fe	-0.51*	-0.40	-0.10	0.11
Sediment Mn	0.45	0.56*	0.43	0.44
Sediment Zn	-0.05	0.12	0.31	0.43
Habitat[b]	0.67*	0.64*	0.48	0.31

[a] Percent of control or reference. [b] Percent of Reference. *Significant correlation at the $p < 0.05$ level.

with either water or sediment chemistry. However, *C. dubia* survival and Asian clam survival ($r = 0.93$, $p < 0.0001$), as well as *Daphnia* reproduction and survival ($r = 0.82$, $p = 0.0001$), were significantly correlated with each other.

Upstream and neutralized AMD impacted stations correlation analysis

Correlations between water column parameters and the benthic macroinvertebrate indices at upstream and neutralized AMD impacted stations were all significant, ranging from –0.54 (conductivity vs. EPT richness and Tricoptera richness) to 0.89 (pH vs. EPT richness) (Table 7).

Table 7. Correlation coefficients between select ecological parameters, chemical and physical data at upstream and neutral AMD impacted stations (n = 29).

Chemical and physical vs. ecological parameters	Al in H_2O	Fe in H_2O	Mn in H_2O	Conductivity	PH	Sediment Al	Sediment Cu	Sediment Fe	Sediment Mn	Sediment Zn	Habitat[a]
Taxon rich.	-0.54*	-0.54*	-0.59*	-0.50*	0.53*	0.24	0.20	0.31	0.37*	0.10	0.35
EPT rich.	-0.59*	-0.65*	-0.80*	-0.61*	0.73*	0.21	0.12	0.11	0.24	-0.09	0.63*
Ephemeroptera rich.	-0.55*	-0.55*	-0.69*	-0.57*	0.49*	0.11	0.02	0.03	0.11	-0.21	0.54*
Plecoptera rich.	-0.59*	-0.69*	-0.78*	-0.66*	0.66*	0.27	0.23	0.13	0.32	-0.06	0.63*
Tricoptera rich.	-0.49*	-0.50*	-0.66*	-0.39*	0.80*	0.16	0.05	0.17	0.22	0.08	0.49*
EPT rich. – Hydrop. rich.	-0.61*	-0.65*	-0.82*	-0.65*	0.69*	0.18	0.12	0.08	0.22	-0.13	0.63*
EPT rich. – Leuctr. rich.	-0.60*	-0.64*	-0.80*	-0.61*	0.74*	0.19	0.09	0.08	0.20	-0.14	0.65*
EPT rich. – (Hydrop. rich. + Leuctr. rich.)	-0.63*	-0.64*	-0.82*	-0.65*	0.68*	0.15	0.07	0.06	0.19	-0.16	0.65*

rich- richness, Hydrop.- Hydropsychidae, Leuctr- Leuctridae, Sed.- Sediment. *Significant correlation at the $p < 0.05$ level. [a]Percent of reference.

Only one correlation was significant between the sediment chemistry parameters and the benthic macroinvertebrate indices, sediment Mn vs. taxon richness ($r = 0.37$). Habitat assessment score was significantly correlated with seven of the eight benthic macroinvertebrate indices excluding taxon richness ($r = 0.35$, $p = 0.06$).

Many correlations between *C. dubia* survival and the chemical/physical parameters were significant except for water column Mn and pH at upstream and neutralized AMD impacted stations (Table 8). Asian clam survival was significantly correlated with all the water column parameters except pH. Neither sediment chemistry parameters nor habitat assessment were correlated with *C. dubia* or Asian clam survival. *Ceriodaphnia* and Asian clam survival were significantly correlated with each other ($r = 0.58$, $p = 0.001$). Daphnid reproduction and survival were not significantly correlated with any of the chemical/physical parameters.

Table 8. Correlation coefficients between toxicological parameters, chemical and physical data at upstream and neutral AMD impacted stations (n = 29).

Toxicological vs. chemical and physical parameters	*Ceriodaphnia dubia* survival	Asian clam survival	*Daphnia magna* reproduction[a]	*Daphnia magna* survival[a]
Al in H_2O	-0.54*	-0.54*	0.35	0.21
Fe in H_2O	-0.55*	-0.64*	0.24	0.08
Mn in H_2O	-0.26	-0.39*	0.32	0.20
Conductivity	-0.45*	-0.40*	0.18	0.05
pH	0.14	0.25	-0.22	-0.30
Sediment Al	-0.24	-0.10	-0.28	-0.07
Sediment Cu	-0.22	0.02	-0.10	0.12
Sediment Fe	-0.14	0.05	-0.31	0.06
Sediment Mn	0.08	0.14	-0.12	0.20
Sediment Zn	-0.09	0.03	-0.24	0.24
Habitat[A]	0.17	0.22	0.01	-0.27

[a] Percent of Control, *Significant correlation at the $p < 0.05$ level.

Discussion

The results of this study had three specific trends: (1) only benthic macroinvertebrate diversity indices consistently differentiated upstream reference stations from AMD impacted stations; (2) impaired habitat, elevated metals in the water column, and depressed pH levels downstream of AMD inputs were associated with reduced benthic macroinvertebrate richness and high mortality to Asian clams and *C. dubia*; and (3) there was considerable variability in sediment metal concentrations and toxicity among station categories, which were not generally correlated with benthic macroinvertebrate richness, in situ toxicity, water column toxicity, or water column chemistry. These results indicate that benthic macroinvertebrate communities are more sensitive to AMD impacts than the toxicity testing endpoints used in this study. They also suggest that the primary mode of impairment to the benthic macroinvertebrate communities is metal-contaminated waters, both within and beyond the zone of pH depression, not metal-contaminated sediments from precipitates of neutralized AMD.

The observed benthic macroinvertebrate responses to AMD and heavy metal contamination were similar to those found in other studies [26-28]. Even in Reed's Creek, where in general, both water and sediment chemistry were not significantly different below AMD inputs as compared to upstream, a reduction in the diversity of Ephemeroptera, Plecoptera, and Tricoptera were observed. However, in Reed's Creek, 48-hour acute water column toxicity tests using *C. dubia*, 10-day chronic sediment toxicity tests using *D. magna*, and 30 day in situ Asian clam survival detected no differences between upstream and downstream stations. Cladocerans have been found to be very sensitive to heavy metal contamination, even in neutral waters, and in some cases, determined to be more sensitive than some genera of the order Ephemeroptera [10, 29-33]. Asian clam in situ toxicity has been determined to be sensitive to AMD inputs and test end points are found to be predicative of the resident benthic macroinvertebrate communities [11]. These data reinforce the importance of using multiple toxicity techniques in biological assessments, as no single "most sensitive" species nor the small battery of single species toxicity test organisms utilized in this investigation revealed environmental effects that were observed at higher levels of biological organization in Reed's Creek [34-35].

Further evidence that batteries of toxicity tests are required to conduct watershed evaluations lie in the need to connect laboratory with field data through in situ experiments. In situ tests utilizing Asian clams are useful as they bridge the gap between laboratory and field evaluations, and the clams' role as sediment residing filter-feeders expose them to both water column and sediment bound toxicants [36]. However, while performing these experiments in small headwater streams, flow may become a confounding variable. Small headwater streams may not contain running water for the entire extent of the 30-day test period. As a result increased mortality at these locations can introduce confounding evidence at stations where flow maybe limited. In such cases, laboratory tests become a valuable factor in the watershed evaluation. Synchronizing in situ Asian clam experiments with sediment and water column toxicity tests, as was the case in this investigation, can help diagnose false positives as a result of flow limitations, while also quantifying both sediment and water column exposures.

The particular combination of in situ and laboratory toxicity tests used in this investigation also helped to emphasize that, regardless of water column pH and sediment metal contaminant concentrations, ecological effects at the community level were consistently correlated with water chemistry and habitat availability. Although the effects of low pH on benthic communities have been well documented, the toxic effects of Al and Fe in neutral waters have not. A recent review by Goodyear and McNeil [37] found a wealth of data investigating the effects of Cu, Zn, lead (Pb) and cadmium (Cd) on benthic macroinvertebrate communities, but found infrequent investigations of Al and Fe. In an investigation of the AMD impact in Puckett's Creek, Soucek et al observed acute water column toxicity to *C. dubia*, with a $LC_{50} < 2$ % mine effluent as a result of high water column concentrations of Al and Fe in association with low pH [9]. However, toxicity to *C. dubia* persisted even in neutral waters a mile downstream of this mine effluent input into the stream as a result of the slow precipitation kinetics of Al [33]. An investigation of metal-contaminated sediments in this stream found that Fe precipitates in the absence of dissolved Fe caused toxicity to *D. magna*, presumably through physical abrasion [10]. The precipitation of Al and Fe can also limit habitat availability by filling crevices with flocculants, much like sedimentation, as evidenced by the positive correlation coefficients between habitat score and the benthic macroinvertebrate richness in this study. These lines of evidence suggest that Al and Fe can create persistent toxicity in neutral waters both in dissolved and solid forms, impairing benthic communities downstream of mining activities.

Sediment toxicity tests were insensitive to AMD inputs in this investigation. In fact, to our knowledge, no other investigation has attributed toxicity to invertebrates to elevated concentrations of Al or Fe in stream sediments. Most investigations of mining related sediment toxicity have concentrated on Cu, Zn, Pb and Cd [37]. The variability found in the sediment metals of this investigation contributes greatly to the fact that few significant differences were found between station category sediment chemistry. In addition, Fe as oxyhydroxides (FeOOH), is thought to sorb other potentially toxic metals, especially in oxic sediments found in steep headwater streams [38]. Other studies investigating the toxicity or bioavailability of metal-contaminated sediments have found that water column or pore water concentrations of heavy metals are more predictive of benthic macroinvertebrate community structure than are whole sediment metals concentrations [10, 13, 30, 38-39]. In Puckett's Creek, Soucek et al. found significant correlations between sediment Fe concentrations and sediment toxicity, which was also correlated with pH. Sediments in that study were found to have high percent water content [10]. This would suggest pore water concentrations of free Fe ions in association with acidic pH were the likely source of toxicity.

The sediment test approach used in the current investigation may have compromised the toxicity of these metal contaminated sediments. In collection, the sediments were briefly exposed to air, potentially oxidizing any free metal ions. Substituting reference water, in this case Sinking Creek water, for site water to overlay the sediments, may have diluted the pore water metal concentrations and neutralize pH at the soil water interface. Also, some argument has been made that *D. magna*, a zooplankter, is not a sediment dweller and therefore not exposed to sediment bound metals. Also, Schmidt et al [40] found that sediment toxicity tests utilizing *D. magna* or *C. tentans*, a sediment dwelling Dipteran, were not predictive of benthic macroinvertebrate community responses to Al and Fe dominated AMD seepage or sediments.

In conclusion, integrative assessments of Al and Fe dominated AMD impacted watersheds should focus on benthic macroinvertebrate community structure, water column metals, and in situ and water column toxicity tests. The use of multiple toxicity tests and organisms both in situ and in the laboratory may provide data that can diagnose false positives resultant of factors (i.e., lack of flow, predation) other then those of interest (i.e., heavy metals, acid). Also, the use of multiple toxicity tests can elucidate the mode of toxicity, further focusing future research efforts at the causative agents of toxicity. These data also suggest that sediment Al and Fe either are not bioavailable to *D. magna* or that the testing methods utilized in the current study compromised the toxicity of the sediments.

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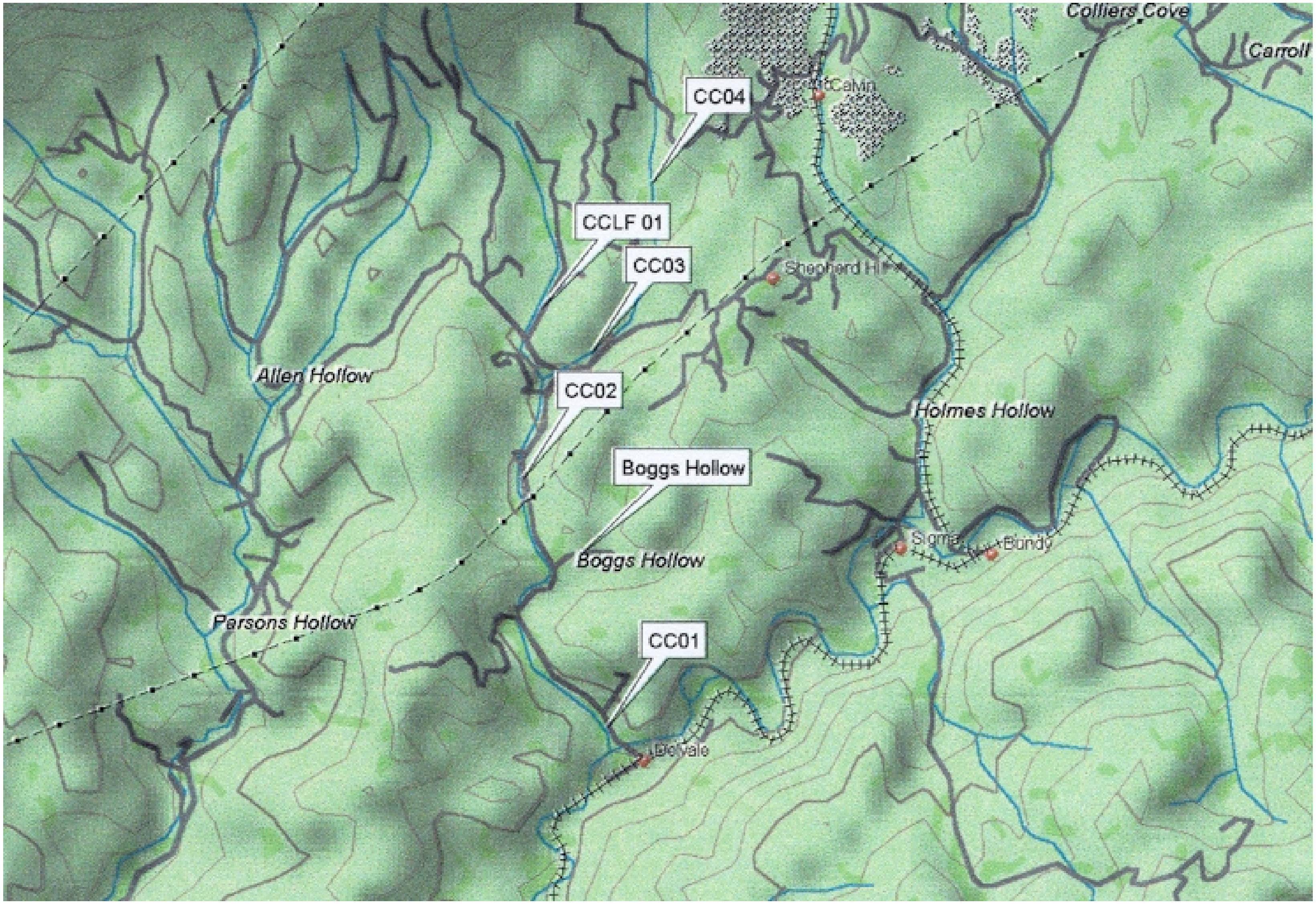
APPENDIX A, Cox Creek

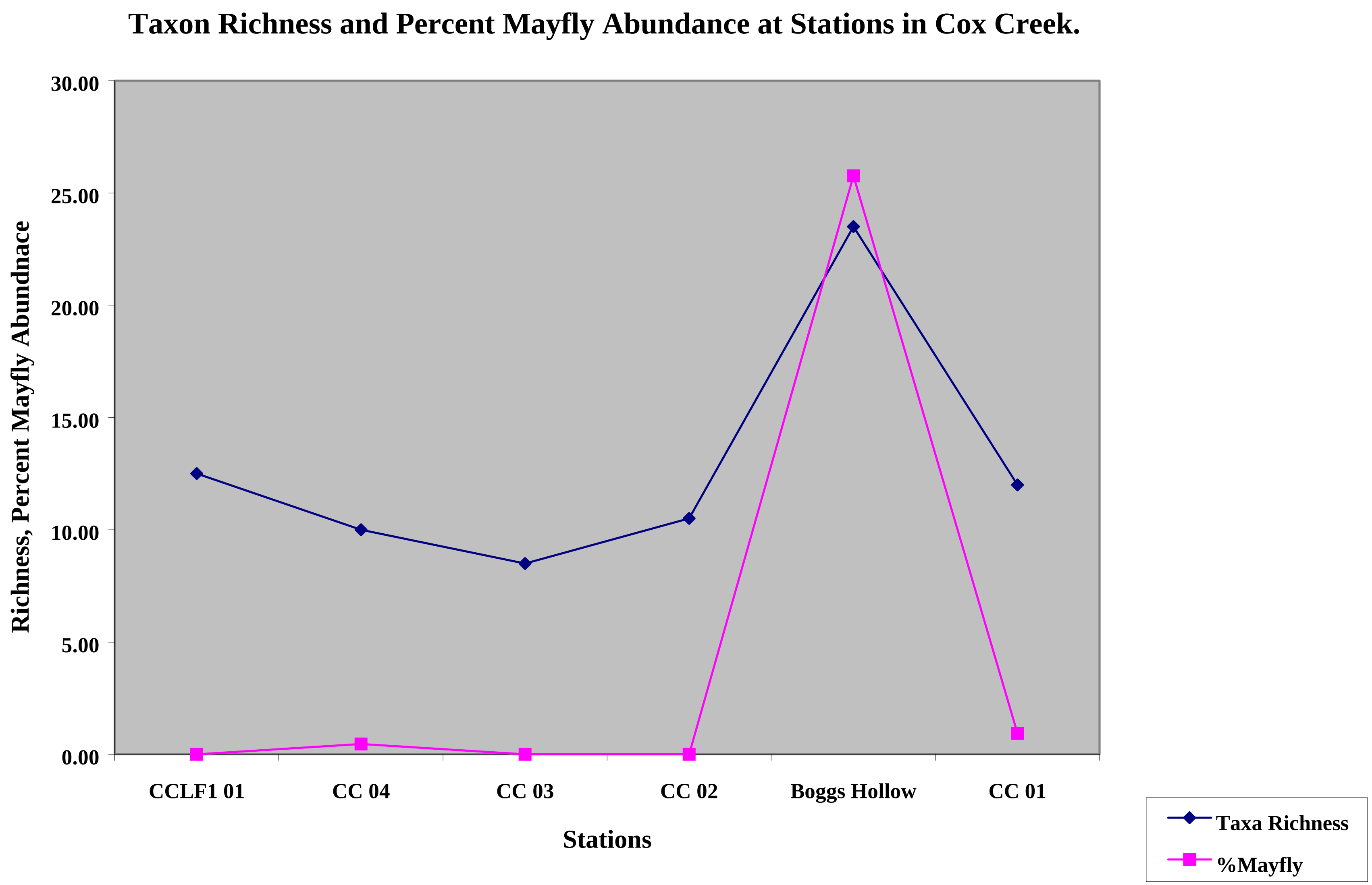
Map of Cox Creek Stations



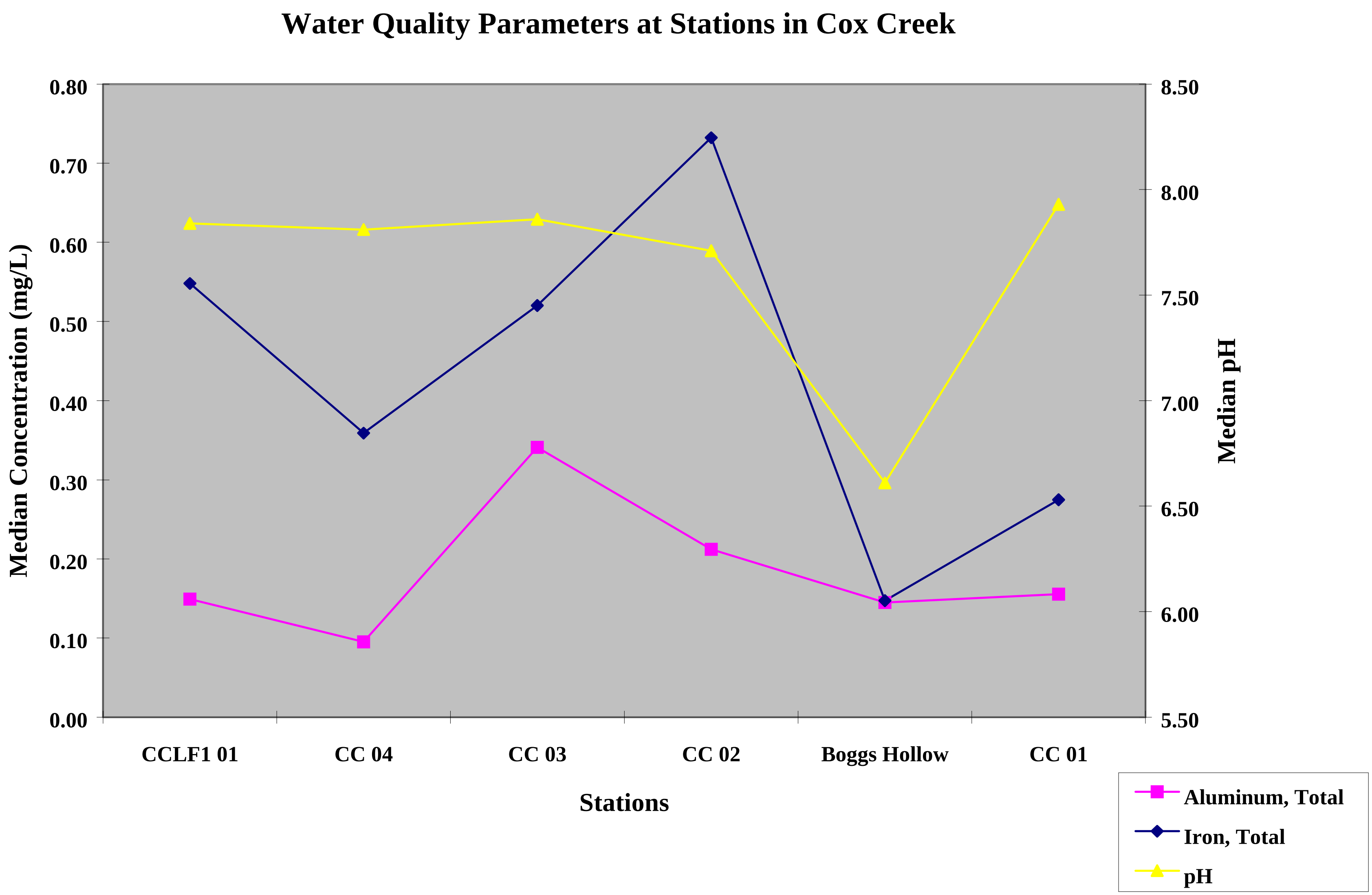
Picture of Agricultural Impacted Stations in Cox Creek

Picture of CC 01 at the Bottom of Cox Creek

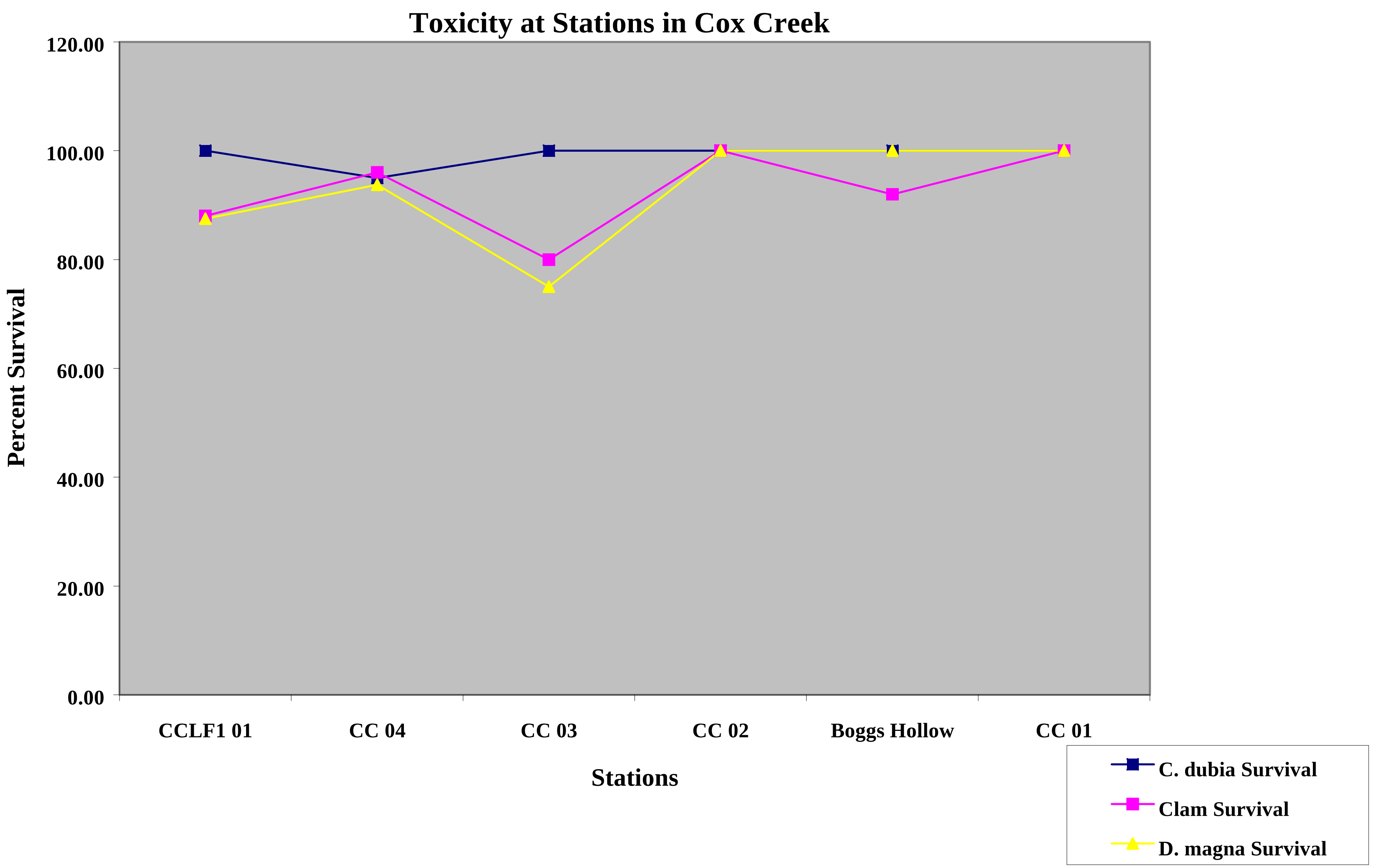
A.1. Graphs of Ecotoxicological Parameters for Cox Creek



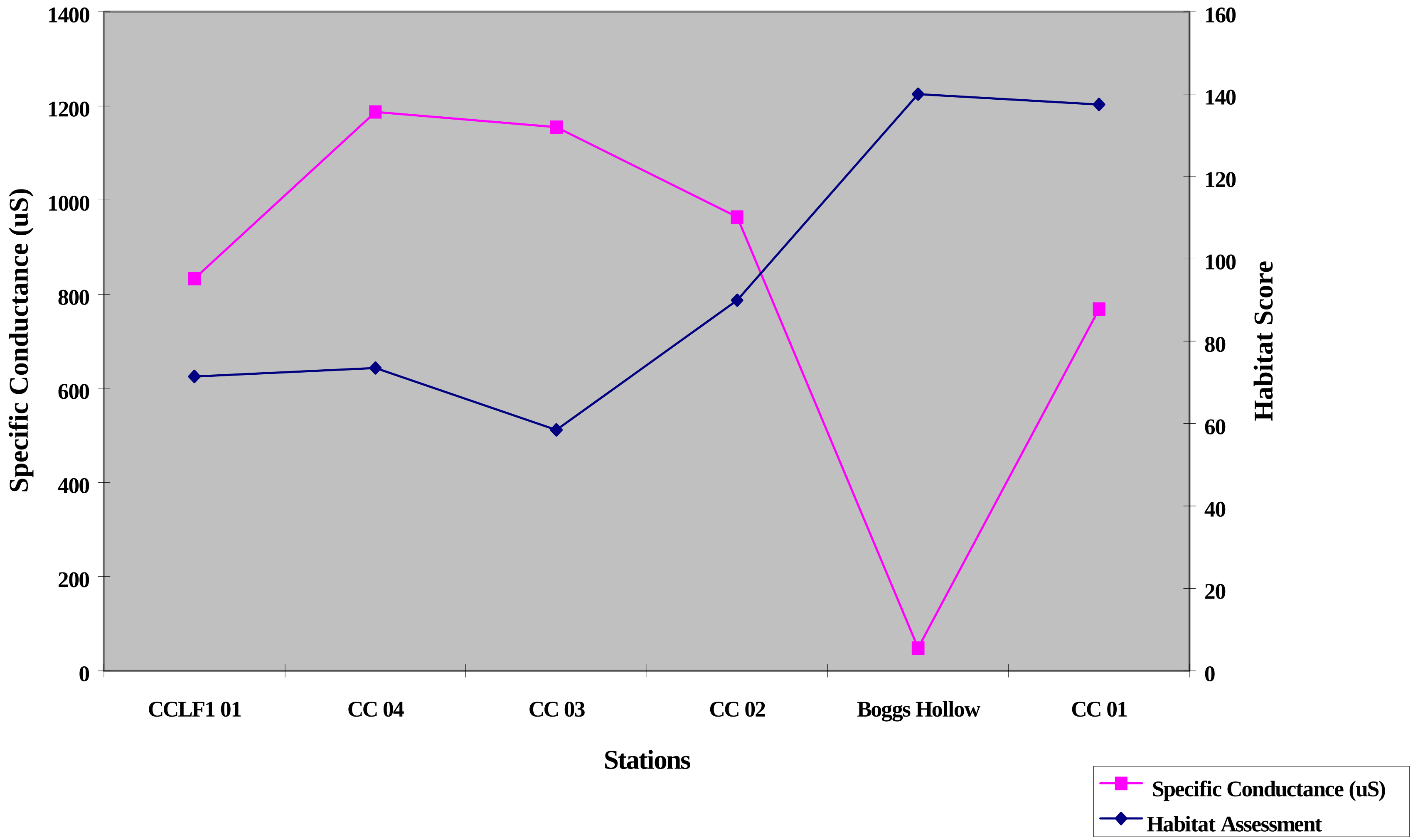
Taxon Richness and Percent Mayfly Abundance at Stations in Cox Creek.
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Richness, Percent Mayfly Abundnace
CCLF1 01
CC 04
CC 03
CC 02
Boggs Hollow
CC 01
Stations
Taxa Richness
%Mayfly

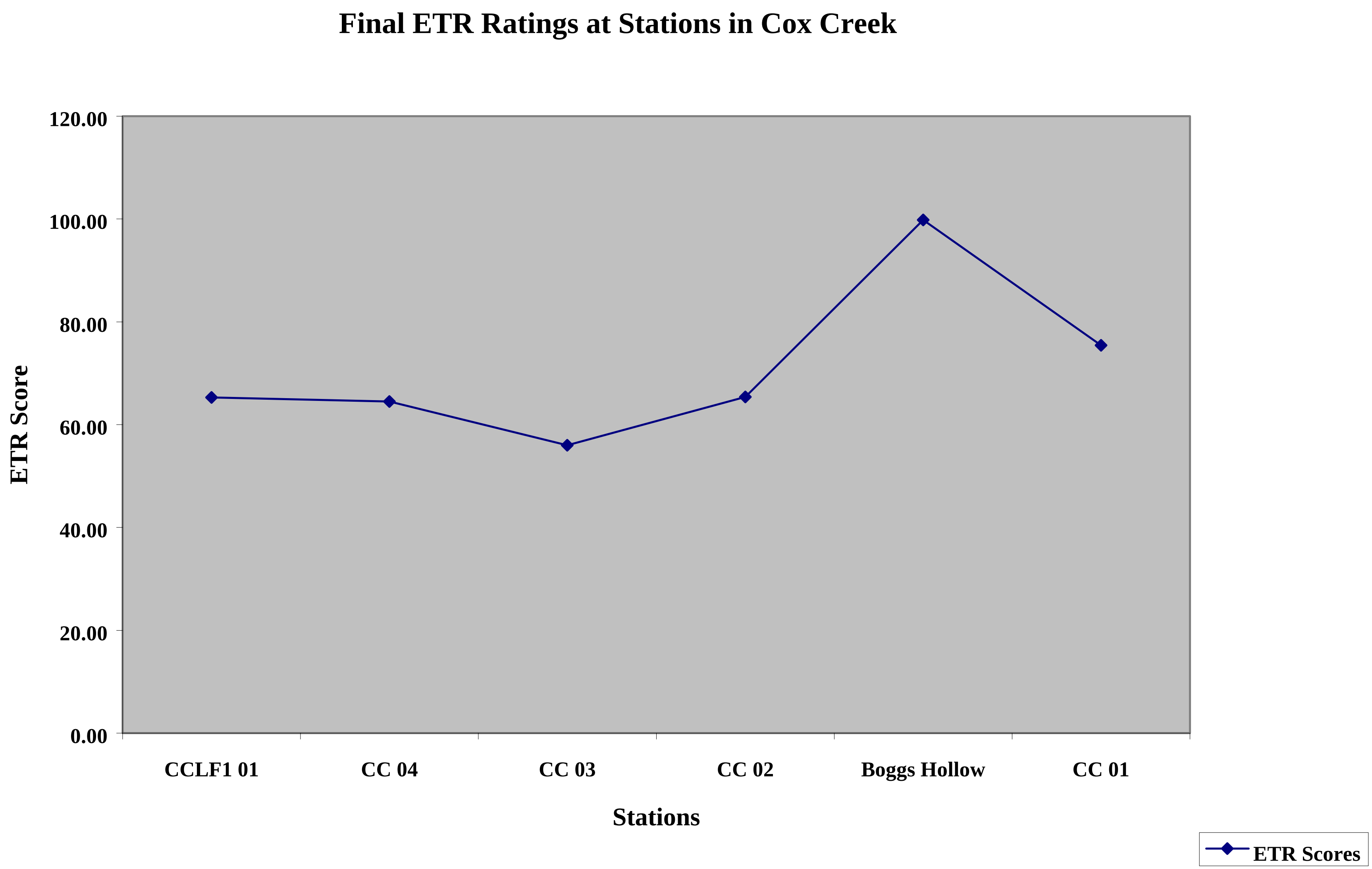


Water Quality Parameters at Stations in Cox Creek
Median Concentration (mg/L)
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Median pH
8.50
8.00
7.50
7.00
6.50
6.00
5.50
CCLF1 01
CC 04
CC 03
CC 02
Boggs Hollow
CC 01
Stations
Aluminum, Total
Iron, Total
pH


Toxicity at Stations in Cox Creek
Percent Survival
120.00
100.00
80.00
60.00
40.00
20.00
0.00
CCLF1 01
CC 04
CC 03
CC 02
Boggs Hollow
CC 01
Stations
C. dubia Survival
Clam Survival
D. magna Survival

Mean Habitat Assessment Score and Median Conductivity at Stations in Cox Creek




Final ETR Ratings at Stations in Cox Creek
120.00
100.00
80.00
60.00
40.00
20.00
0.00
ETR Score
CCLF1 01
CC 04
CC 03
CC 02
Boggs Hollow
CC 01
Stations
ETR Scores

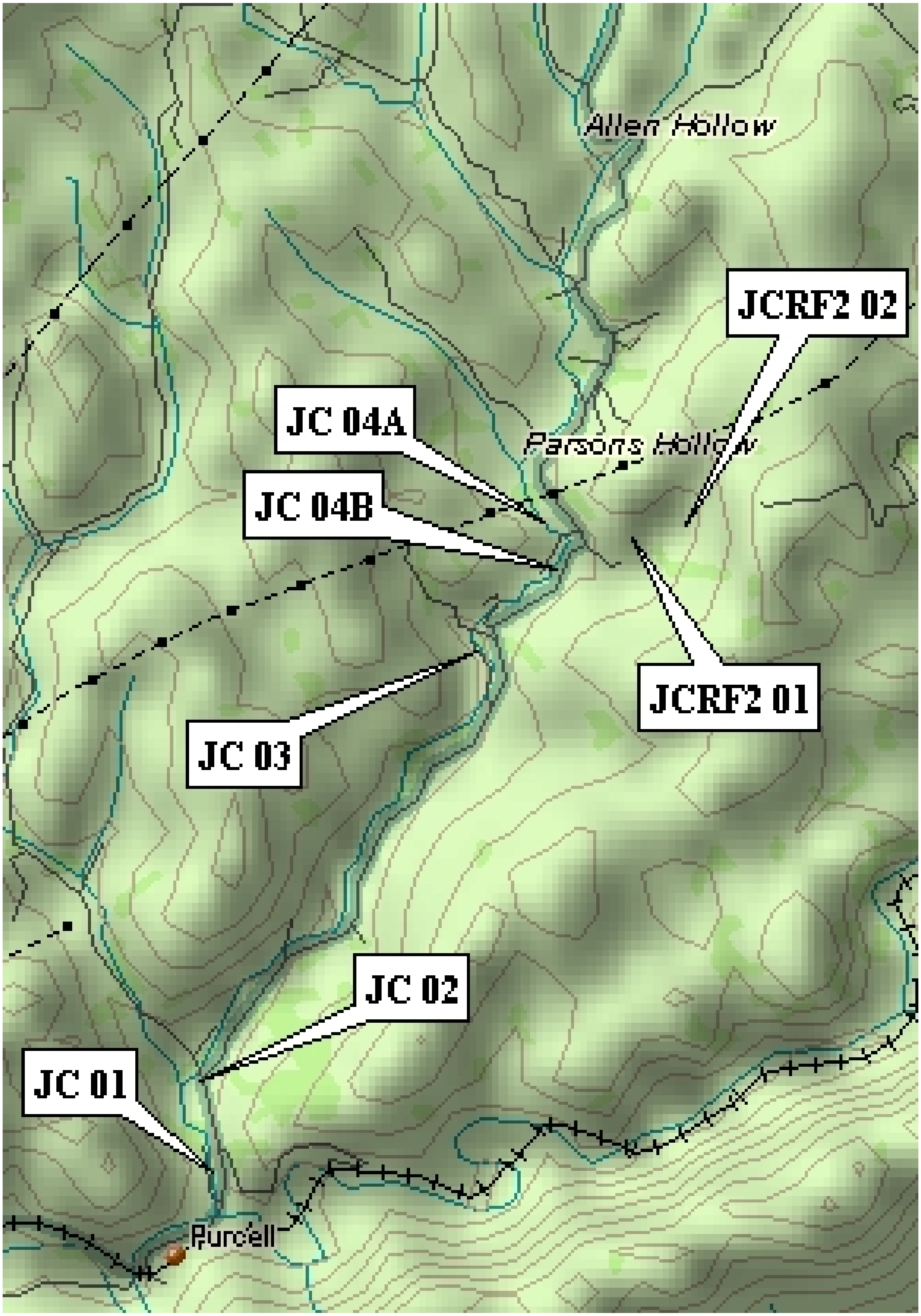
A.2. Benthic Macroinvertebrate Data, Cox Creek

DESC {Powell River Bugs 2000}	CCLF 01	CCLF 01	CC 04	CC 04
ASELLIDAE				3
CAMBARIDAE	2	1		
NEMOURIDAE	2			
Caenis				2
CORDULEGASTRIDAE	1	1		
Gomphus	1	1	3	14
Boyeria	6	2		1
CALOPTERYGIDAE	1	3	3	1
COENAGRIONIDAE	2	2		1
Nigronia				1
Cheumatopsyche	2			1
LIMNEPHILIDAE	1			
DYTISCIDAE				1
ELMIDAE	1		7	65
Hexatoma	1			
SIMULIIDAE	1	1		
TANYPODINAE			2	1
CERATOPOGONIDAE	2	1		3
Atherix			1	
EMPIDIDAE	1			4
SPHAERIIDAE		2		
DESC {Powell River Bugs 2000}	CC 03	CC 03	CC 02	CC 02
CAMBARIDAE				1
NEMOURIDAE				3
CORDULEGASTRIDAE				4
GOMPHIDAE		1		
Progomphus			3	6
Gomphus	1			
Boyeria	1	3	2	3
CALOPTERYGIDAE		1	2	1
COENAGRIONIDAE			1	3
SIALIDAE				1
Cheumatopsyche	3	3	16	
DYTISCIDAE		1		
ELMIDAE	1	3	5	6
TIPULIDAE			1	
Hexatoma	1			
SIMULIIDAE	45	12		3
CHIRONOMINAE/ORTHOCLADIINAE	24	49	31	38
CERATOPOGONIDAE	1	9	2	2

DESC {Powell River Bugs 2000}	Boggs Hollow	Boggs Hollow	CC 01	CC 01
CAMBARIDAE	7	4		1
NEMOURIDAE	5	4	3	4
PERLIDAE	3	1	2	1
Remenus	1	2		
Isoperla	2	2		
LEUCTRIDAE	82	47	4	9
CAPNIIDAE	1	4		
Caenis	3			
Drunella	1			
Ephemerella	10	5		
AMELETIDAE	8	4		
LEPTOPHLEBIIDAE	5	1		
BAETIDAE	43	14	1	
Stenonema	10	1		
ISONYCHIIDAE		1		
GOMPHIDAE			1	
Boyeria			2	
Nigronia	1	1		1
Hydropsyche			1	
Cheumatopsyche			9	18
RHYACOPHILIDAE	3	1	2	9
LEPIDOSTOMATIDAE	3			
LIMNEPHILIDAE		2	1	
Ectopria	2			
ELMIDAE		2	3	
TIPULIDAE			1	
Tipula	1	3		
Hexatoma	1			
DIXIDAE	1	2		
SIMULIIDAE		1	4	2
CHIRONOMINAE/ORTHOCLADIINAE	6	87	11	28
TANYPODINAE	2	7		
ATHERICIDAE			9	
Atherix	6	6		

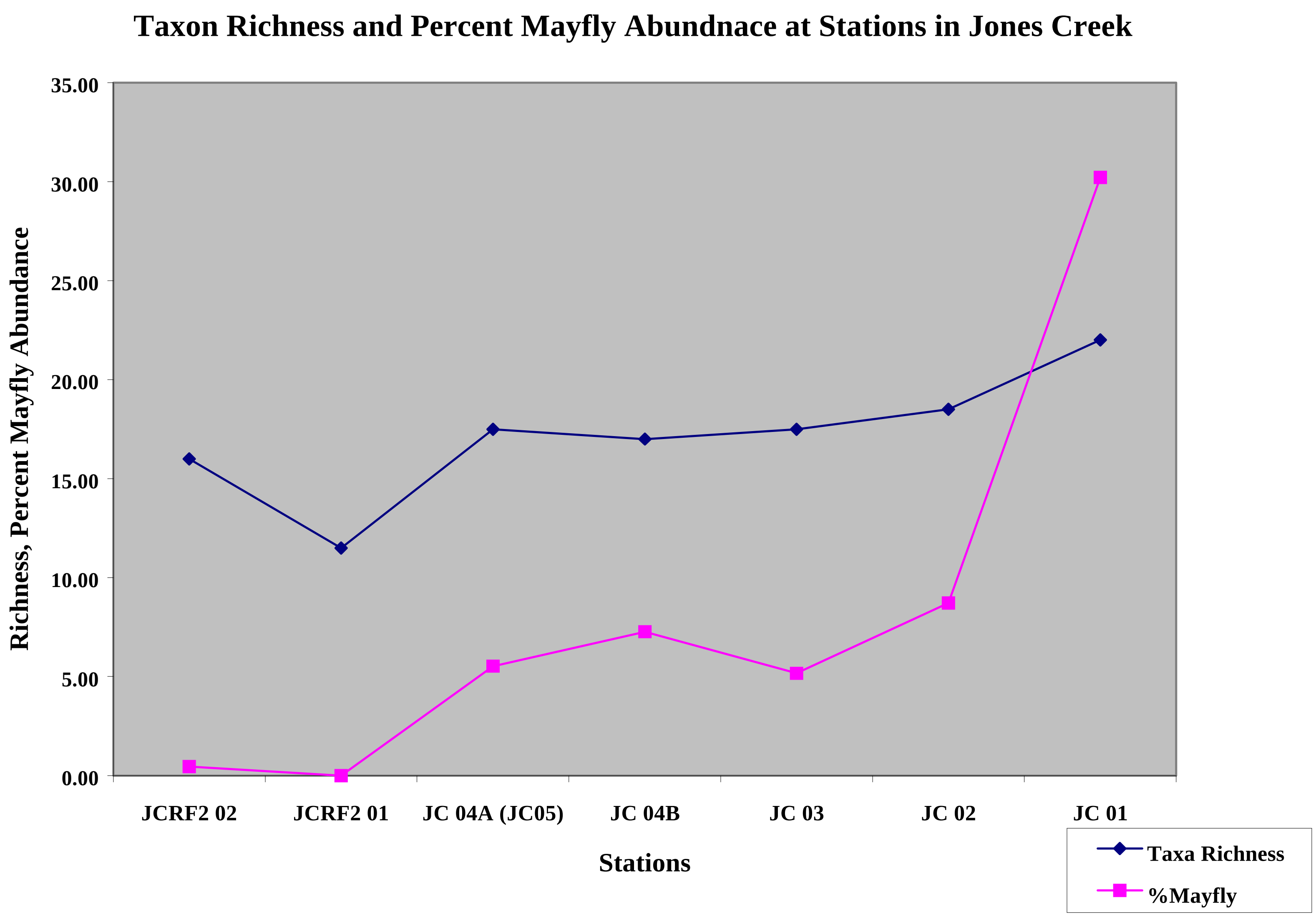
APPENDIX B, JONES CREEK

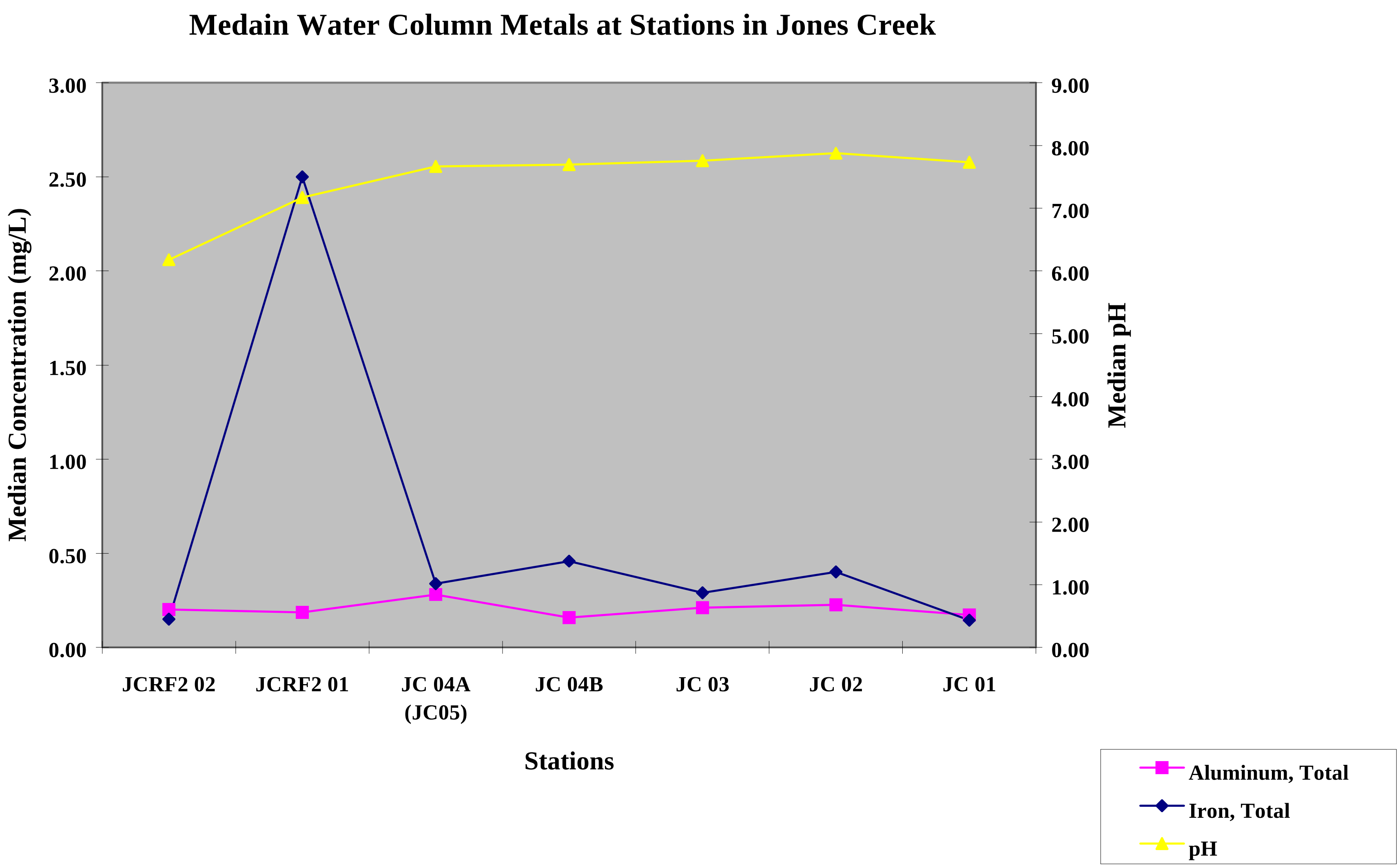
Jones Creek Stations



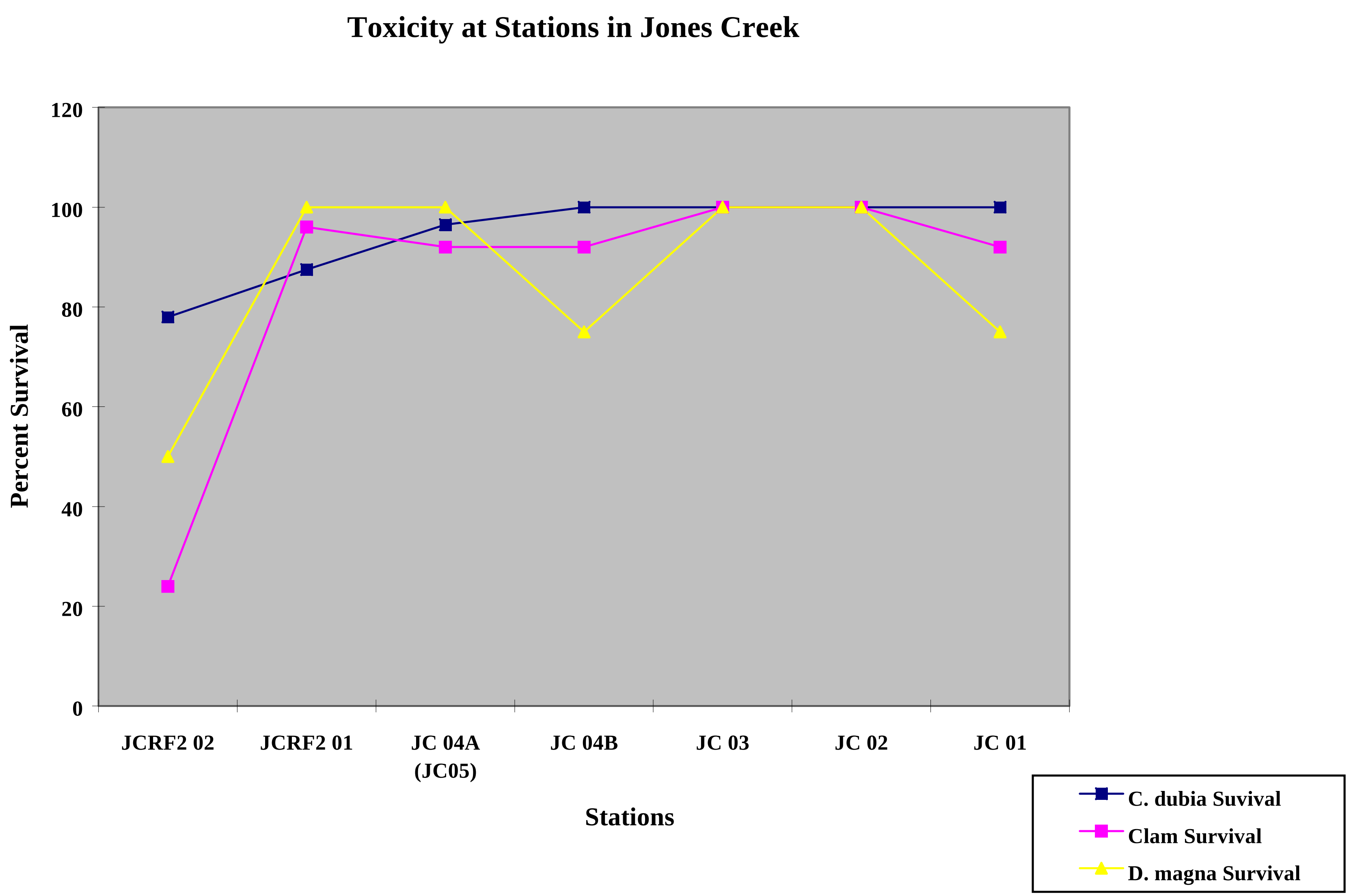
Robins Chapel Mine Blow Out in Jones Creek

B.1. Graphs of Ecotoxicological Parameters for Jones Creek

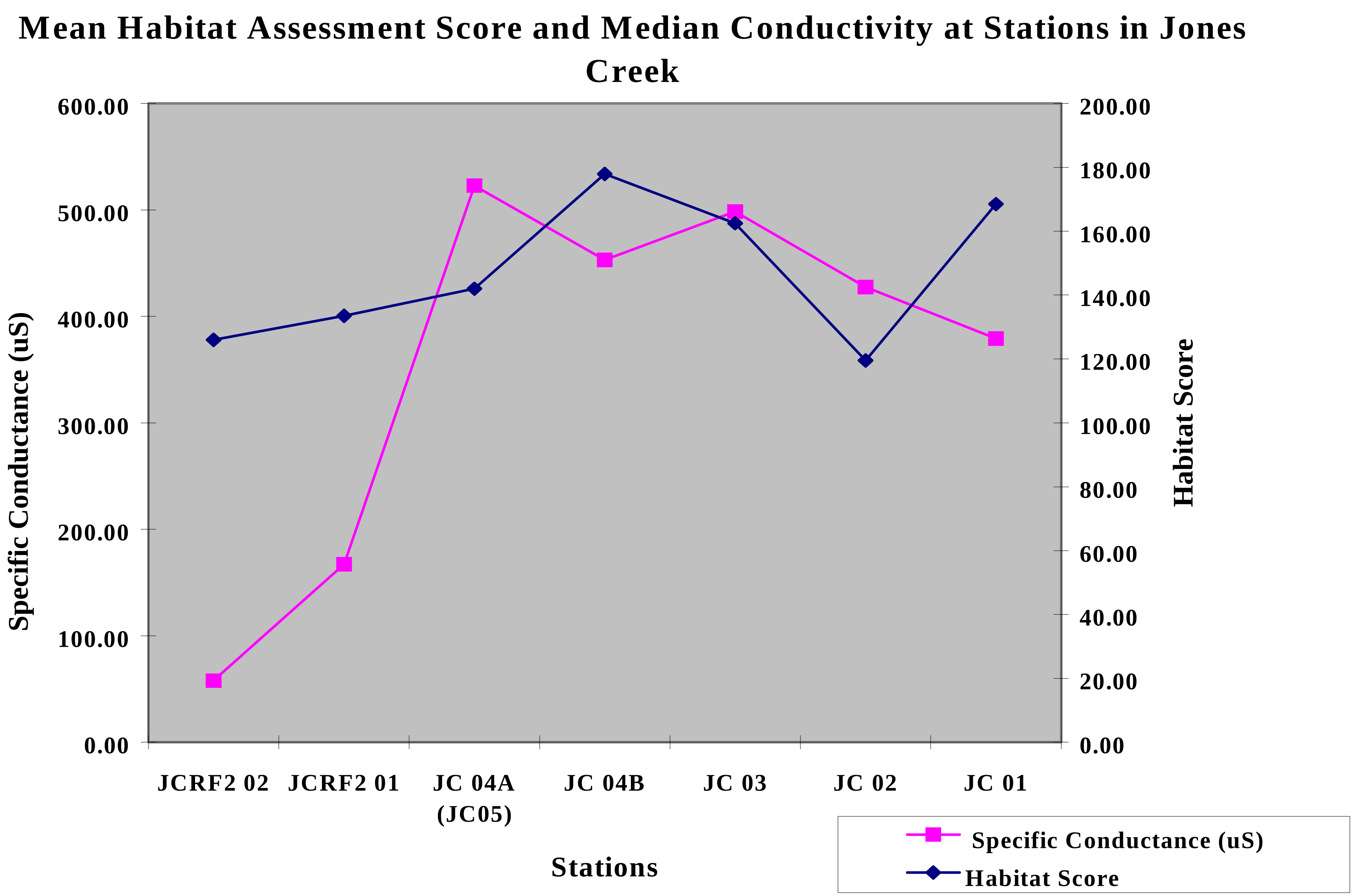

Taxon Richness and Percent Mayfly Abundnace at Stations in Jones Creek
Richness, Percent Mayfly Abundance
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
JCRF2 02
JCRF2 01
JC 04A (JC05)
JC 04B
JC 03
JC 02
JC 01
Stations
Taxa Richness
%Mayfly



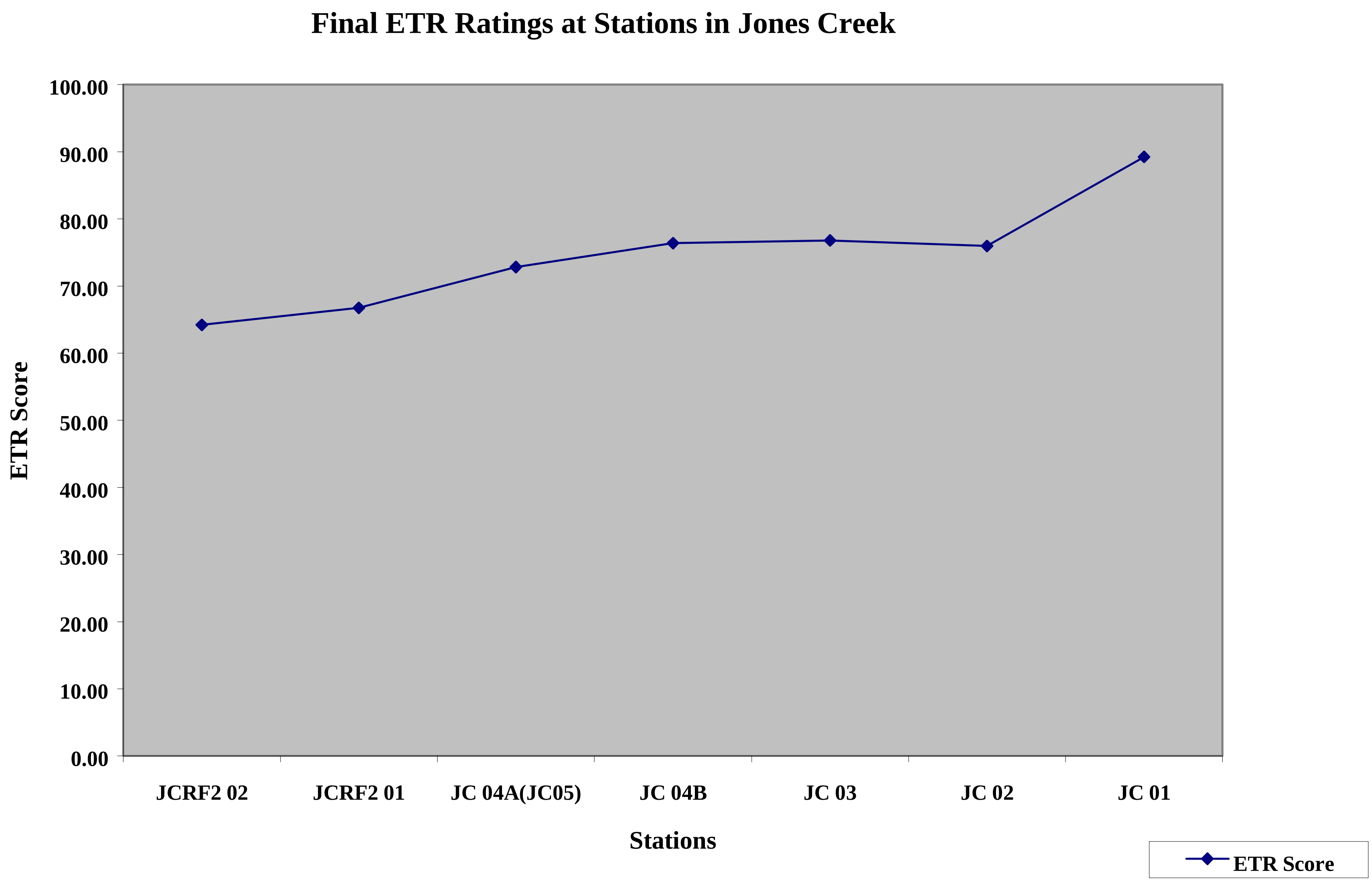
Medain Water Column Metals at Stations in Jones Creek
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Median Concentration (mg/L)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Median pH
JCRF2 02
JCRF2 01
JC 04A (JC05)
JC 04B
JC 03
JC 02
JC 01
Stations
Aluminum, Total
Iron, Total
pH



Toxicity at Stations in Jones Creek
120
100
80
60
40
20
0
Percent Survival
JCRF2 02
JCRF2 01
JC 04A (JC05)
JC 04B
JC 03
JC 02
JC 01
Stations
C. dubia Suvival
Clam Survival
D. magna Survival



Mean Habitat Assessment Score and Median Conductivity at Stations in Jones Creek
600.00
500.00
400.00
300.00
200.00
100.00
0.00
Specific Conductance (uS)
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Habitat Score
JCRF2 02
JCRF2 01
JC 04A (JC05)
JC 04B
JC 03
JC 02
JC 01
Stations
Specific Conductance (uS)
Habitat Score


Final ETR Ratings at Stations in Jones Creek
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
ETR Score
JCRF2 02
JCRF2 01
JC 04A(JC05)
JC 04B
JC 03
JC 02
JC 01
Stations
ETR Score

B.2. Benthic Macroinvertebrate Data, Jones Creek

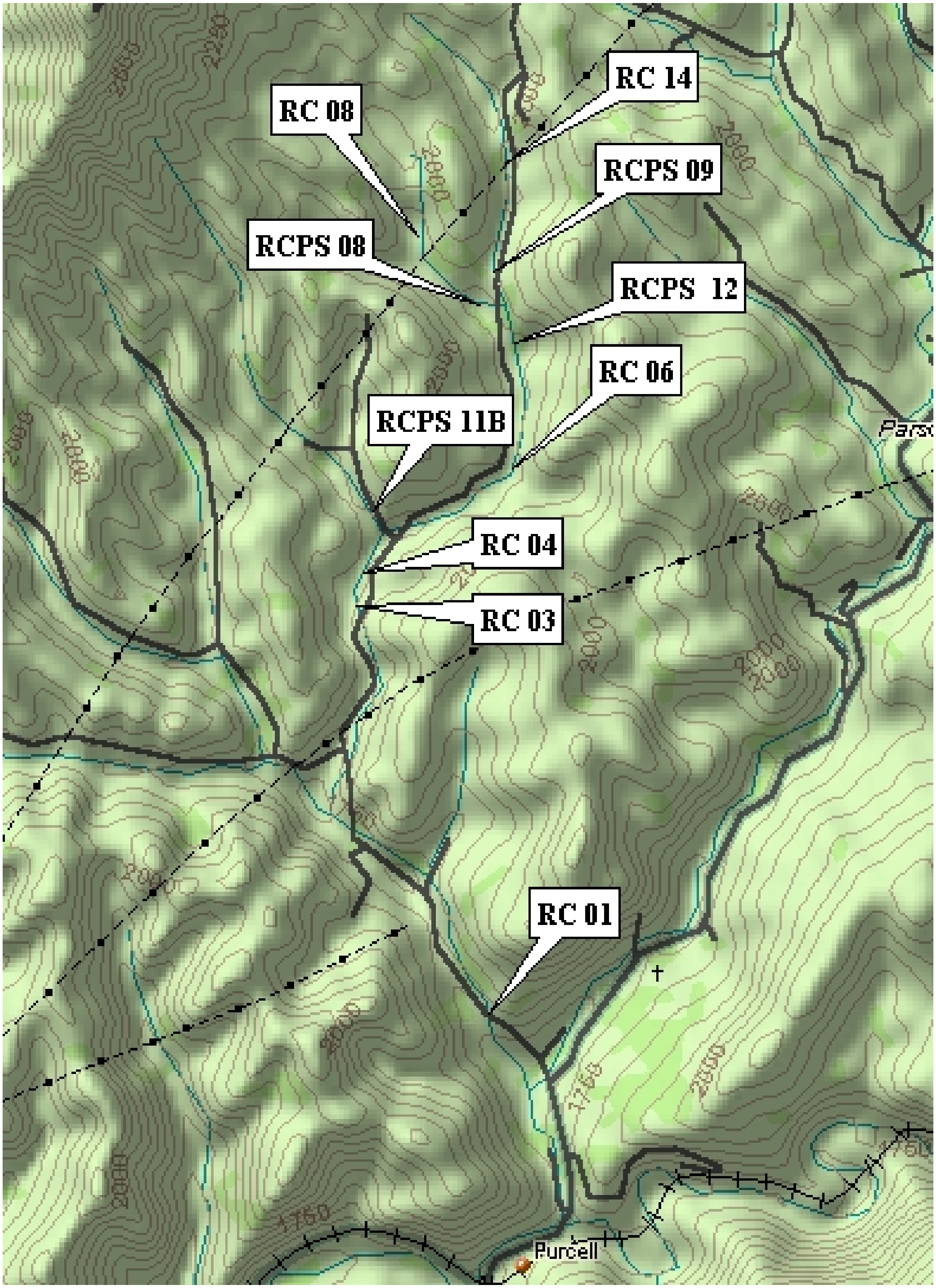
DESC {Powell River Bugs 2000}	JCRF2 02	JCRF2 02	JCRF2 01	JCRF2 01
CAMBARIDAE			4	
NEMOURIDAE	4	2	1	1
Strophopteryx			1	
LEUCTRIDAE	10	15	5	4
LEPTOPHLEBIIDAE	1			
BAETIDAE		1		
Boyeria		1		
CORDULIIDAE	5			
LIBELLULIDAE	2			
Chauliodes		1		
Hydropsyche	2			
Cheumatopsyche		7	2	1
LEPIDOSTOMATIDAE		1		
DYTISCIDAE	25	4		
HYDROPHILIDAE			3	1
ELMIDAE				1
TIPULIDAE	2			
Tipula		1	21	6
Hexatoma		1		
Limnophila	1			
Molophilus		1		
CULICIDAE	1			
SIMULIIDAE	23	80	4	1
TANYPODINI	98	23		
TANYPODINAE			1	
CHIRONOMINAE	65	53	72	38
CERATOPOGONIDAE			4	
TABANIDAE	2	1	1	
Atherix		3	10	8
EPHYDRIDAE				1
SPHAERIIDAE	2	5		

DESC {Powell River Bugs 2000}	JC04A	JC04A	JC 04B	JC 04B	JC 03	JC 03
ASELLIDAE	1					
CAMBARIDAE	4	6	5	1		7
NEMOURIDAE		1			4	1
PERLIDAE					1	1
Isoperla		2				
LEUCTRIDAE			6	10	16	12
Ephemera	2	2	4	3		1
CAENIDAE	5			8		1
Caenis		1				
BAETIDAE	11	2	1	6	14	
Stenonema			4			1
ISONYCHIIDAE				1	3	1
Stylogomphus	2	1	28	5		12
Gomphus	1					
Boyeria	3			4	1	1
Sialis		1	2			
Chauliodes						
Nigronia	1		9	3	2	
Hydropsyche	3	23	9	1	46	1
Cheumatopsyche	24	94	76	34	55	147
RHYACOPHILIDAE			2	1	2	3
Chimarra						1
Dolophilodes					1	
Pycnopsyche	1					
Psephenus						6
DRYOPIDAE				1		
ELMIDAE	7	17	1		2	13
Tipula	1		5			2
Antocha	1					
SIMULIIDAE	10	4	5	2	3	2
TANYPODINI						
CHIRONOMINAE/ORTHOCLADIINAE		70				
TANYPODINAE	1	2		2	2	
CHIRONOMINAE	123		41	93	46	5
CERATOPOGONIDAE	1					
Atherix				1	2	
EMPIDIDAE		2	3	3		
SPHAERIIDAE	1					

DESC {Powell River Bugs 2000}	JC 02	JC 02	JC 01	JC 01
CAMBARIDAE	4	10	12	
PERLIDAE	2	3	3	2
Isoperla	2			
Rasvena			11	46
LEUCTRIDAE	33	77		
Leuctra			8	10
Paracapnia			9	7
Ephemera	2	9		
Litobrancha recurvata			1	
CAENIDAE		1		
BAETISCIDAE	11		37	45
AMELETIDAE		1		
BAETIDAE		5	4	4
Heptagenia			16	14
Ephoron				1
ISONYCHIIDAE	4	6		
Stylogomphus	6	5		
Boyeria	2			
Erythemis			1	
Hetaerina			2	1
Mesovelia			33	5
VELIIDAE			4	5
Palmacorixa			2	
NOTONECTINAE			1	
SIALIDAE				1
Nigronia		1		
Hydropsyche	1	1	1	
Cheumatopsyche	36	10		
Rhyacophila			1	
Neureclipsis			8	7
MOLANNIDAE			4	3
DYTISCIDAE				
GYRININAE			1	1
Psephenus	22	17		
ELMIDAE	10	6		
CURCULIONIDAE			32	4
Tipula	26	4		
Limnophila				1
Pseudolimnophila			12	51
Psorophora			3	
SIMULIIDAE	1			
TANYPODINI				1
TANYPODINAE	4	4		
CHIRONOMINAE	71	52		
EMPIDIDAE	1	1		
MUSCIDAE		1		

APPENDIX C, REEDS CREEK

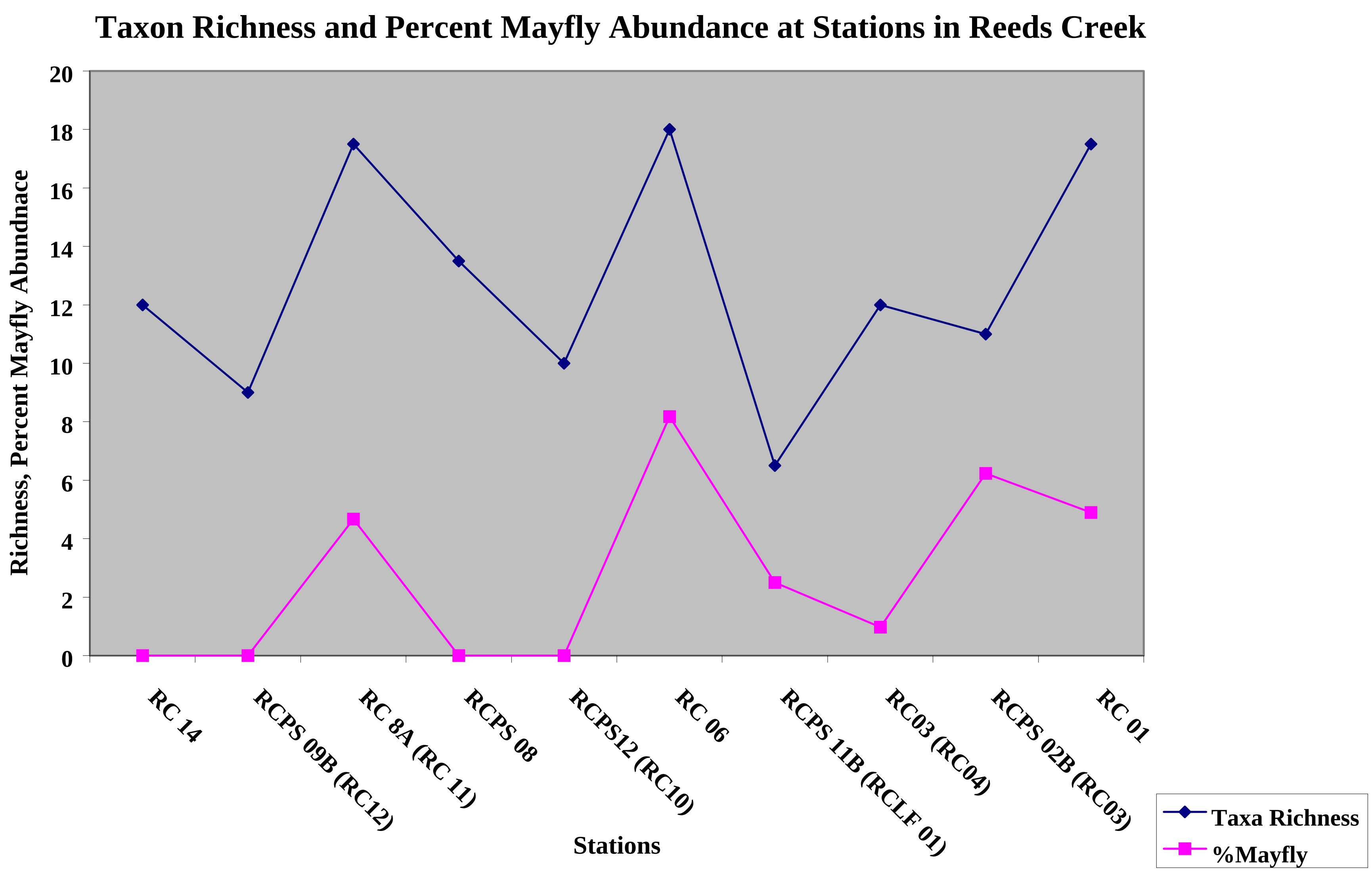
Reeds Creek Stations



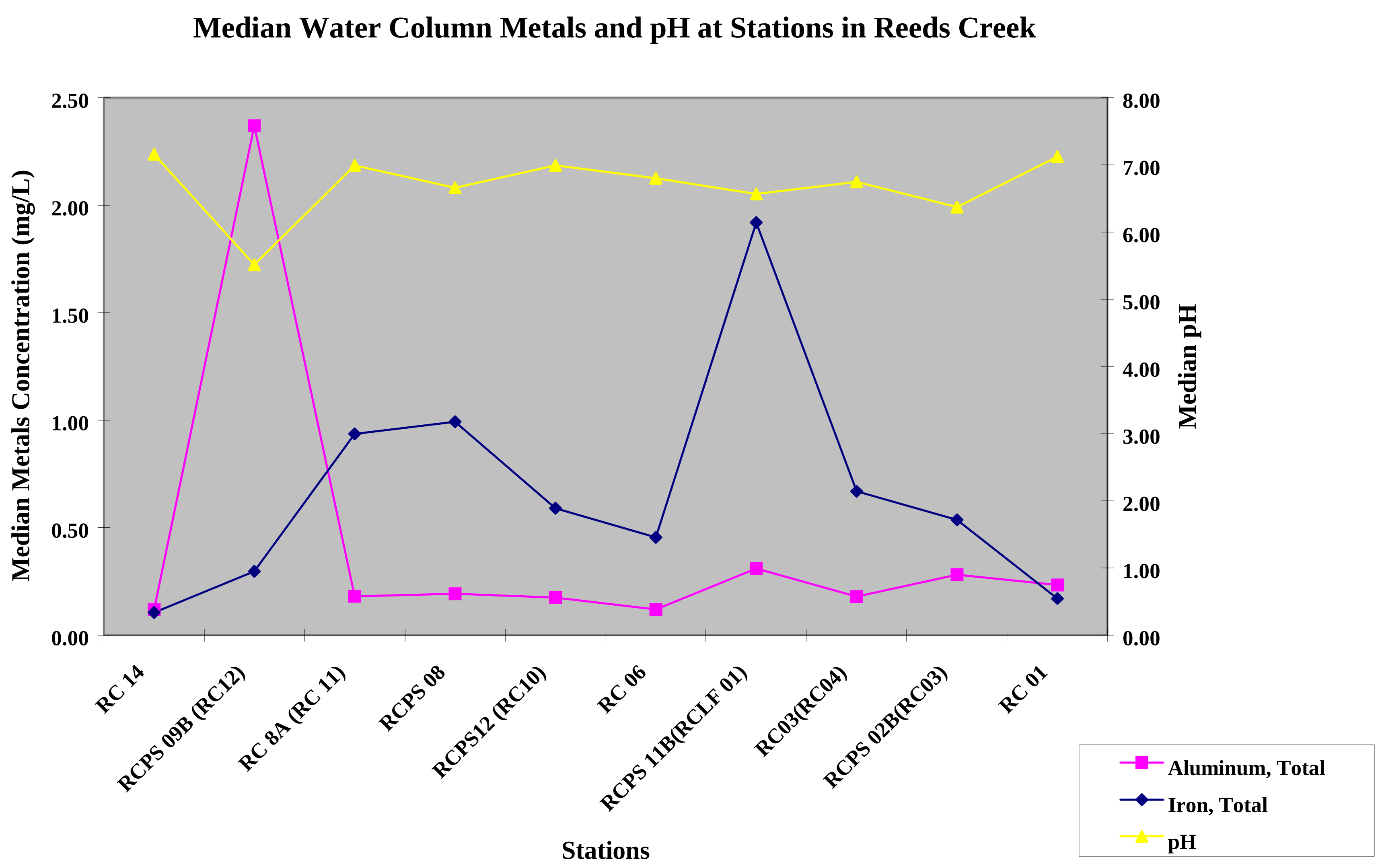
Picture of RC 03

Picture of McPherson Coal Bond Forfeiture

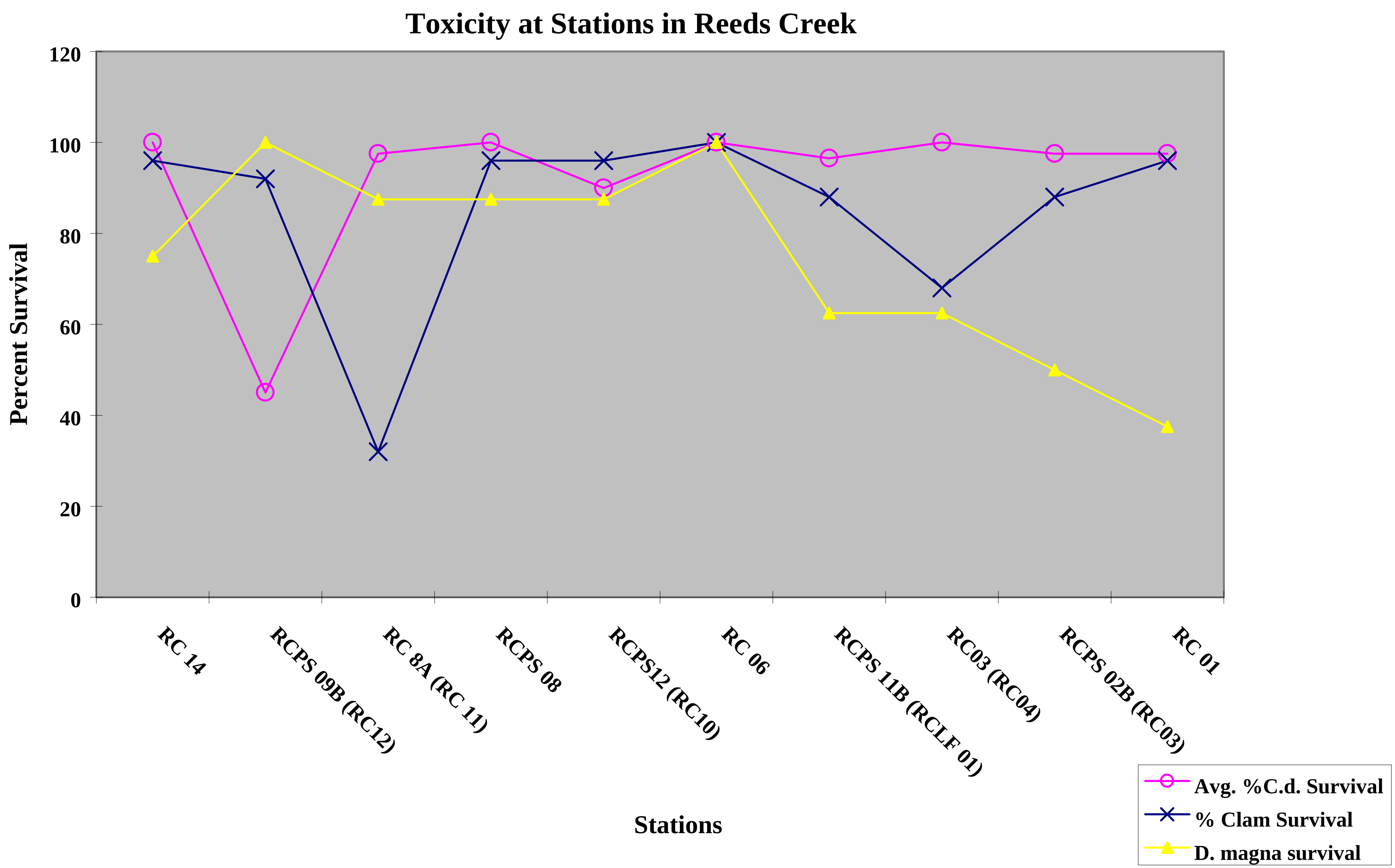
C.1. Graphs of Ecotoxicological Parameters for Reeds Creek

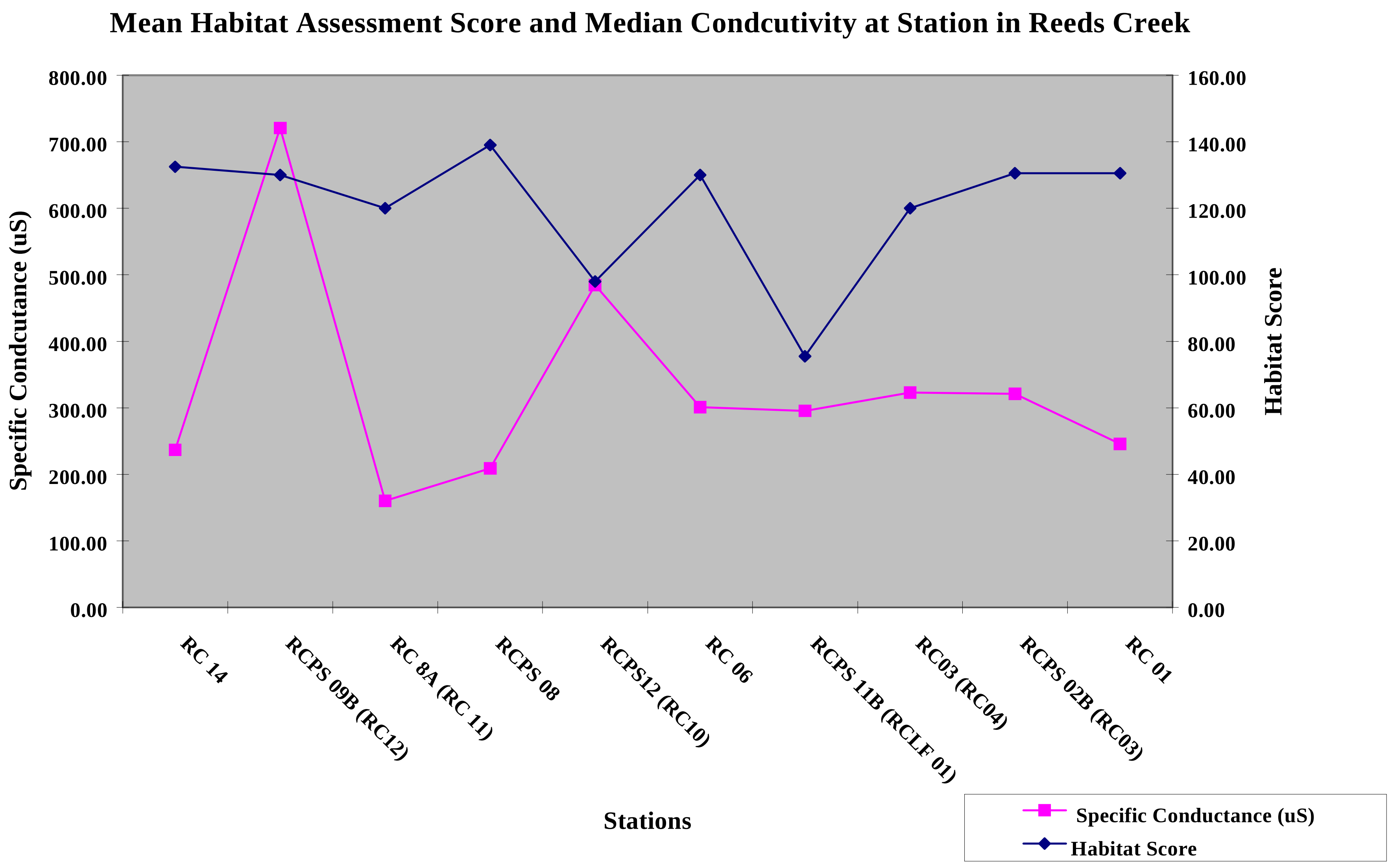


Taxon Richness and Percent Mayfly Abundance at Stations in Reeds Creek
Richness, Percent Mayfly Abundnace
20
18
16
14
12
10
8
6
4
2
0
RC 14
RCPS 09B (RC12)
RC 8A (RC 11)
RCPS 08
RCPS12 (RC10)
RC 06
RCPS 11B (RCLF 01)
RC03 (RC04)
RCPS 02B (RC03)
RC 01
Stations
Taxa Richness
%Mayfly



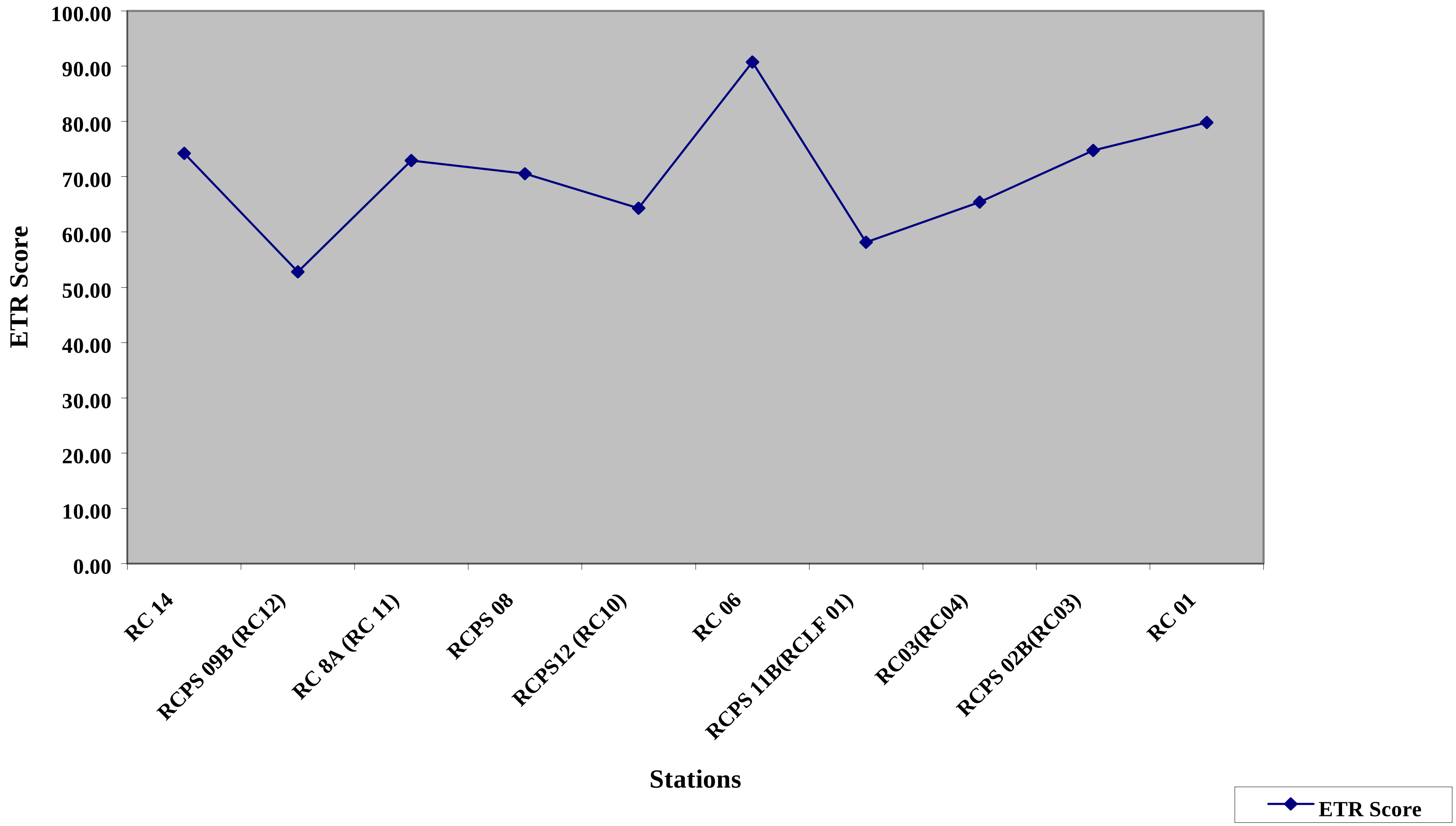
Median Water Column Metals and pH at Stations in Reeds Creek
Median Metals Concentration (mg/L)
Median pH
0.00
0.50
1.00
1.50
2.00
2.50
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
RC 14
RCPS 09B (RC12)
RC 8A (RC 11)
RCPS 08
RCPS12 (RC10)
RC 06
RCPS 11B(RCLF 01)
RC03(RC04)
RCPS 02B(RC03)
RC 01
Stations
Aluminum, Total
Iron, Total
pH


Toxicity at Stations in Reeds Creek
120
100
80
60
40
20
0
Percent Survival
RC 14
RCPS 09B (RC12)
RC 8A (RC 11)
RCPS 08
RCPS12 (RC10)
RC 06
RCPS 11B (RCLF 01)
RC03 (RC04)
RCPS 02B (RC03)
RC 01
Stations
Avg. %C.d. Survival
% Clam Survival
D. magna survival



Mean Habitat Assessment Score and Median Condcutivity at Station in Reeds Creek
Specific Condcutance (uS)
Habitat Score
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
RC 14
RCPS 09B (RC12)
RC 8A (RC 11)
RCPS 08
RCPS12 (RC10)
RC 06
RCPS 11B (RCLF 01)
RC03 (RC04)
RCPS 02B (RC03)
RC 01
Stations
Specific Conductance (uS)
Habitat Score

Final ETR Ratings at Stations in Reeds Creek



C.2. Benthic Macroinvertebrate Data, Reeds Creek

DESC {Powell River Bugs 2000}	RC14	RC14	RC8A	RC8A
CAMBARIDAE	4	1	2	
NEMOURIDAE	1		1	
Strophopteryx	1	1	2	
LEUCTRIDAE	13	32	9	10
LEPTOPHLEBIIDAE			6	
BAETIDAE			4	2
CORDULEGASTRIDAE	8	21	1	3
Sialis		1		2
Nigronia	1	2	1	
Cheumatopsyche	1		1	4
Wormaldia		1		1
Dolophilodes	1		1	
LIMNEPHILIDAE		4		1
ELMIDAE				1
Tipula			6	
Antocha			2	
Hexatoma	1		1	1
Limnophila	2	31	7	8
DIXIDAE		2		
SIMULIIDAE			1	2
CHIRONOMINAE/ORTHOCLADIINAE	6	14	52	128
TANYPODINAE	2			19
CERATOPOGONIDAE			1	3
Atherix			21	5
PTYCHOPTERIDAE			1	10

DESC {Powell River Bugs 2000}	RCPS09B	RCPS09B
CAMBARIDAE		1
LEUCTRIDAE	7	1
CORDULEGASTRIDAE		1
Sialis		4
PHRYGANEIDAE	2	1
LIMNEPHILIDAE	1	
POLYCENTROPODIDAE	1	
Tipula	3	4
Limnophila	1	
CHIRONOMINAE/ORTHOCLADIINAE	3	1
TANYPODINAE	1	
STRATIOMYIIDAE	2	
Atherix		1
EMPIDIDAE	1	

DESC {Powell River Bugs 2000}	RCPS08	RCPS08	RCPS12	RCPS12
CAMBARIDAE		2	2	1
NEMOURIDAE	1			
Taeniopteryx		1		
LEUCTRIDAE	35	7	21	8
CORDULEGASTRIDAE	3	2		2
Gomphus	1			
Sialis		2	7	2
Nigronia	1			
LIMNEPHILIDAE			1	
POLYCENTROPODIDAE	1			
DYTISCIDAE	1			
HYDROPHILIDAE	1	3		
TIPULIDAE			4	
Tipula	2	3		7
Antocha	1	1	1	
Limnophila				2
DIXIDAE	1			
SIMULIIDAE			1	
CHIRONOMINAE/ORTHOCLADIINAE	4	10	26	28
TANYPODINAE	25	1	3	
CERATOPOGONIDAE				1
TABANIDAE	1			1
Atherix	6	2		2
SCIOMYZIDAE				1
EMPIDIDAE		1		

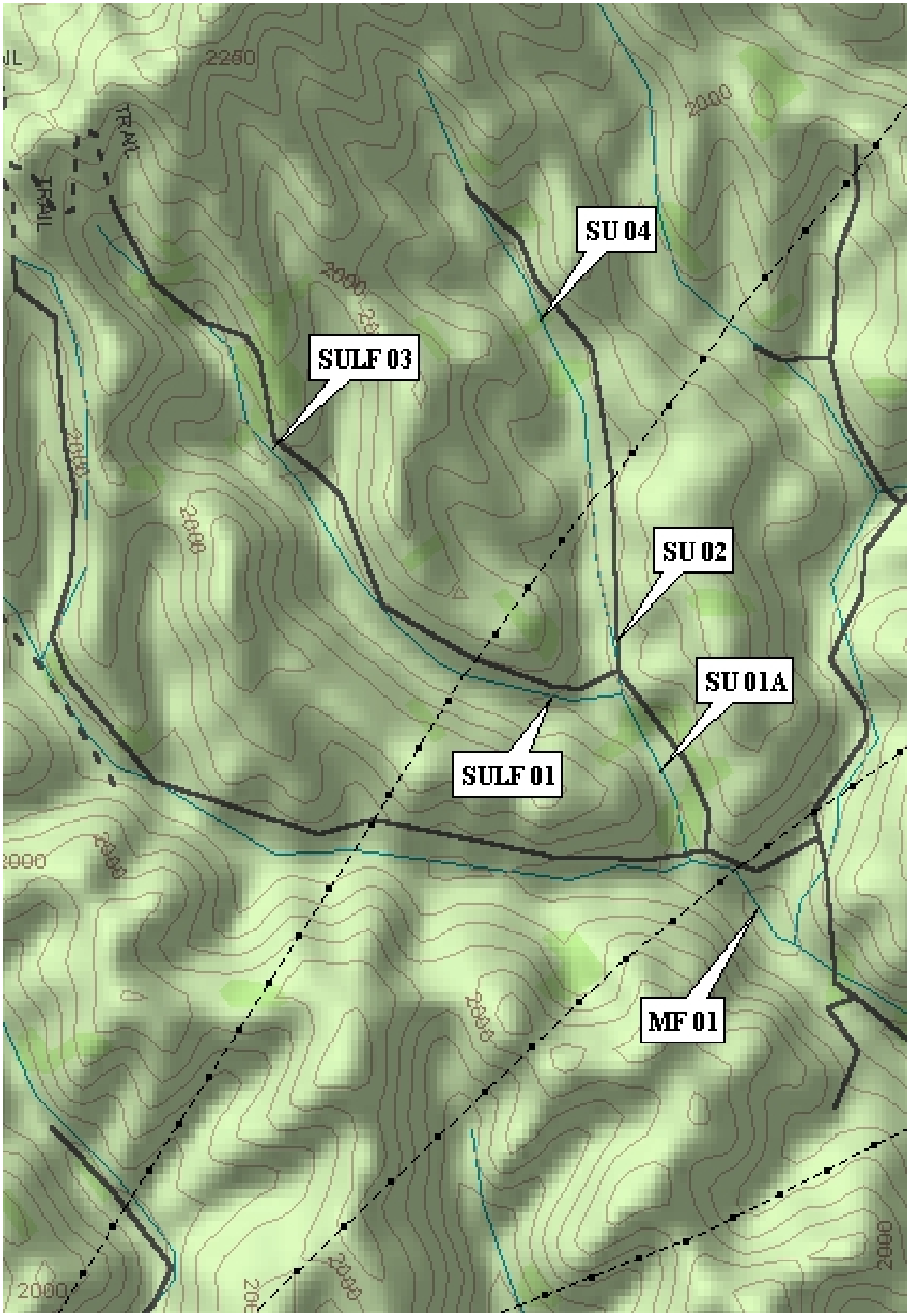
DESC {Powell River Bugs 2000}	RC06	RC06	RCPS10	RCPS10
CAMBARIDAE	5	5	3	2
NEMOURIDAE				3
PERLIDAE	1			
LEUCTRIDAE	26	9		
EPHEMERIDAE	8	1		
CAENIDAE				1
Caenis	11	5		
BAETIDAE	1			
CORDULEGASTRIDAE		1		1
Stylogomphus	13	13	1	
Gomphus	2			
Dromogomphus	3			
Calopteryx	2	3		
Nigronia	4			
Cheumatopsyche		1		
PHRYGANEIDAE	1			1
POLYCENTROPODIDAE		1		
NOCTUIDAE				1
DYTISCIDAE	5			
HYDROPHILIDAE		1		
ELMIDAE	2	4		
TANYDERIDAE	1			
Tipula	1			7
DIXIDAE		2		
SIMULIIDAE	1			
CHIRONOMINAE/ORTHOCLADIINAE	90	63	1	3
TANYPODINAE	3	2	1	1
CERATOPOGONIDAE	1	1		
PLANORBIDAE	1			

DESC {Powell River Bugs 2000}	RC04	RC04	RC03	RC03
CAMBARIDAE	3	9		1
LEUCTRIDAE	8	30	1	
CAPNIIDAE				1
Caenis		3	1	3
LEPTOPHLEBIIDAE				1
Stylogomphus		12	8	1
SIALIDAE		3		
Sialis			1	
Nigronia	2	1		2
Hydropsyche	1			2
Cheumatopsyche	1	3	4	10
Chimarra				2
PHRYGANEIDAE				1
LIMNEPHILIDAE				2
PYRALIDAE	1			
ELMIDAE	3	3		3
Tipula	3	20	1	
Antocha		1		
Limnophila	2			
CHIRONOMINAE/ORTHOCLADIINAE	23	54	11	15
TANYPODINAE	6	14	1	
CERATOPOGONIDAE				1
ATHERICIDAE		1		

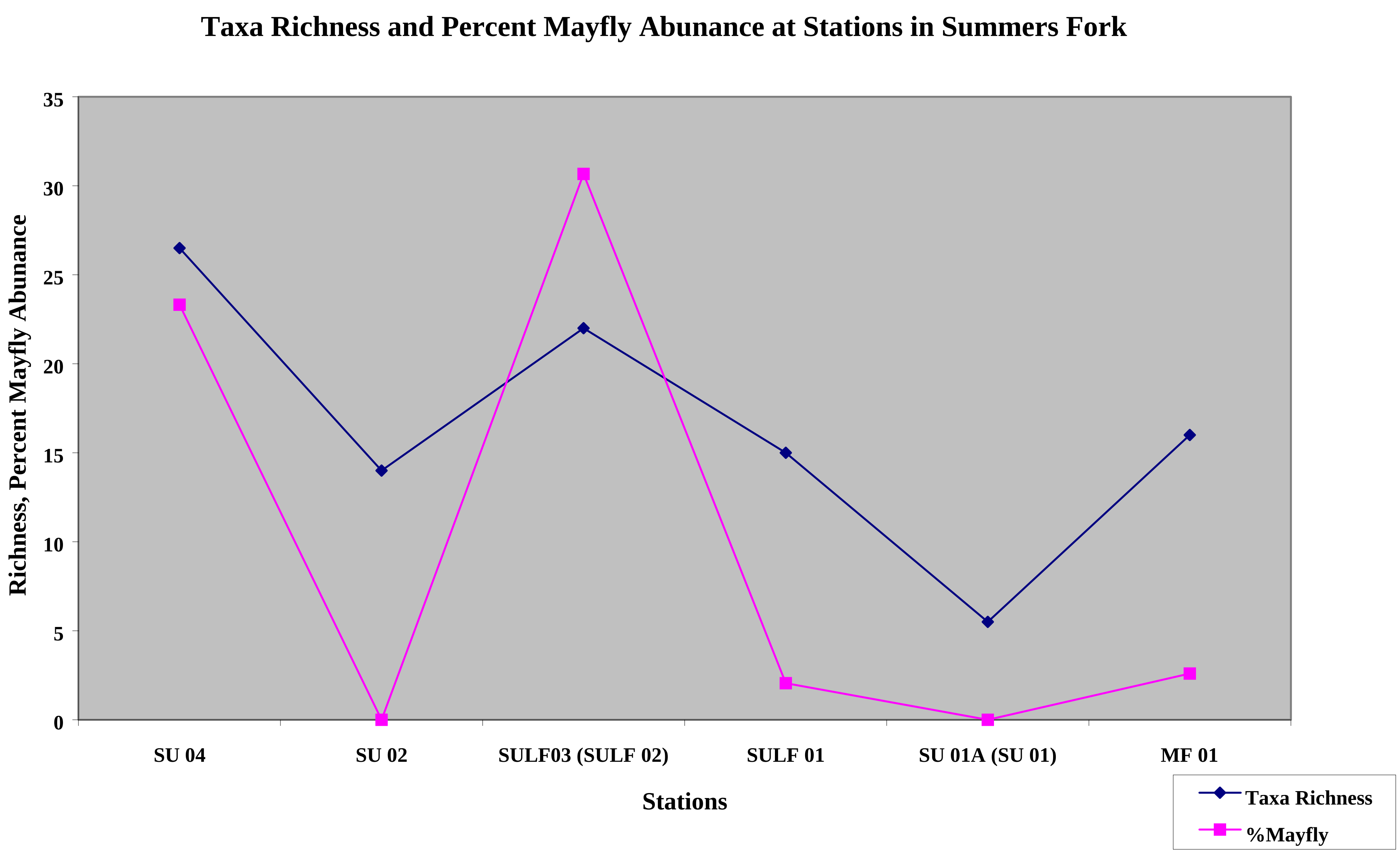
DESC {Powell River Bugs 2000}	RC01	RC01
CAMBARIDAE		5
PERLIDAE	4	2
PERLODIDAE	1	
Isoperla		1
LEUCTRIDAE	76	86
Ephemera		2
BAETIDAE	3	13
Stenonema		2
Stylogomphus		13
Gomphus	22	
Nigronia	1	
Hydropsyche	1	1
Cheumatopsyche	50	23
RHYACOPHILIDAE	9	
Chimarra		4
Dolophilodes	23	11
GLOSSOSOMATIDAE	1	
Psephenus	7	4
ELMIDAE	6	9
Tipula		1
SIMULIIDAE		2
CHIRONOMINAE/ORTHOCLADIINAE	18	19
TANYPODINAE	2	1
Atherix		2
EMPIDIDAE	1	

APPENDIX D, SUMMERS FORK

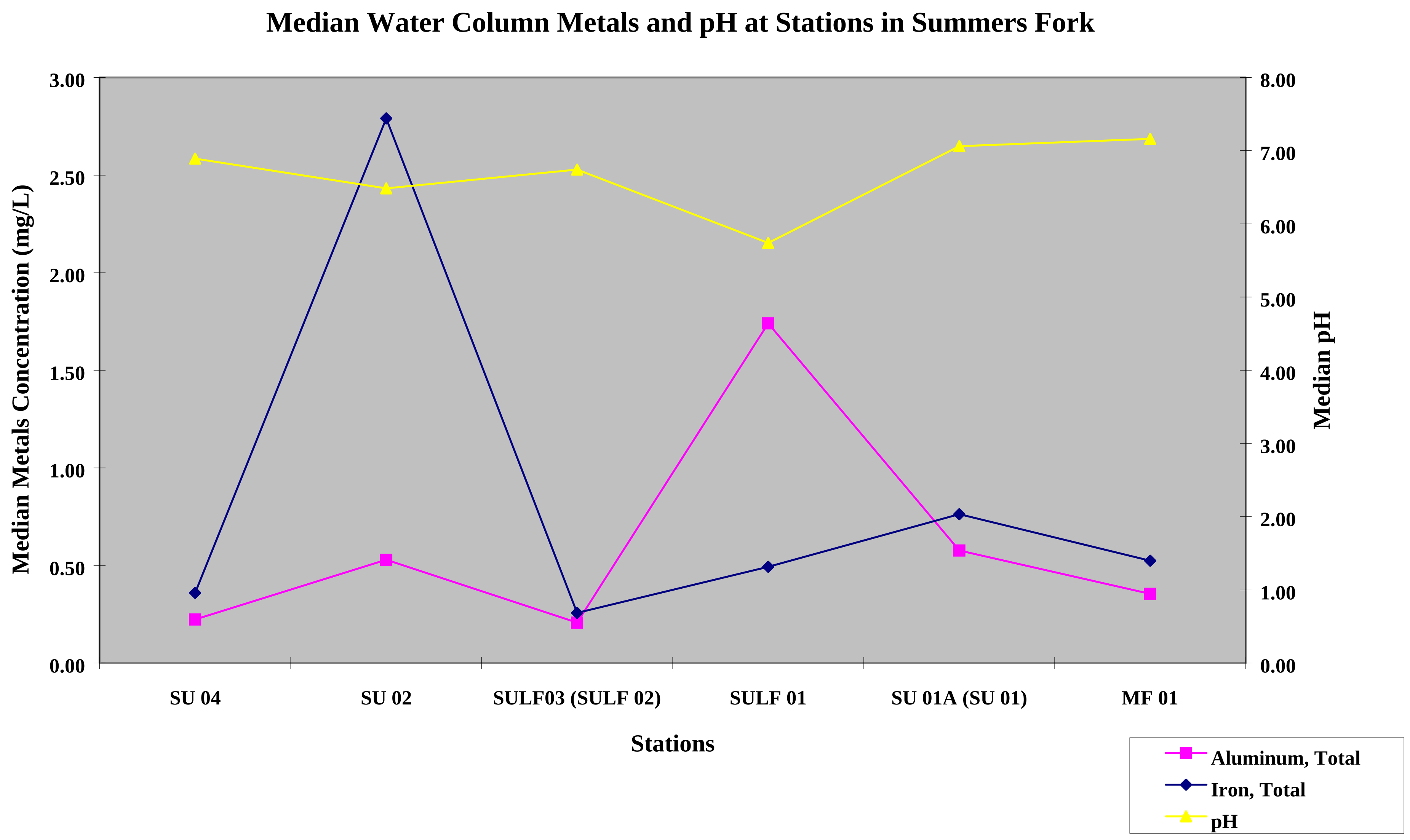
Summers Fork Stations

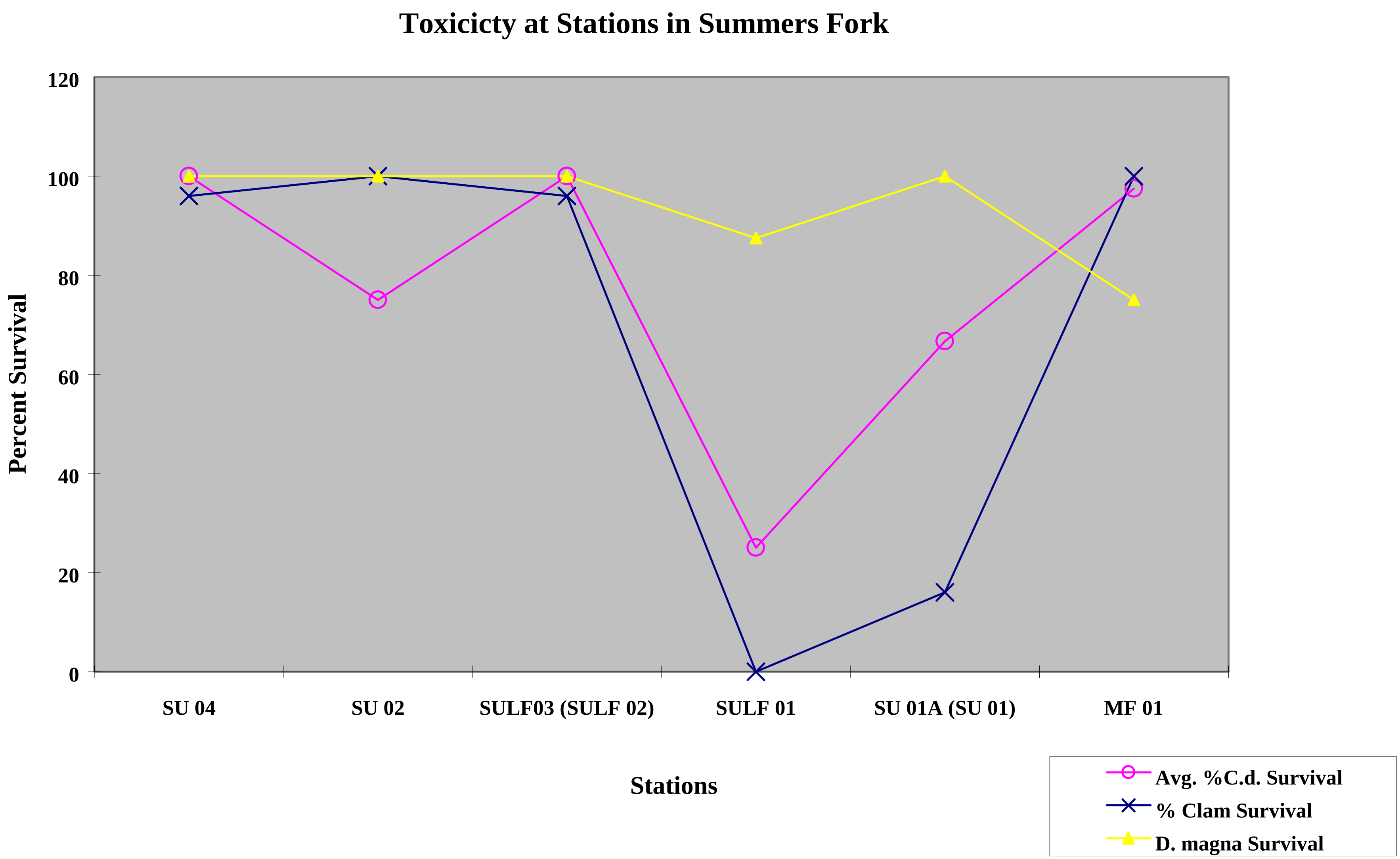


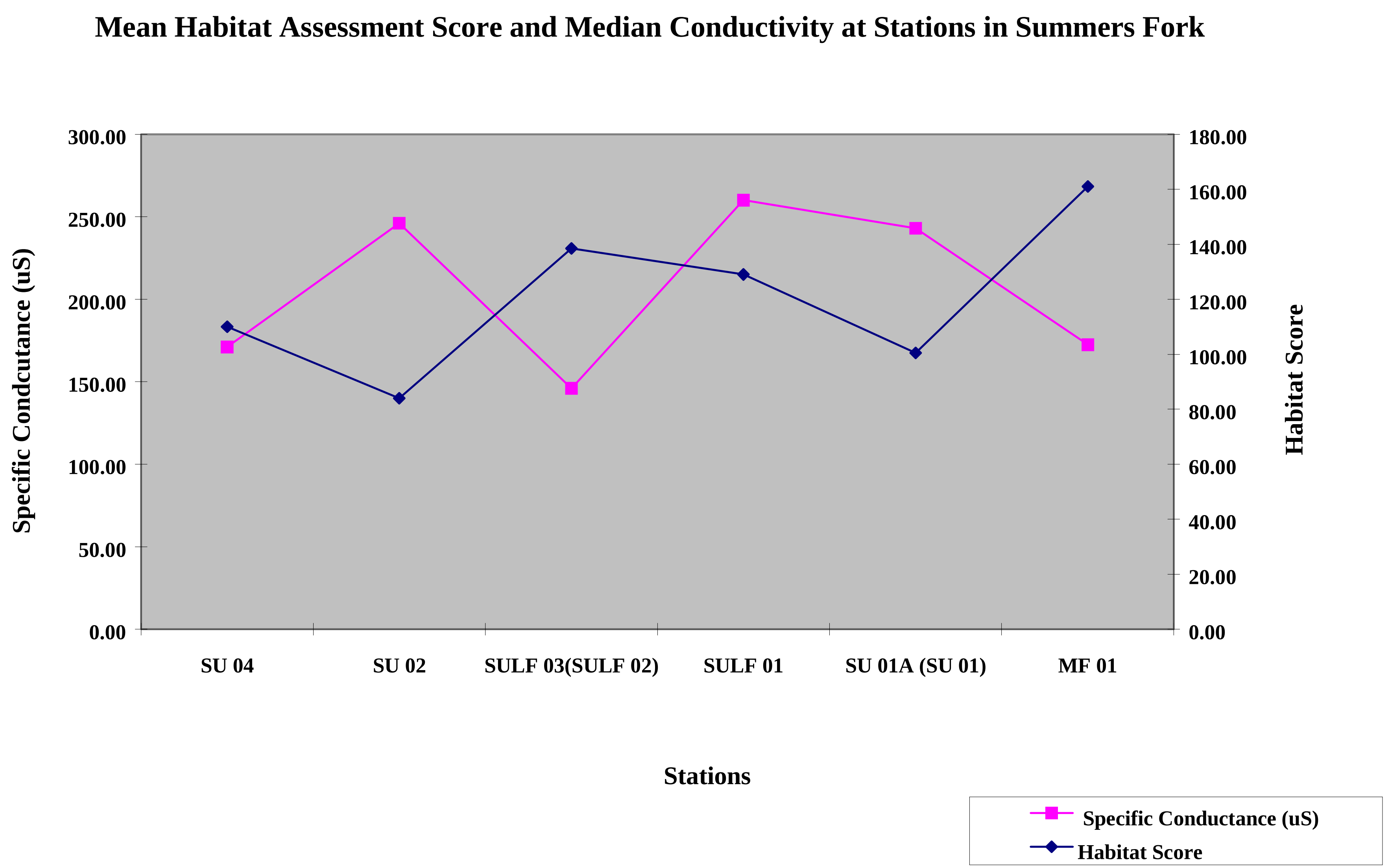
D.1. Graphs of Ecotoxicological Parameters for Summers Fork

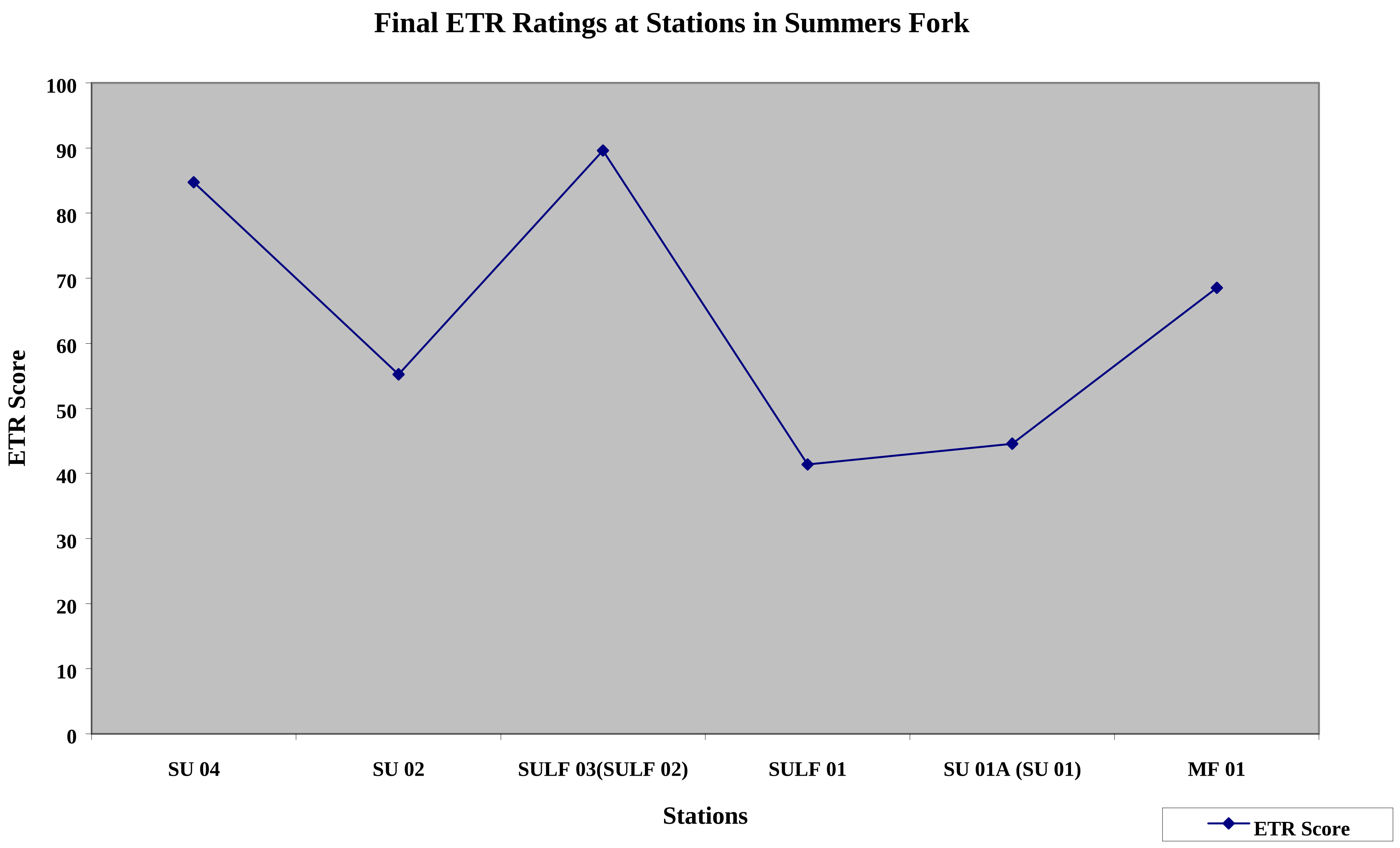


Taxa Richness and Percent Mayfly Abunance at Stations in Summers Fork
Richness, Percent Mayfly Abunance
35
30
25
20
15
10
5
0
SU 04
SU 02
SULF03 (SULF 02)
SULF 01
SU 01A (SU 01)
MF 01
Stations
Taxa Richness
%Mayfly


Median Water Column Metals and pH at Stations in Summers Fork
Median Metals Concentration (mg/L)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Median pH
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
SU 04
SU 02
SULF03 (SULF 02)
SULF 01
SU 01A (SU 01)
MF 01
Stations
Aluminum, Total
Iron, Total
pH


Toxicicty at Stations in Summers Fork
Percent Survival
120
100
80
60
40
20
0
SU 04
SU 02
SULF03 (SULF 02)
SULF 01
SU 01A (SU 01)
MF 01
Stations
Avg. %C.d. Survival
% Clam Survival
D. magna Survival


Mean Habitat Assessment Score and Median Conductivity at Stations in Summers Fork
Specific Condcutance (uS)
Habitat Score
300.00
250.00
200.00
150.00
100.00
50.00
0.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
SU 04
SU 02
SULF 03(SULF 02)
SULF 01
SU 01A (SU 01)
MF 01
Stations
Specific Conductance (uS)
Habitat Score


Final ETR Ratings at Stations in Summers Fork
100
90
80
70
60
50
40
30
20
10
0
ETR Score
SU 04
SU 02
SULF 03(SULF 02)
SULF 01
SU 01A (SU 01)
MF 01
Stations
ETR Score

D.2. Benthic Macroinvertebrate Data, Summers Fork

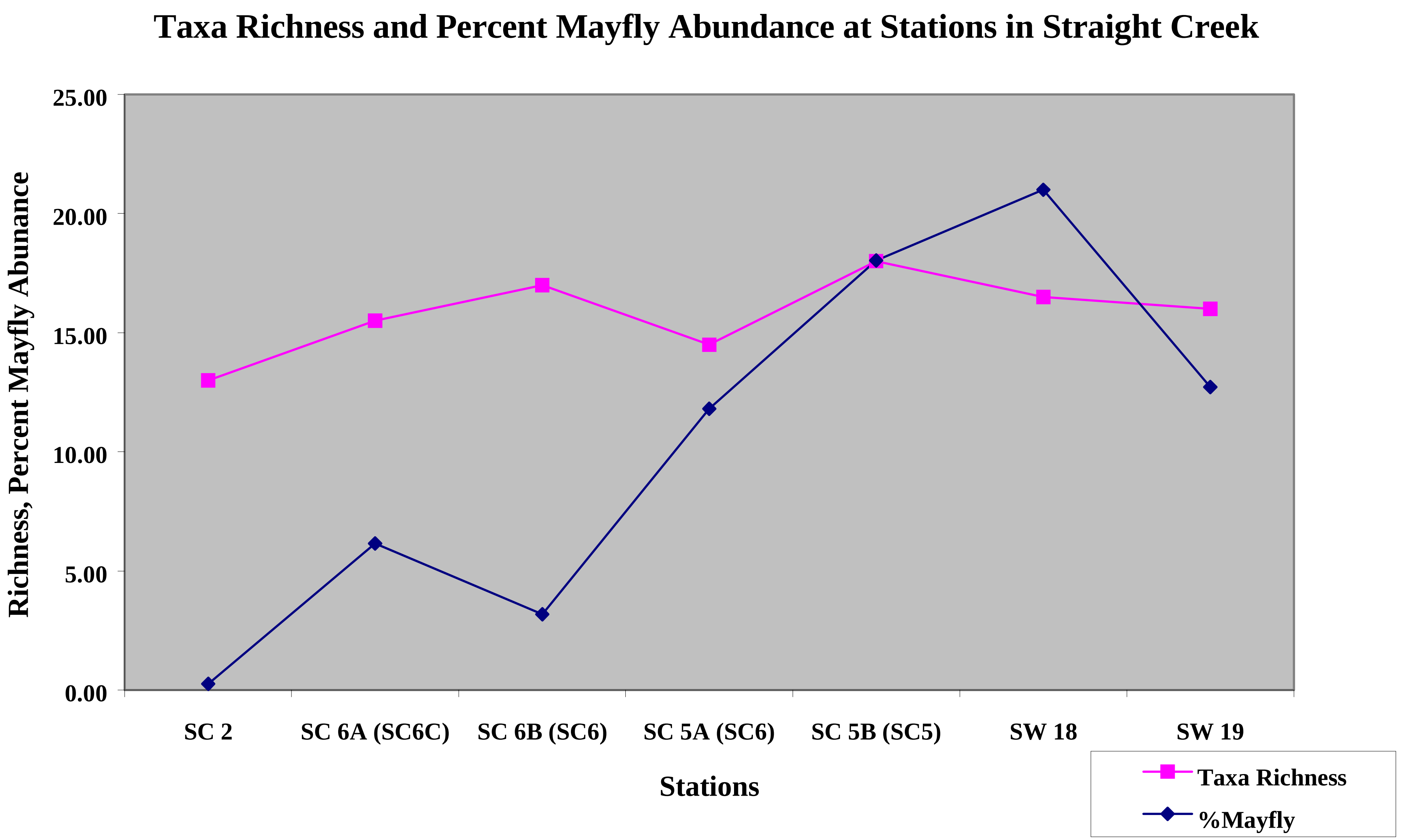
DESC {Powell River Bugs 2000}	SU 04	SU 04	SU 02	SU 02	SULF 03	SULF 03
CAMBARIDAE	12	7	4	11	16	
NEMOURIDAE	2	5		1	2	2
PERLIDAE	1	1				1
PERLODIDAE		1				1
Remenus					3	1
LEUCTRIDAE	18	25	11	9	55	121
CAPNIIDAE		1				
Ephemerella					2	
Eurylophella	5					3
AMELETIDAE	2				13	1
LEPTOPHLEBIIDAE					3	4
BAETIDAE	70	22			59	19
Stenonema					34	
CORDULEGASTRIDAE	4	9		7	4	1
Stylogomphus	1			1	2	
Gomphus	2		2	1		
Dromogomphus		2				
Boyeria	1				4	4
SIALIDAE			7	4		
Nigronia	1	2		1		
Cheumatopsyche	3	3			4	4
Dolophilodes		1				7
PHRYGANEIDAE	4		10	15		
Pycnopsyche		1		1	4	
PYRALIDAE	1		1			
DYTISCIDAE			2			
HYDROPHILIDAE	5	1		1		
Psephenus						1
Ectopria					5	12
ELMIDAE	2	1			2	2
Tipula	5	1	2	4		
Antocha	1	1	1	1		
Hexatoma	1		1		1	
Limnophila	19	14	2		3	2
Molophilus	1					
DIXIDAE	2					1
SIMULIIDAE						1
CHIRONOMINAE/ORTHOCLADIINAE	36	86		2	8	23
TANYPODINAE	4	6	2	6		1
CERATOPOGONIDAE	1			4	1	
STRATIOMYIIDAE	1					
TABANIDAE	1				1	
Atherix	1	26			1	5
EPHYDRIDAE		1				
PLEUROCERIDAE	1					
SPHAERIIDAE	3					

DESC {Powell River Bugs 2000}	SULF 01	SULF 01	SU 01A	SU 01A
PERLIDAE				1
LEUCTRIDAE	9	8		
Leuctra			4	3
BAETIDAE	1	1		
HEPTAGENIIDAE	1			
CORDULEGASTRIDAE	2			
SIALIDAE	2	1		
Sialis				2
Nigronia	1	5		1
Cheumatopsyche		1	1	2
PHRYGANEIDAE	13	9		1
LEPIDOSTOMATIDAE	1			
LIMNEPHILIDAE		4		
PYRALIDAE	4	1		1
NOCTUIDAE	4			
Ectopria	1			
ELMIDAE		1		
Tipula	4	2		1
CHIRONOMINAE/ORTHOCLADIINAE	26	26		2
CERATOPOGONIDAE	3	4		
TABANIDAE	4	3		
EMPIDIDAE		1		
PTYCHOPTERIDAE		1		

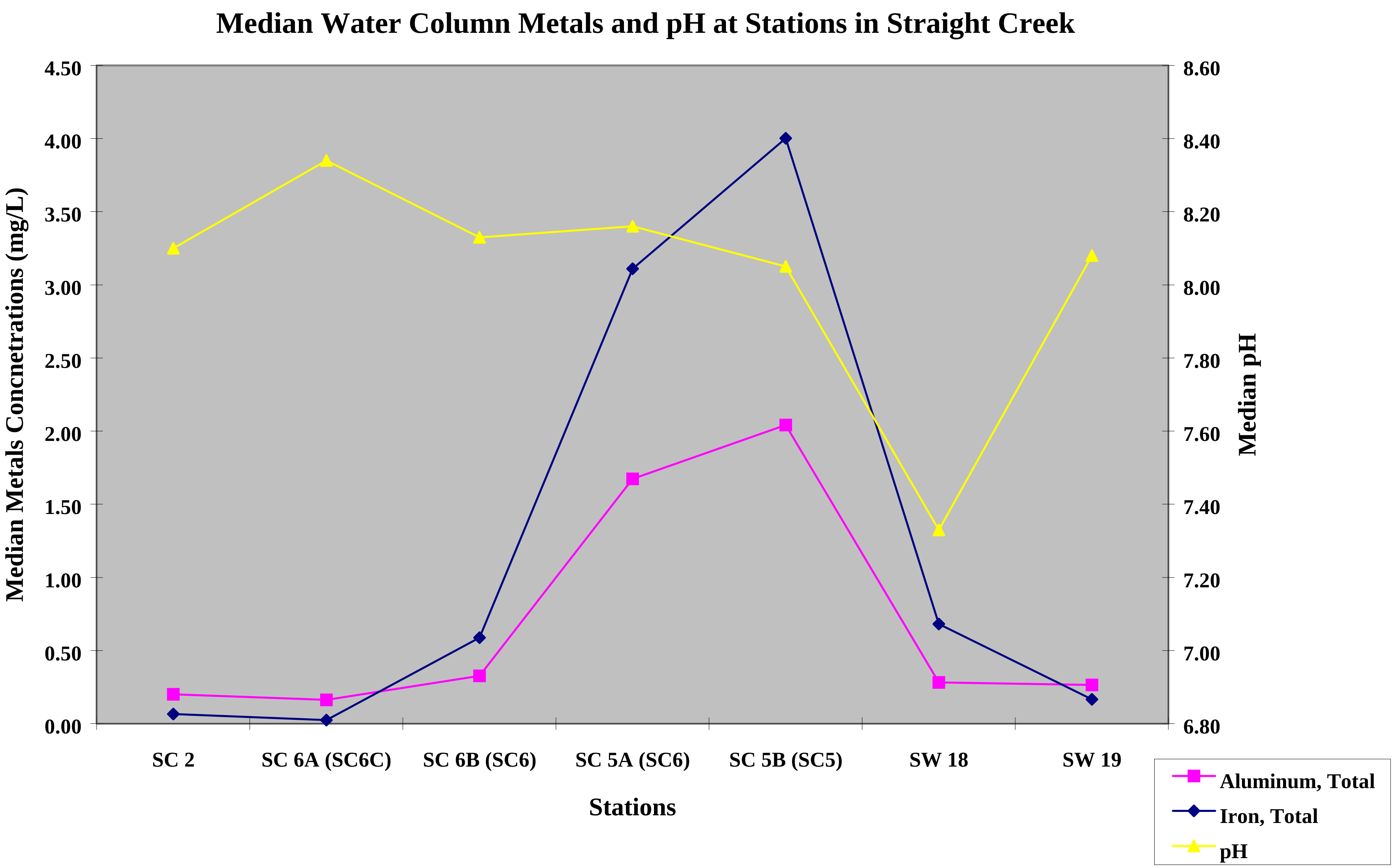
DESC {Powell River Bugs 2000}	MF01	MF01
CAMBARIDAE	4	3
NEMOURIDAE	4	1
Isoperla		1
LEUCTRIDAE	59	21
Ephemera		1
BAETIDAE	1	2
HEPTAGENIIDAE	1	
Stylogomphus	9	1
Sialis	1	
Hydropsyche		1
Cheumatopsyche	17	18
GLOSSOSOMATIDAE	1	1
LIMNEPHILIDAE	1	
POLYCENTROPODIDAE	1	
Psephenus	13	5
ELMIDAE	1	
Tipula	3	3
DIXIDAE		1
SIMULIIDAE	1	
CHIRONOMINAE/ORTHOCLADIINAE	31	17
TANYPODINAE		1
EMPIDIDAE		1

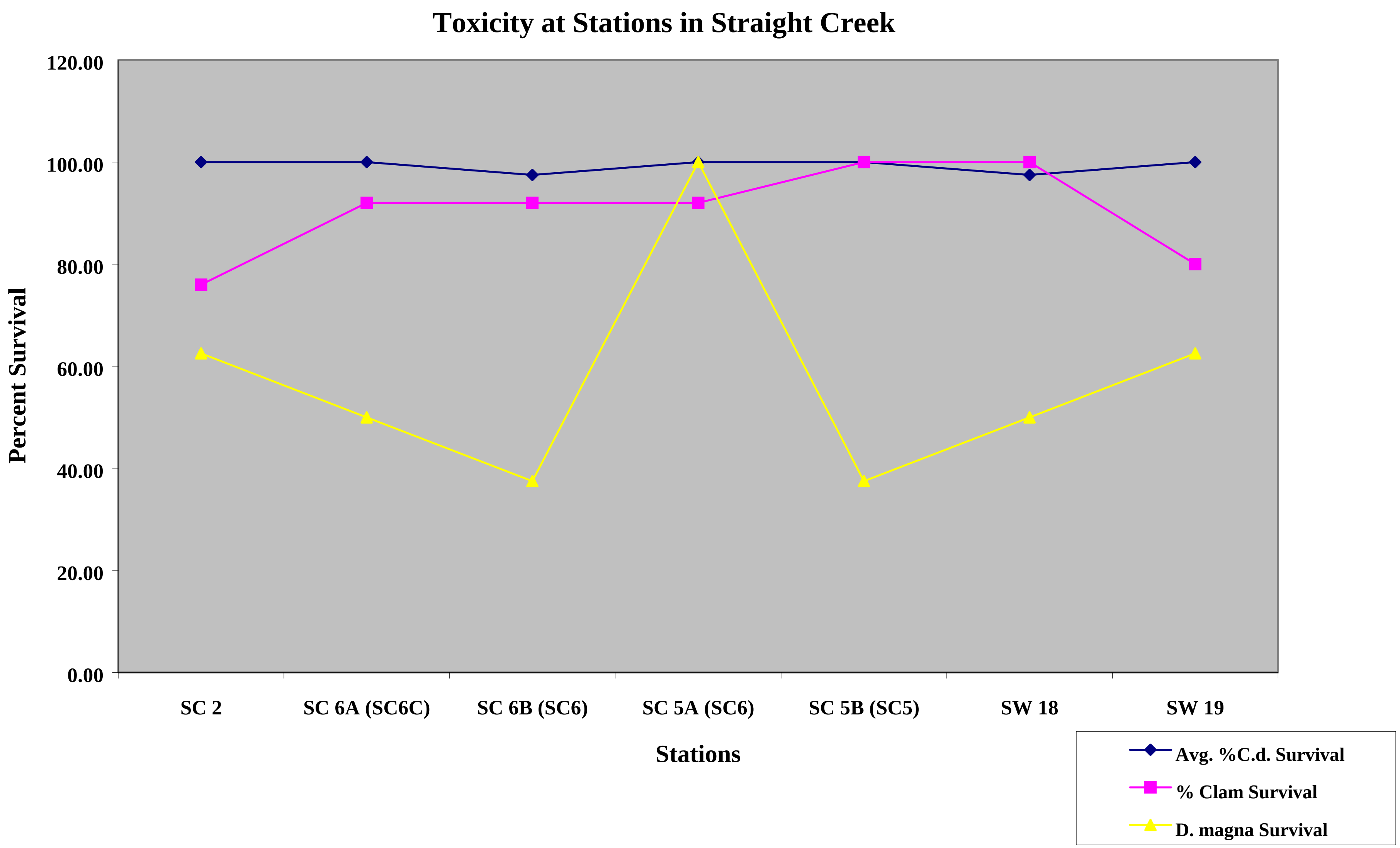
APPENDIX E, STRAIGHT CREEK

E.1. Graphs of Ecotoxicological Parameters for Straight Creek



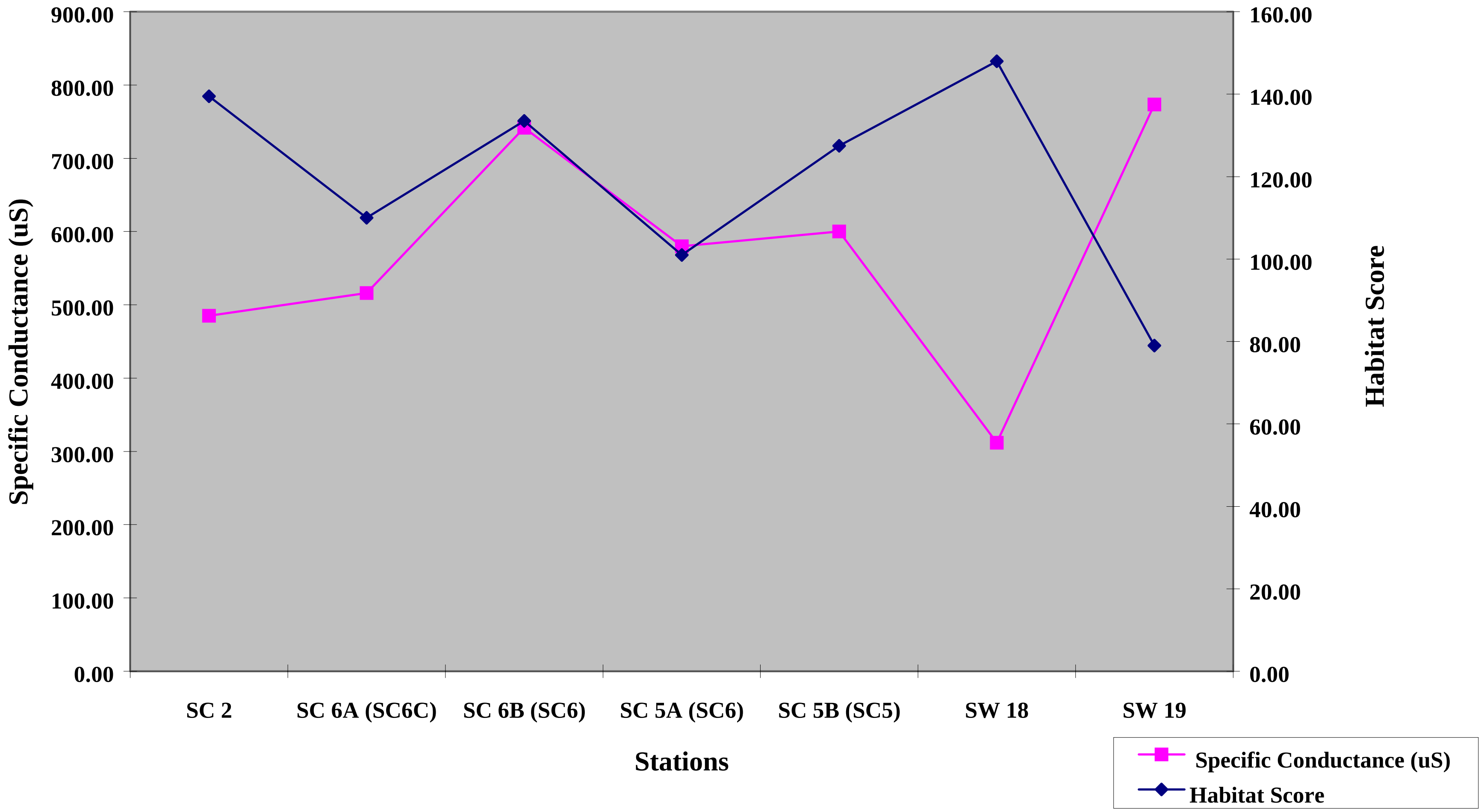
Taxa Richness and Percent Mayfly Abundance at Stations in Straight Creek
Richness, Percent Mayfly Abunance
25.00
20.00
15.00
10.00
5.00
0.00
SC 2
SC 6A (SC6C)
SC 6B (SC6)
SC 5A (SC6)
SC 5B (SC5)
SW 18
SW 19
Stations
Taxa Richness
%Mayfly


Median Water Column Metals and pH at Stations in Straight Creek
Median Metals Concnetrations (mg/L)
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
Median pH
6.80
7.00
7.20
7.40
7.60
7.80
8.00
8.20
8.40
8.60
SC 2
SC 6A (SC6C)
SC 6B (SC6)
SC 5A (SC6)
SC 5B (SC5)
SW 18
SW 19
Stations
Aluminum, Total
Iron, Total
pH

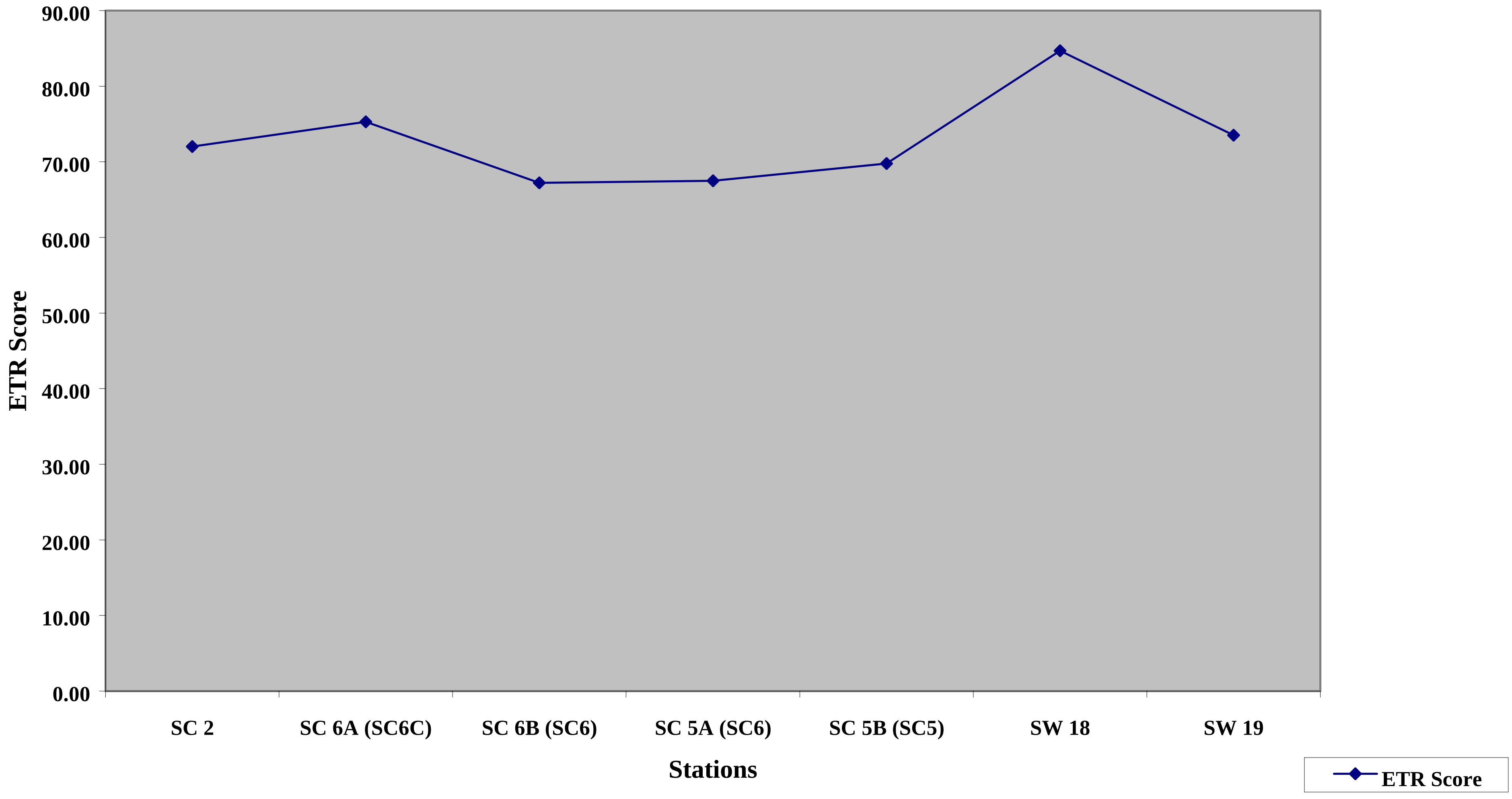


Toxicity at Stations in Straight Creek
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Percent Survival
SC 2
SC 6A (SC6C)
SC 6B (SC6)
SC 5A (SC6)
SC 5B (SC5)
SW 18
SW 19
Stations
Avg. %C.d. Survival
% Clam Survival
D. magna Survival

Mean Habitat Assessment and Median Conductivity at Stations in Straight Creek



Final ETR Ratings at Stations in Straight Creek



E.2. Benthic Macroinvertebrate Data, Straight Creek

DESC {Powell River Bugs 2000}	SC 2	SC 2	SC 6A	SC 6A
CAMBARIDAE		3	3	5
LEUCTRIDAE				1
Caenis			1	
BAETIDAE	1		15	9
ISONYCHIIDAE				1
Cordulegaster		1		
Stylogomphus			4	5
Boyeria	1	1	3	2
Corydalus			3	9
Nigronia	1	5		
Hydropsyche	1		1	1
Cheumatopsyche	84	80	101	63
Psephenus	3	4	18	31
ELMIDAE	3	1		1
Tipula	1	30	2	2
Antocha	1	1	12	10
PSYCHODIDAE	2			
SIMULIIDAE	3		1	
CHIRONOMINAE/ORTHOCLADIINAE	82	82	43	49
TANYPODINAE	8	13	5	18
EMPIDIDAE	1			
ANCYLIDAE	1		1	2

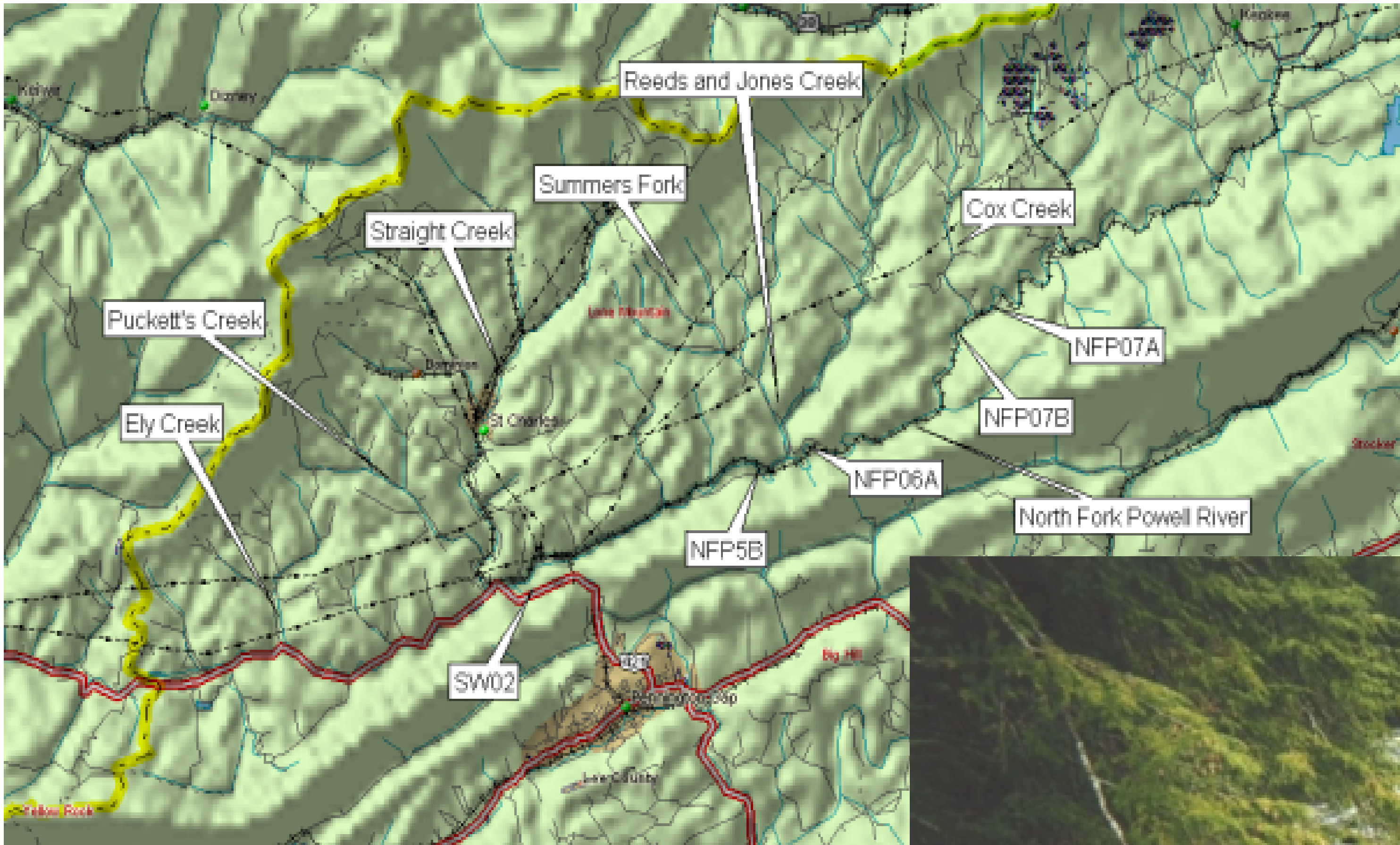
DESC {Powell River Bugs 2000}	SC 5B	SC 5B	SW 19	SW 19
CAMBARIDAE	6	4	5	3
LEUCTRIDAE	1	1	2	
CAENIDAE			1	
Eurylophella			1	
AMELETIDAE	2			
BAETIDAE	36	16	17	34
HEPTAGENIIDAE	1			
ISONYCHIIDAE	1			1
Stylogomphus		9	5	
Hagenius			1	
Boyeria			1	
CALOPTERYGIDAE			1	
Sialis	1	1	2	
Corydalus	1		5	18
Nigronia		1	1	
Hydropsyche		1	1	
Cheumatopsyche	12	64	86	89
HYDROPHILIDAE		1		
Psephenus	3	10	11	8
ELMIDAE	1	9	9	2
Tipula	2	3		1
Antocha	1	5	3	10
Molophilus		1		
SIMULIIDAE	3	2	2	
CHIRONOMINAE/ORTHOCLADIINAE	48	62	57	40
TANYPODINAE	21	12	4	4
CERATOPOGONIDAE				1
TABANIDAE		1		
PLANORBIDAE	1			
SPHAERIIDAE	1			

DESC {Powell River Bugs 2000}	SW18	SW18
CAMBARIDAE	4	7
LEUCTRIDAE		5
CAENIDAE	24	13
BAETIDAE	23	20
HEPTAGENIIDAE		9
Stenonema	2	
ISONYCHIIDAE	2	
Stylogomphus	14	19
Boyeria		1
Corydalus	1	1
Nigronia	1	3
Hydropsyche	1	
Cheumatopsyche	46	27
PSEPHENIDAE		1
Psephenus	6	
ELMIDAE	7	1
TIPULIDAE	8	
Antocha	2	3
SIMULIIDAE	1	
CHIRONOMINAE	93	83
TANYPODINAE	9	4
CERATOPOGONIDAE		2

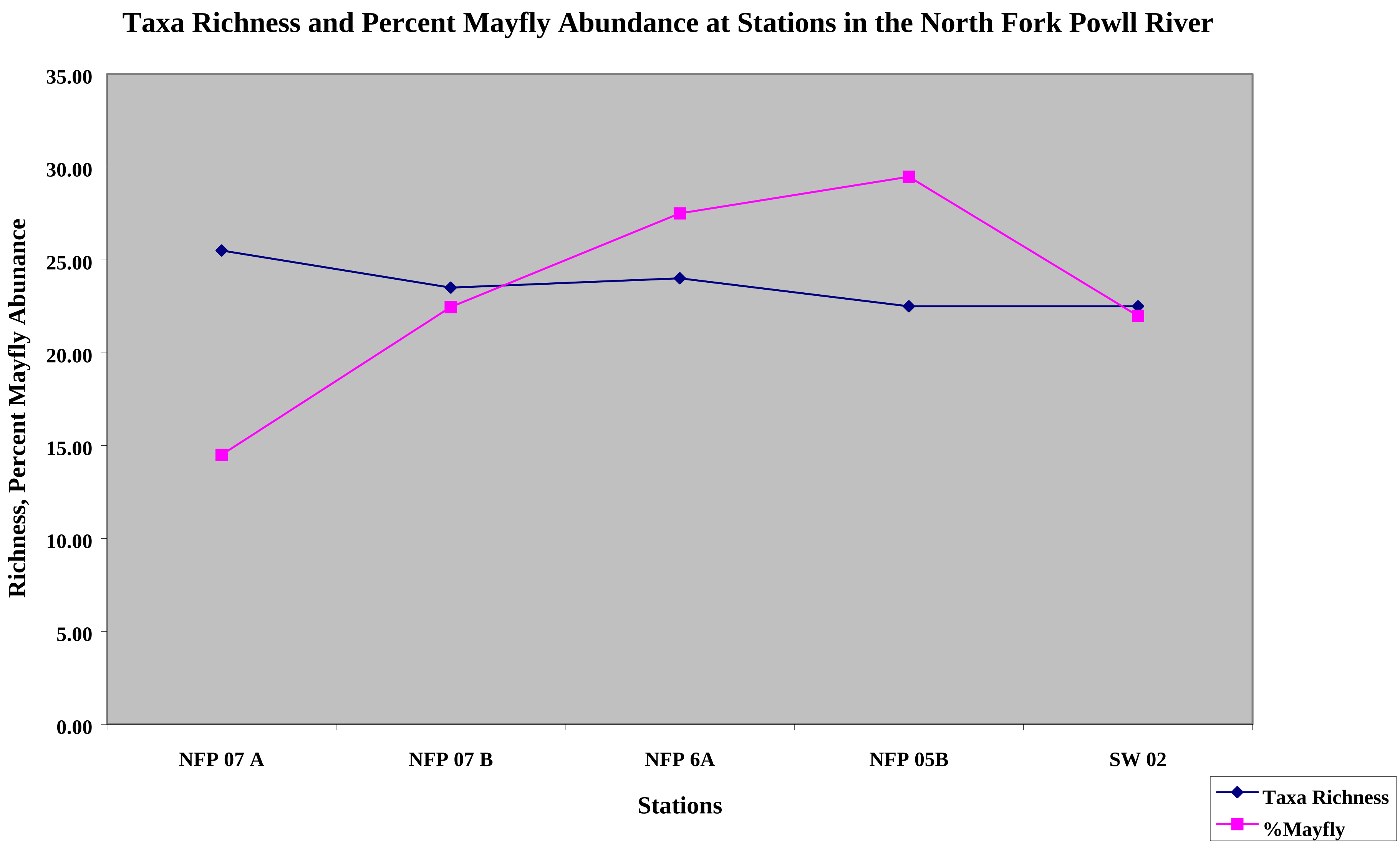
DESC {Powell River Bugs 2000}	SC 6B	SC 6B	SC 5A	SC 5A
CAMBARIDAE	6	5	4	7
LEUCTRIDAE	1		1	
Hexagenia			1	
CAENIDAE	1	3		
Eurylophella		2		
AMELETIDAE				1
BAETIDAE	9		29	19
ISONYCHIIDAE	1			
Stylogomphus	2	5	3	2
Boyeria	1		1	
SIALIDAE	1			
Sialis			1	
Corydalus	1			
Nigronia			1	
Hydropsyche	1	1		2
Cheumatopsyche	97	52	16	7
PSEPHENIDAE	39	18		
Psephenus			11	5
ELMIDAE	1	1	12	
TIPULIDAE		1		
Tipula	6			
Antocha	12	3	1	
Molophilus	1			
SIMULIIDAE	1	1	2	4
CHIRONOMINAE/ORTHOCLADIINAE	78	111	75	152
TANYPODINAE	7	20	54	6
CERATOPOGONIDAE			1	
TABANIDAE	1			
Atherix			1	2
EMPIDIDAE			1	
PHYSIDAE	1			

APPENDIX F, NORTH FORK POWELL RIVER STATIONS

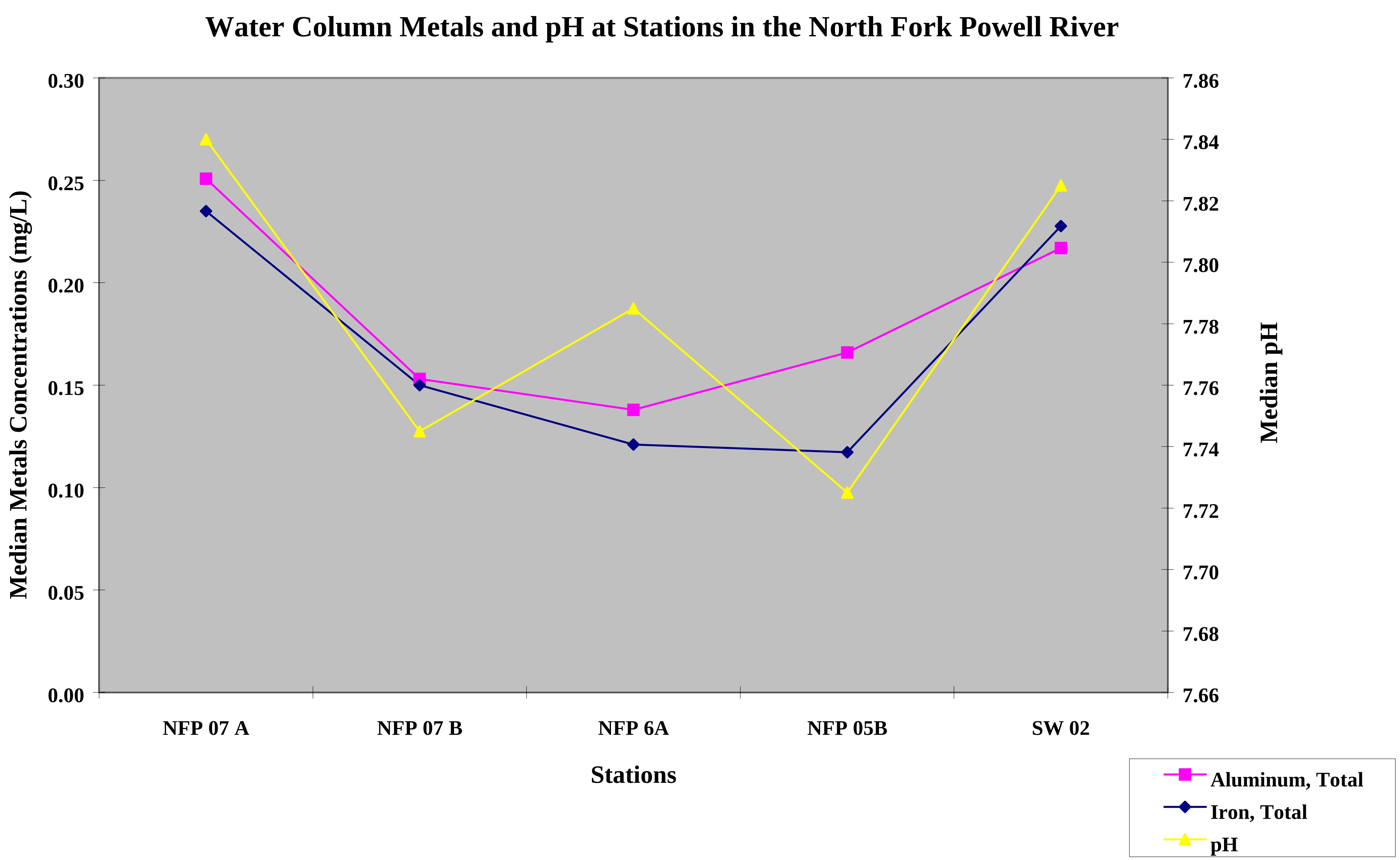
Map of North Fork Powell River Stations and Sub-watersheds

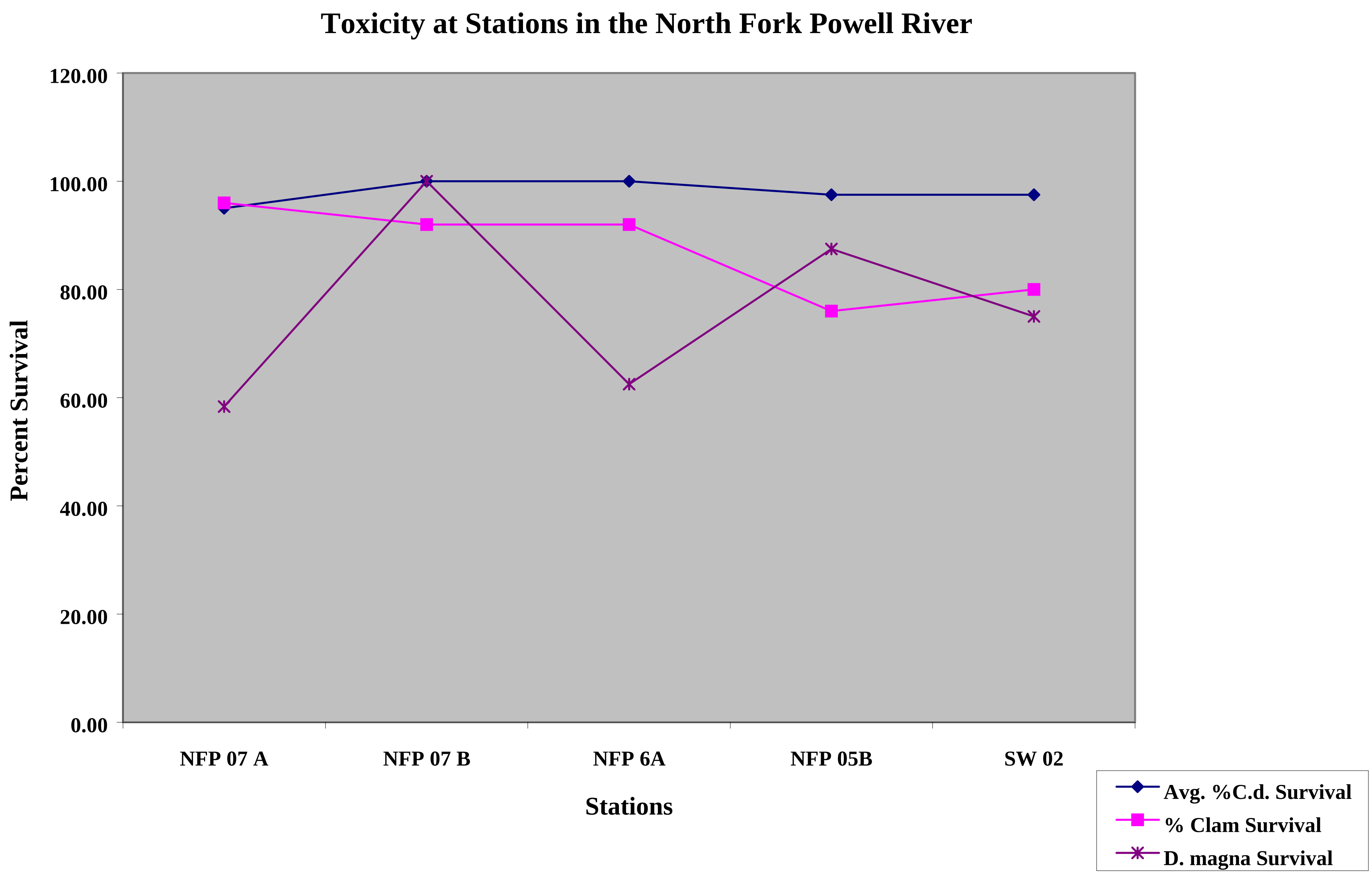


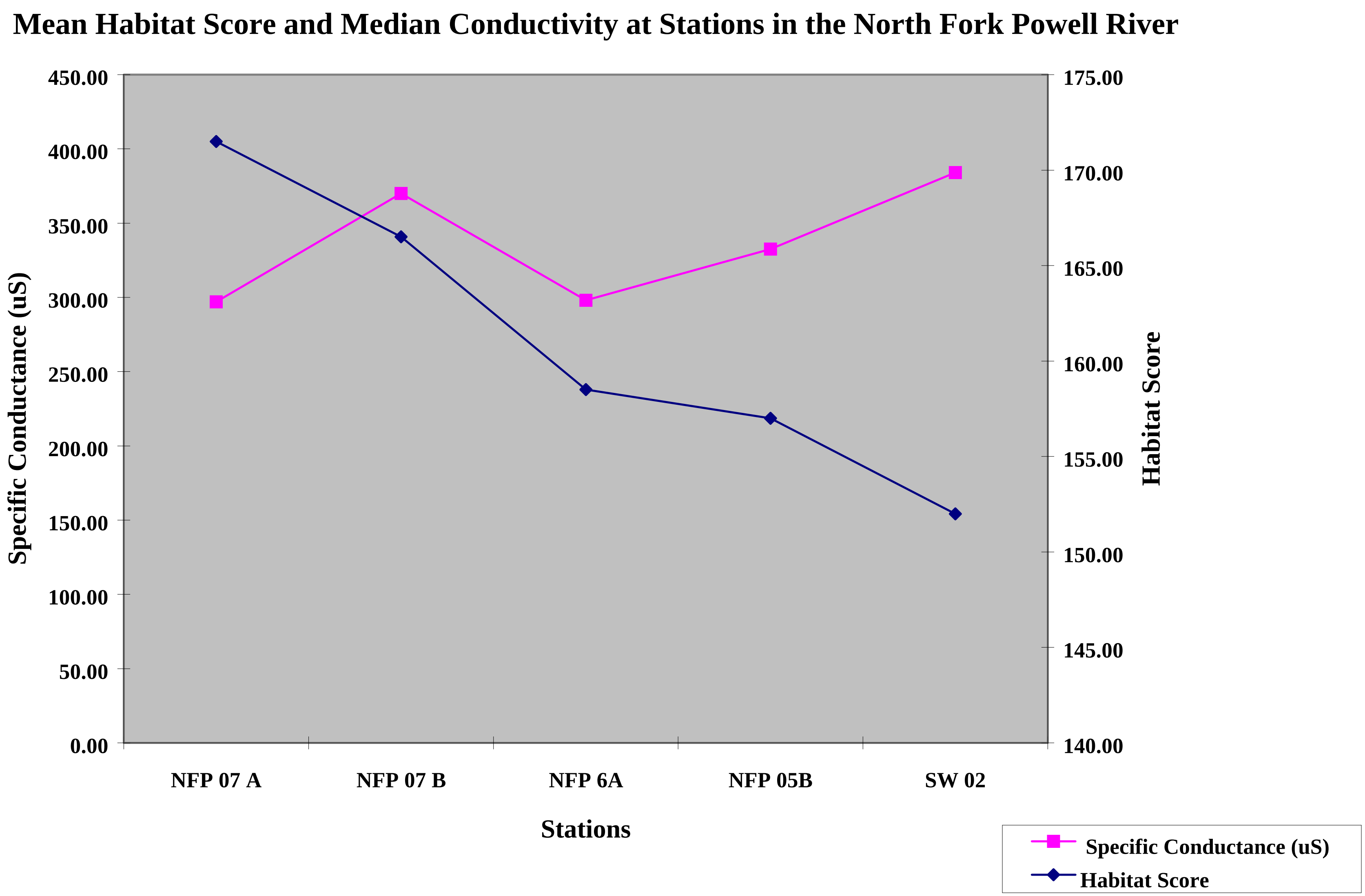
F.1. Graphs of Ecotoxicological Parameters for North Fork Powell River Stations



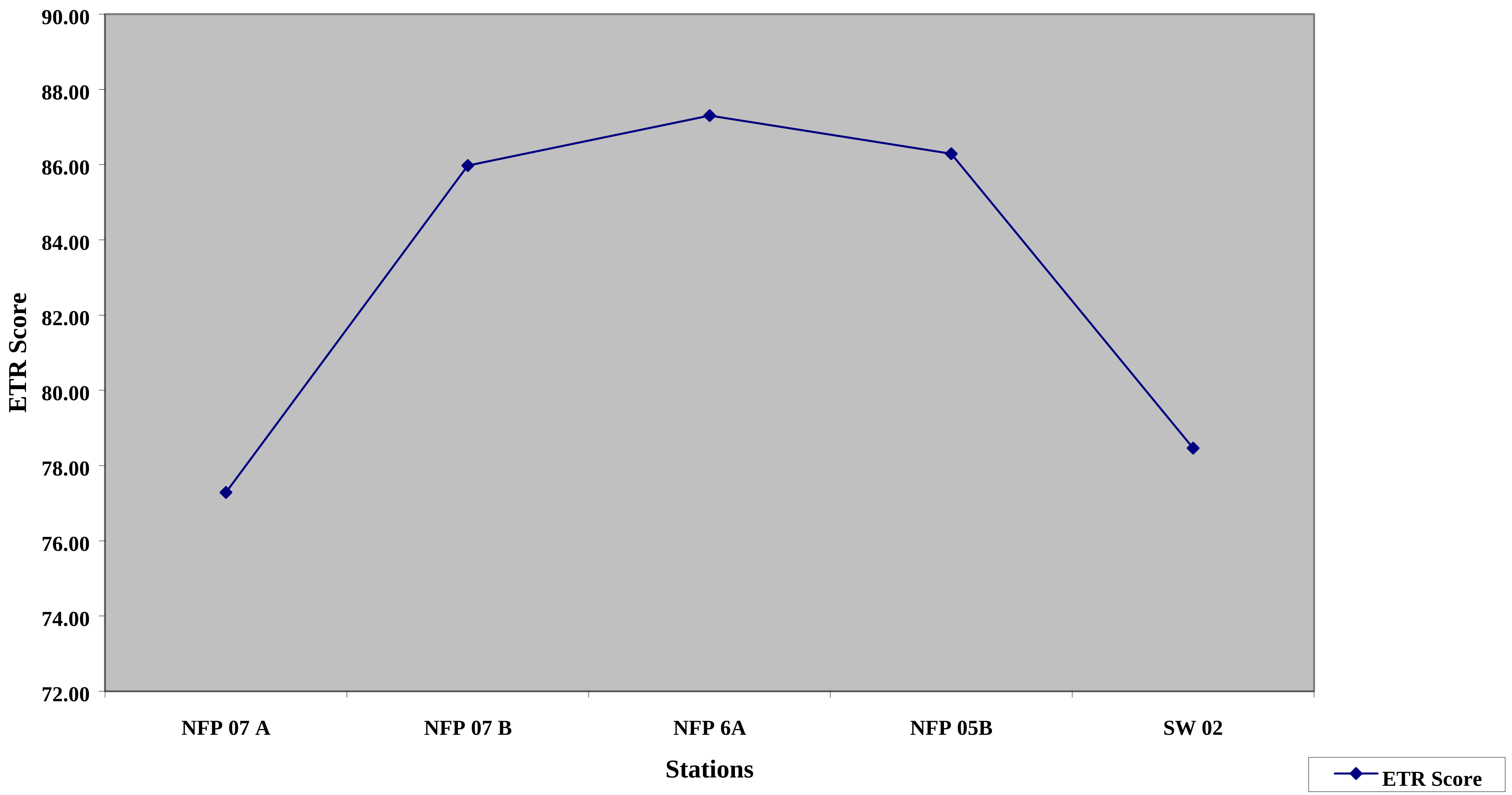
Taxa Richness and Percent Mayfly Abundance at Stations in the North Fork Powll River
Richness, Percent Mayfly Abunance
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
NFP 07 A
NFP 07 B
NFP 6A
NFP 05B
SW 02
Stations
Taxa Richness
%Mayfly


Water Column Metals and pH at Stations in the North Fork Powell River
Median Metals Concentrations (mg/L)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Median pH
7.86
7.84
7.82
7.80
7.78
7.76
7.74
7.72
7.70
7.68
7.66
NFP 07 A
NFP 07 B
NFP 6A
NFP 05B
SW 02
Stations
Aluminum, Total
Iron, Total
pH


Toxicity at Stations in the North Fork Powell River
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Percent Survival
NFP 07 A
NFP 07 B
NFP 6A
NFP 05B
SW 02
Stations
Avg. %C.d. Survival
% Clam Survival
D. magna Survival


Mean Habitat Score and Median Conductivity at Stations in the North Fork Powell River
Specific Conductance (uS)
Habitat Score
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
175.00
170.00
165.00
160.00
155.00
150.00
145.00
140.00
NFP 07 A
NFP 07 B
NFP 6A
NFP 05B
SW 02
Stations
Specific Conductance (uS)
Habitat Score

Final ETR Ratings at Stations in the North Fork Powell River



F.2. Benthic Macroinvertebrate Data, North Fork Powell River Stations

DESC {Powell River Bugs 2000}	NFP 07A	NFP 07A	NFP 7B	NFP 7B
CAMBARIDAE		1		
NEMOURIDAE	1			
PERLIDAE	6	15	7	5
LEUCTRIDAE	5	5	7	9
EPHEMERIDAE				1
CAENIDAE	2		7	6
Caenis		8		
Timpanoga	1			
Ephemerella	1			3
Eurylophella		4	4	5
AMELETIDAE		2		
BAETIDAE	3	3	18	7
HEPTAGENIIDAE			1	11
Stenonema	5	10	7	
ISONYCHIIDAE	14	5	23	
Stylogomphus	6	6	8	8
Gomphus				2
Hagenius		3		
Boyeria	1	2	4	4
CORDULIIDAE				1
Sialis		2		
Corydalus	5			
Nigronia	10	8	8	
Hydropsyche	12	1		
Cheumatopsyche	13	1	10	4
RHYACOPHILIDAE	5		1	1
Chimarra	26	9	7	1
LIMNEPHILIDAE			1	
HYDROPHILIDAE		1		
PSEPHENIDAE			3	5
Psephenus	9	3		
Ectopria				1
ELMIDAE	12	19	23	28
Tipula	4	1		
Antocha	1	1	1	
TANYPODINI	3	2		
CHIRONOMINAE/ORTHOCLADIINAE	48	76	49	89
TANYPODINAE			4	1
EMPIDIDAE		2	12	3
PLANORBIDAE				1
PLEUROCERIDAE	7	4	3	3
Corbicula		6	2	3

DESC {Powell River Bugs 2000}	NFP6A	NFP6A	NFP5B	NFP5B
CAMBARIDAE	1			15
NEMOURIDAE				1
PERLIDAE	16	30	7	9
LEUCTRIDAE	5	6	14	11
Ephemera			4	2
CAENIDAE		2	3	
Drunella				1
AMELETIDAE	1			
Habrophlebia			33	5
BAETIDAE	10	10		
Baetis (complex)			24	7
Stenonema	2	7		
Anthopotamus			17	23
ISONYCHIIDAE	41	37		
Isonychia bicolor			10	22
Stylogomphus	4	1		
Gomphus	4	3		
Dromogomphus			2	
Boyeria	1			
Celithemis			1	
COENAGRIONIDAE	1			
CORIXINAE			1	
NOTONECTIDAE			1	2
Corydalus	1	4	2	11
CHAULIODINAE			4	10
Nigronia	1	4		
HYDROPTILINAE				20
Hydropsyche	19	6		
Cheumatopsyche	24	21		
RHYACOPHILIDAE	2	1		
Chimarra	12	7		
Oecetis			2	
LIMNEPHILIDAE			16	7
Platycentropus			12	7
PSEPHENIDAE	4	10		
Psephenus	1			
Helichus	10	12		
ELMIDAE			10	
CURCULIONIDAE			23	47
ORDER DIPTERA			1	1
TANYDERIDAE			4	
Tipula abdominalis		1		

DESC {Powell River Bugs 2000}	NFP6A	NFP6A	NFP5B	NFP5B
PSYCHODIDAE			1	3
DIXIDAE			5	
Psorophora	1			
TANYTARSINI	2	1		
CHIRONOMINAE/ORTHOCLADIINAE	17	15		
TABANIDAE			3	
Tabanus	18	12		
EPHYDRIDAE				1
Rhaphium		8		1
AMNICOLINAE	1	2		
PLEUROCERIDAE				
Fusconaia	1			
SPHAERIIDAE				
Corbicula				

DESC {Powell River Bugs 2000}	SW 02	SW 02
CAMBARIDAE	13	20
Ephemera	3	1
CAENIDAE	20	12
Eurylophella	1	
BAETIDAE	7	24
HEPTAGENIIDAE	15	7
ISONYCHIIDAE	1	
Stylogomphus	6	7
Dromogomphus	1	
Hagenius	8	
Boyeria	6	
CALOPTERYGIDAE	1	2
SIALIDAE	1	2
Nigronia	1	1
Hydropsyche		
Cheumatopsyche	12	20
GLOSSOSOMATIDAE		1
DYTISCIDAE		2
Psephenus	11	4
ELMIDAE	15	12
TIPULIDAE	1	
Tipula	1	1
Antocha	3	
TANYPODINAE	16	14
CHIRONOMINAE	67	61
EPHYDRIDAE		1
DOLICHOPODIDAE	1	
ANCYLIDAE	1	5
SPHAERIIDAE	1	
Corbicula		4

Curriculum Vita

Travis Schmidt

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EDUCATION

M.S. Student, Biology, Aquatic Ecotoxicology, Anticipated graduation, Summer 2001. Virginia Polytechnic Institute. Blacksburg, VA. GPA: 3.87/4.00.

B.S. Biology, option in Ecology, May 1998. The Pennsylvania State University. University Park, PA. Personally financed 100% of my education. GPA: 3.42/4.00.

Study Abroad, Tropical Field Ecology, Costa Rica, December to January 1998, The Pennsylvania State University. University Park, PA.

ACTIVITIES/HONORS/MEMBERSHIPS

Dean's List last four semesters, GPA: 3.83/4.00. Penn State Outing Club, 1994-1998. Penn State Varsity Track & Field 1993-1994. Scholastic All-American, 1993. Phi Sigma Honor Society, Society of Environmental Toxicology and Chemistry, American Society for Surface Mining Reclamation.

PEER REVIEWED JOURNAL ARTICLES

Schmidt, T.S., Soucek, D.J., Cherry, D.S., Accepted. Modification of an Ecotoxicological Rating to Bioassess Small Acid Mine Drainage Impacted Watersheds, Exclusive of Benthic Macroinvertebrate Analysis. 7 tables. Environ Toxicol Chem.

Soucek, D.J., Schmidt, T.S., Cherry, D.S. In Situ Studies with Asian Clams *(Corbicula fluminea)*Detect Acid Mine Drainage and Nutrient Inputs in Low Order Streams. Can J Fish Aquat Sci 58 (3): 602-608.

Soucek, D.J., Schmidt, T.S., Cherry, D.S., Zipper, C.E., Accepted. Individual and Community Impacts of Acid Mine Drainage and Urban Runoff in the Powell River, Virginia, USA. 4 Tables and 2 Figures. J Aquat Ecosyst Stress and Recovery.

Soucek, D.J., Denson, B.C., Schmidt, T.S., Cherry, D.S., Zipper, C.E., In Review. Impaired Common Stonefly (*Acroneuria*) Populations Associated with Aluminum Contamination in Neutral pH Surface Waters. Arch Environ Contam Toxicol.

PEER REVIEWED JOURNAL ARTICLES IN DEVELOPMENT

Schmidt, T.S., Soucek, D.J., Cherry, D.S., In Prep. Integrative Assessment of Benthic Macroinvertebrate Community Impairment From Metal-Contaminated Waters in Tributaries of the Upper Powell River, Virginia. 8 tables and 1 Figure. Environ Toxicol Chem.

PEER REVIEWED CONFERENCE PAPERS

Schmidt, T.S., Soucek, D.J., Cherry, D.S., Currie, R.J., Latimer, H.A., and Trent, G.C. 2000. Integrative Bioassessment Techniques to Predict Ecotoxicological Impairment by Acid Mine Drainage. Proc Amer Soc Surface Mining Reclam, June 2000, Tampa, FL.

Cherry, D.S., Schmidt, T.S., Soucek, D.J., 2000. Preliminary Review of the Integrative Biological Assessment of Sites in Five Sub-watersheds, and in the North Fork Powell River. In, North Fork of the Powell River Ecosystem Restoration, 2nd Interim Feasibility Study. Design Conference October 23- 27, 2000, Big Stone Gap, Virginia.

PRESENTATIONS/PUBLISHED ABSTRACTS

Schmidt, T.S., Soucek, D.J., Cherry, D.S., Submitted. Delineating Coal Mine Drainage Impacted Watersheds: Importance of Reference Site Selection. Abstract submitted for platform presentation at the Society of Environmental Toxicology and Chemistry 22st Annual Meeting, Baltimore, MD November 2001.

Soucek, D.J., Schmidt, T.S., Cherry, D.S., Zipper, C.E., Submitted. Use of In situ Experiments to Eliminate Confounding Variables in Community Studies. Abstract submitted for platform presentation at the Society of Environmental Toxicology and Chemistry 22st Annual Meeting, Baltimore, MD November 2001.

Schmidt, T.S., Soucek, D.J., Cherry, D.S., Currie, R.J., Latimer, H.A., and Trent, G.C., 2000. A Modified Ecotoxicological Rating to Delineate Acid Mine Drainage Impacted Sub-watersheds. Platform presentation at the Society of Environmental Toxicology and Chemistry 21st Annual Meeting, Nashville, TN November 12-16, 2000.

Soucek, D.S., Schmidt, T.S., Cherry, D.S., 2000. Transplanted Asian Clams *(Corbicula Fluminea)*as dual-purpose bioassessment tools in low order streams. Poster presentation at the Society of Environmental Toxicology and Chemistry 21st Annual Meeting, Nashville, TN November 12-16, 2000.

PRESENTATIONS/PUBLISHED ABSTRACTS Con't

Schmidt, T.S., Soucek, D.J., Cherry, D.S., Currie, R.J., Latimer, H.A., and Trent, G.C., 2000. Integrative Bioassessment Techniques to Predict Ecotoxicological Impairment by Acid Mine Drainage. Poster presented at the National Meeting of the American Society for Mining and Reclamation, Tampa, FL, June 2000.

GRANTS FUNDED

Cherry, D.S., Soucek, D.J., Schmidt, T.S., Currie, R.J. Continuation of the Integrative Bioassessment of the Straight, Reeds, Jones and Cox Sub-basins Impacted by Abandoned Mined Land and Acid Mine Discharges in the Powell River Watershed. Funded June 2000 by David Miller and Associates, Inc. Vienna, VA, and Virginia Department of Mines, Minerals, and Energy, Division of Mined Land Reclamation. Amount Received
$59,478.

TECHNICAL REPORTS

Cherry, D.S., Schmidt, T.S., Soucek, D.J., 2001. Integrative bioassessment of the Cox, Jones, Reeds, Summers, Straight sub-watersheds and the North Fork Powell River, impacted by abandoned mined land and acid mine drainage in the Powell River Watershed: Final Report. Submitted to David Miller and Associates and the Virginia Division of Mine Land Reclamation, Big Stone Gap, VA.

Cherry, D.S., Soucek, D.J., Schmidt, T.S., 2001. Metal accumulation in sediment, Asian clams (*Corbicula fluminea*), and periphyton at selected freshwater mussel preserves in the Clinch and Powell Rivers, Virginia. Submitted to The Nature Conservancy, Clinch Valley Program, Abingdon, VA. Virginia/Tennessee Field Office.

Cherry, D.S., Currie, R.J., Schmidt, T.S., Merricks, T.C., 2001. Chronic Effluent Toxicity Test Report. Submitted to Brush Wellman, Inc., Shoemakersville, PA.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J., Merricks, T.C., Quarterly Chronic Biomonitoring Results of a Water Flea, (*Ceriodaphnia dubia*) and Fathead Minnow (*Pimephales promelas*) To Pine Bluff Mill Effluent, International Paper Company, Pine Bluff, Arkansas.

Cherry, D.S., Currie, R.J., Schmidt, T.S., Kennedy, A.J., Merricks, T.C., 2001. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 3 (round 2, single species tests): Conesville, Muskingham and Pickway Plants. Submitted to AEP, Columbus, OH.

TECHNICAL REPORTS Con't

Cherry, D.S., Currie, R.J., Schmidt, T.S., Kennedy, A.J. 2001. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 2 (round 2, single species tests): Conesville, Muskingham and Pickway Plants. Submitted to AEP, Columbus, OH.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2001. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 1 (round 2, single species tests): Amos, Big Sandy and Sporn Plants. Submitted to AEP, Columbus, OH.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Kennedy, A.J., Hull, M.S., 2001. Chronic Effluent Toxicity Test Report. Submitted to Brush Wellman, Inc., Shoemakersville, PA.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2000. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 5: Rockpost and Tanner Creek Plants. Submitted to AEP, Columbus, Ohio.

Cherry, D.S., Currie, R.J., Hull, M.S., Soucek, D.J., Schmidt, T.S., Kennedy, A.J. 2000. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 4: Glen Lyn and Clinch River Plants. Submitted to AEP, Columbus, Ohio.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2000. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 3: Cardinal and Mitchell Plants. Submitted to AEP, Columbus, Ohio.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2000. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 2: Conesville, Muskingum River and Picway Plants. Submitted to AEP, Columbus, Ohio.

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2000. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 1: Amos, Big Sandy, and Sporn Plants. Submitted to AEP, Columbus, Ohio.

TECHNICAL REPORTS Con't

Cherry, D.S., Currie, R.J., Soucek, D.J., Schmidt, T.S., Hull, M.S., Kennedy, A.J. 2000. Toxicity Test Laboratory Performance Evaluation (DMR-QA Study 20) for Acute and/or Chronic Tests. US EPA Round Robin Test Evaluations.

WORK EXPERIENCES

Research Assistant, Virginia Tech, Department of Biology. *Ecotoxicology.* Conducted research involving the use of integrative bioassessment techniques to investigate acid mine drainage impacts in the North Fork Powell River watershed, VA. Contributed to research investigating the potential impairment of aquatic fauna in the North Fork Powell, the Powell, and Clinch Rivers from AMD and urban runoff. Other responsibilities include the daily maintenance/care of standard toxicity testing organisms, and toxicity testing for various contracted industries. Cherry Lab web page designer http://128.173.184.249/cherry/default.html. Advisor: Dr. Don Cherry. May 1999 to Aug 2001.

Teaching Assistant, Virginia Tech Department of Biology.
Principles of Biology Laboratory. Fall 1999 and Spring 2000.
General Biology Laboratory. Fall 1999 and Spring 2000.

Research Technician, The Ohio State University, Aquatic Ecology Lab, Department of Evolution, Ecology and Organismal Biology. *Fishery Recruitment.* Assisted a doctoral candidate in a study of recruitment mechanisms of fishes in Lake Erie. Responsibilities included identifying and quantifying larval/juvenile fishes and zooplankton, otolith analysis, diet analysis, chlorophyll *a* analysis. Supervisor: Stuart Ludsin, M.S. and Dr. Roy Stein. September 1998 to April 1999.

Research Technician, University of Notre Dame, Department of Biology. *InvasiveSpecies Ecology.* Assisted a doctoral candidate investigating life history attributes of aquatic invaders. Responsibilities included building library of native, exotic, and potential invader species to the Great Lakes basin, building and maintaining fish behavior experiments, and field sampling. Supervisor: Cynthia Kolar, M.S. and Dr. David Lodge. May to August 1998.

Research Technician, The Pennsylvania State University, Department of Biology. *Forested Wetland Ecology*. Assisted two doctoral candidates on separate projects. The primary project was an ecological study of a federally endangered wetland plant species. Also assisted on an attempt to understand how atmospheric deposition influenced treehole communities. Responsibilities included field sampling of wetland characteristics/hydrology, plants, water chemistry analyses, and design and maintenance of experiments. Supervisor: Dr. Kendra Cipollini and Dr. William Dunson, Emeritus. May 1996 to January 1998.

FIELD SKILLS

Qualitative and quantitative macroinvertebrate sampling, *Corbicula in situ* toxicity testing, water quality measures of surface/interstitial waters, habitat assessments using US EPA RBP. Fish tagging, seining, gill netting, bottom trawling, trolling, electro-fishing.

COMPUTER/LAB SKILLS

Web page design and management, Microsoft Word, Word Perfect, EXCEL, SAS, conduction of standard water quality measures, otolith analysis, fish, zooplankton, and macroinvertebrate identification and quantification, water column and sediment acute and chronic toxicity testing. Culturing of fishes: *Stizostedion vitreum, Esox lucius, Perca flavescens, Gymnocephalus cernuus, Notropis atherinoides*; standard test organisms: *Daphnia magna, Ceriodaphnia dubia, Chironomus tentans.*

REFERENCES

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