Determining the Ecotoxicological Recovery of Black Creek (Wise County) and Ely Creek (Lee County) after Watershed Restoration of Abandoned Mine Lands and Acid Mine Drainage: Final Report

This report is submitted to Virginia Department of Mines, Minerals and Energy to fulfill the requirements of Applied Science Grant Funding.

by

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Acid Mine Drainage (AMD) from abandoned mines impacts water resources throughout coal mining areas of the eastern USA. In the Powell River watershed of southwestern Virginia, the two subwatersheds most severely impacted by AMD from abandoned mines were Ely Creek in Lee County and Black Creek in Wise County (Cherry et al 1995, l997, Cherry and Currie 1997). In recent years, however, both subwatersheds have been remediated. In the Ely Creek subwatershed, holding ponds and artificial wetland systems (Successive Alkalinity Producing Systems, SAPS) were constructed by the US Army Corps of Engineers with Virginia DMME assistance to remediate AMD. The Black Creek subwatershed has undergone extensive re-mining for coal with active remediation of the Abandoned Mine Land (AML) and AMD seeps as part of the mining activities. Large volumes of acid-producing materials were isolated from hydrologic influence, and new holding ponds were developed to encourage settling of precipitates from those AMD seeps that remain. Virginia Department of Mines, Minerals and Energy Abandoned Mine Land Program conducted additional activities in the Black Creek subwatershed intended to remediate AMD impacts. The purpose of this research was to evaluate the ecotoxicological status of the Ely Creek and Black Creek subwatersheds, now that active remediation of the AML/AMD sites has occurred.

Similar research methods were employed in both subwatersheds aquatic resources were sampled to assess water and sediment quality and benthic macroinvertebrate status; samples of water and sediments were evaluated for toxicity to standard test organisms using laboratory bioassays; *in situ* ecotoxicological assessments were conducted using Asian clams (*Corbicula fluminea*); and Ecotoxicological Ratings (ETRs) were compiled for primary sampling points. Sampling and ecotoxicological analyses were conducted at locations that had been assessed by previous studies (Cherry et al 1995, l997, Cherry and Currie 1997) in most cases; however, some sampling stations were added and others removed in both subwatersheds as needed, in the judgment of the investigators. Analysis of recovery was performed through comparison of current conditions to those which had been documented prior to remediation by earlier studies.

Overall water quality and aquatic life in the Ely Ck subwatershed have been improved, and both sediment and water-column toxicities have been reduced, as a result of the remediation, but some problems remain. Several small seeps in the upper subwatershed, above the SAPS remediation remain evident, but acidity and metals in the stream below the SAPS are well below pre-remediation levels and benthic macroinvertebrates have returned to sites where they were previously absent. Conductivity levels remain comparable to pre-remediation, a result to be expected since SAPS systems are not intended to affect conductivity. Sensitive components of the benthic macroinvertebrate community (mayflies and stoneflies) were not found at sampling sites immediately below the SAPS remediation and were uncommon at all sampled sites within the subwatershed, including reference sites. Non-ideal sampling conditions, including extreme dry weather during the spring, summer and fall of 2007, may have contributed to the failure of sensitive benthic macroinvertebrates to return to the areas below the SAPS remediation.

The situation in the Black Ck subwatershed is more complicated. Although several of the AMD seeps that were exerting toxic influence in the 1990s have been remediated, others remain in place. Water quality within the upper mainstem, where intermittent low-pH events had been documented prior to remediation, appears to have improved as all measured pH values were within the range of 6.9–8.2; mainstem conductivity, however, has increased, especially in the lower mainstem where levels in the range of 1000 – 1500 µS/cm, or higher, rarely occurred prior to remediation but occurred consistently in the current study. Measured values of water-column dissolved metals (Al and Fe), although remaining at very high levels in several of the unremediated seeps, were at acceptable levels within the mainstem, as would be expected given the mainstem's circumneutral-to-alkaline pH. Sediment metals, especially Fe, however, remain at high levels relative to what would be expected in non-AMD-impacted water bodies. Both *in situ* and laboratory bioassays revealed that the non-remediated seeps remain highly toxic, but that toxicity is not being transferred to the mainstem waters and sediments throughout most of the creek's length. There does appear to be a toxicity problem at the lower end of the creek, just above where it enters the Powell River, as all *in situ* Asian clams placed at this location died and sediments from these locations performed poorly as a medium for the laboratory test organism, *Daphnia magna.* Throughout the mainstem, including these lower sites, water-column toxicity testing with *Ceriodaphnia dubia* revealed no significant toxicity and benthic macroinvertebrates were similar to the reference site. The most sensitive benthic macroinvertebrate components – mayflies and stoneflies – were absent at all sites except the reference, where their numbers were very low. The benthic macroinvertebrate data, however, are not considered to be a reliable indicator of ecosystem status for this study because of the extremely dry conditions that were present at the time of assessment; measured benthic macroinvertebrate numbers were extremely depressed at all sites, including the reference.

A complete report of findings follows. The report is presented in two parts: Section A for Ely Ck (begins on page 04), and Section B for Black Ck (begins on page 58).

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Section A.

Ecotoxicological Recovery Status of the Ely Creek Subwatershed Four Years After Acid Mine Drainage Remediation

by

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EXECUTIVE SUMMARY

A two year research project (December 2006-March 2008) was conducted to determine the effect of acid mine drainage (AMD) remediation activities conducted by US Army Corps of Engineers in the Ely Creek subwatershed, Lee Co. VA. Remediation activities were the construction of successive alkalinity producing systems (SAPS) to neutralize AMD being discharged by pre-1977 abandoned mine lands. A study in the 1990's by Cherry and Currie (1997) identified Ely Ck as the most impacted AMD subwatershed in the N. Fork/Powell River watershed. Both the earlier and current studies include water quality sampling and analyses, field assessments of benthic macroinvertebrate communities, laboratory toxicity testing of water and sediments and the development of an Ecotoxicological Rating (ETR) metric. The current study evaluated conditions in 2006-2008 by comparing them to pre-remediation studies that had been conducted in the 1990's.

Overall water quality and aquatic life in the Ely Ck subwatershed have been improved, and both sediment and water-column toxicities have been reduced, as a result of the remediation, but some problems remain. Several small seeps in the upper subwatershed, above the SAPS remediation remain evident, but acidity and metals in the stream below the SAPS are well below pre-remediation levels and benthic macroinvertebrates have returned to sites where they were previously absent. Conductivities remain at levels comparable to pre-remediation, a result to be expected since SAPS systems are not intended to affect conductivity. Sensitive components of the benthic macroinvertebrate community (mayflies and stone flies) were not found at sampling sites immediately below the SAPS remediation and were uncommon at all sampled sites within the subwatershed, both above and below remediation. Non-ideal sampling conditions, including extreme dry weather during the spring, summer and fall of 2007, may have contributed to the failure of sensitive benthic macroinvertebrates to return to the areas below the SAPS remediation.

We studied 19 sampling sites in the Ely Ck subwatershed in 2007 that included Goose Ck, Bean Ck and extended into the confluence of Stone Ck further downstream depending upon drought conditions. There were eight sampling sites in the Ely Ck mainstem, four in Goose Ck, two in Bean Ck, and five in Stone Ck. As the drought conditions worsened in the summer of 2007, the number of sites was reduced to 12 for extensive field evaluations which included water quality, *Ceriodaphnia dubia*, *Daphnia magna*, and Asian clam toxicity testing and benthic macroinvertebrate (bug) surveys at these sites that maintained some amount of continuous stream flow.

There were seven AMD toxicity-related sites in 2006-2007 that could be compared to those in the 1990's based upon ETR calculations and field measurements available. Sampling sites included SW-11 Seep, SW-11Dn, SW-8, SW-7, SW-9, SW-6, SW-3 and SW-4. The worst AMD impacted site, SW-8, had an ETR that improved from 5.2 in 1997 to 33.9 in 2007. Other sites in Ely Ck (SW-11 Seep and SW-11Dn) had slightly higher ETRs in 1997 (37.5-35.8) than in 2007 (30.3-23.3). At the confluence of Ely Ck with Stone Ck (SW-4), the ETR was substantially higher in 2007 (69.6) than in 1997 (36.0). The problem with the ETR comparisons between both eras is that the drought conditions in 2007 severely impacted the abundance and diversity of bug assemblages there. The ETRs at the two reference sites were lower in 2007 than 1997 due to the drought impact upon the bug parameters that also comprise the ETR. In the

Goose Ck reference site, SW-14/16, the ETR differential was 58.0 or failing in 2007 relative to 81.0 in 1997. Also, in Stone Ck which has more flow due to its larger size, the drought still showed some impact as the ETRs were higher (89.2) in SW-1 Up in 1997 and lower (77.2) in 2007. Hence, comparing overall results between the whole Ely Ck subwatershed in 2007 relative to 1997 is basically inconclusive due to the naturally limiting conditions caused by the prolonged drought.

The benthic macroinvertebrate (bug) data were low in richness and percent mayflies at the SW-14/16 reference site (9.50/1.75%) and values in the high impact AMD sites (SW-8, SW-11Dn) had richness (5.0-3.75) and percent mayfly (0%) numbers similar to the reference site. The habitat assessment scores (HAS) were a good indicator of the drought impact on the SW-14/16 reference site as it only scored 131 points out of 200. Reference scores usually are ≥ 160 points but the low flow limited the scoring of four parameters in the HAS by reducing flow over the riffle areas and increasing the incidence of standing pools. These changing habitat conditions are quite detrimental to the incidence of mayflies and other sensitive insect taxa.

Water quality comparisons of pH between 1997 versus 2007 indicated a vast improvement in 2007. The SW-8 site had a pH range of 2.73-3.06 in 1997 versus a substantial improvement to 5.86-6.85 in 2007. For all AMD influenced sites in 1997, the lowest pH values ranged from 2.73-5.00, while in 2007, they ranged from 5.86-6.85. The substantial gain in pH measurements also had a very positive effect upon *Ceriodaphnia dubia* acute toxicity. All seven AMD impacted site had 0% survival in 1997 versus 90-95% in 2007 for six of them. Only the SW-8 site had some intermittent acute toxicity in three separate tests that ranged from 0-90% alive. Asian clam *in situ* mortality was 100% in five AMD sites in 1997 and only two sites in 2007 had the same total mortality.

A portfolio of photographs was included so that the reader could understand the condition of certain sites in Ely Ck and Goose Ck relative to the remediation efforts and the continuing influence of AMD from the distinctive orange precipitate coloration. When traveling down Ely Ck, the first signs of AMD coloration occurred at the new beaver pond just above the SW-11 site area. Then the coloration was enhanced from the new SW-11 Seep to the confluence of Goose Ck into the mainstem. At this point the most prolific impact of AMD was evident from the old SW-8 site area that now receives intermittent discharges from the highwall/ ridge area parallel to the county road on the opposite side. Discharges from the SAPS area below the Goose Ck confluence serve to neutralize some of the acidity and mitigate the orange coloration that continues down past the church at SW-7. At the Bean Ck confluence, the successful SAPS remediation efforts serve to neutralize more orange coloration in the mainstem as it continues to the Stone Ck confluence. The coloration in the mixing zone area of Ely Ck into Stone Ck is now confined to a small length of perhaps several meters at most.

The overall conclusion is that the SAPS remediation system in upper Goose Ck and Bean Ck have been very successful in counteracting the previous three active AMD sites located there. The current problem is that other AMD sites of smaller (SW-11 Seep) and intermittent (SW-8) discharges are currently impacting the Ely Ck mainstem and the lower area of Goose Ck. The SW-11 Seep is always acutely toxic while the SW-8 seep area is intermittently so depending upon dilution from the headwater of Goose Ck at SW-14 and SW-16. The result is the discharge of AMD precipitate into Stone Ck is still there but at a more reduced scale from that which occurred in the 1990's. However, despite the presence of these small and unremediated AMD seeps, overall water quality and aquatic life in Ely Ck, and in Stone Ck below the confluence with Ely Ck, has been improved.

1.0 INTRODUCTION

The Ely Creek subwatershed was considered to be the most heavily impacted basin from acid mine drainage (AMD) in the North Fork Powell River/ Powell River watershed in a study in 1994-1995 by Cherry et al (1995). More comprehensive studies were then conducted at 20 sampling sites in the subwatershed using an intensive series of water quality (pH, conductivity, aluminum concentrations), iron in sediment, toxicity testing (water column, sediments), *in situ* Asian clam toxicity tests and benthic macroinvertebrate assemblages to evaluate the overall condition (Cherry and Currie 1997). Thereafter, an ecotoxicological rating (ETR) procedure was developed to evaluate the 20 sampling sites that synthesized the ten abiotic/ biotic laboratory and field testing parameters into a single composite value up to 100 points with the higher the ETR, the better the condition (Cherry et al 2001). An excellent rating was 90-100 points, followed by good/acceptable (80-89), fair/marginal (70-79), stressed (60-69) to failing/ severely stressed (≤59). This study found that 12 of the 20 sites had low ETR scores ranging from 57.75 to 5.25 which indicated that reclamation efforts were definitely needed.

To summarize how severe AMD impacts were in the Ely Ck subwatershed prior to remediation, the following is offered. Six of these 12 sites were so environmentally impacted by acidic pH and trace metals that benthic macroinvertebrate abundance was zero, that is, not a single bug was found in the sampling efforts. Hence, not even a few tolerant worms, nematodes or chironomids could tolerate the conditions. Three AMD influenced sites (SW-8, SW-9 and SW-10), had acidic pH ranges of 2.73-3.06, 3.66-4.26, and 3.11-3.64, respectively. These extremely low pH ranges were so harsh to the water flea, *C. dubia*, that even though 100% died at nine sites overall, the extremely acidic pH at sites SW-8, SW-9 and SW-10 caused *C. dubia* to die after several minutes of exposure. Of the three major seeps (SW-8, SW-9, SW-10) SW-8 in

Goose Ck had the highest aluminum concentrations (10.10 mg/L) in the water and iron in the sediments (18,392 mg/kg) and the prolific flow into the Ely Ck mainstem created a tainted red/orange coloration/precipitate to occur in the water and sediment; this precipitate was visible to the confluence in Stone Ck and downstream toward the N.F. Powell River. Vivid photographs are available to document this extensive, colorful, iron dissipation down the Ely Ck subwatershed in the final report by Cherry and Currie (1997).

The purpose of this report is to address the success of the reclamation efforts in this subwatershed after four years of potential ecotoxicological recovery. The reclamation efforts were completed by the US Army Corps of Engineers in October 2003 (USACE 2004). The US ACE constructed Successive Alkalinity Producing Systems (SAPS) at the two major AMD sites in Goose Ck (SW-8) and Bean Ck (SW-9). A preliminary study of the area was conducted in 2004 by Simon et al (2006). This report documents the current ecotoxicological status of the Ely Ck subwatershed, based on investigations conducted in late 2006, 2007, and early 2008, and compares current status to pre-remediation status as documented by pre-remediation studies (Cherry et al., 1997 and 2001; Cherry and Currie 1997).

2.0 MATERIALS AND METHODS

2.1. Study Sites

Eighteen sampling stations were selected: 13 in the Ely Creek subwatershed, and five others in the larger streams above and below the Ely Creek confluence with Stone Creek (Table 1, Fig. 1). Two reference stations in Ely Creek (SW-14 and SW-16) appeared to have no AMD or household influences. They were two unnamed tributaries above a major AMD seep that merged into a single stream, Goose Creek. Due to drought condition late in 2007, the combined confluence of these streams was used for further sampling. Stone Creek at SW-1, a third reference station, was above the Ely Creek confluence and appeared to have no AMD or other environmental stresses. Another reference station (SW-15) was in Ely Creek farthest upstream from the AMD confluence but also suffered from insufficient flow. Two other sampling sites at SW-5 and SW-10 in a small tributary in the 1990's were no longer flowing for the current study. An aerial photograph of the SAPS implemented in Ely Ck at SW-8 is shown in Fig. 2.

2.2. Water Quality

Water chemistry was conducted in the laboratory on grab samples collected from the 18 sites in Ely Creek. Unfiltered water samples were collected in acid washed one-liter polyethylene bottles and transported to the lab on ice where they were refrigerated at 4 C until analyzed (less than 24 hours after collection). The pH was measured using an Accumet BASIC AB15 (Fisher Scientific) bench meter. An YSI 55 (YSI Inc., Yellow Springs OH) DO meter was used to measure DO, and an YSI 30 (YSI Inc., Yellow Springs OH) conductivity meter was used to measure the conductivity. Alkalinity and hardness were measured by titration as described in APHA et al., (1995).

Table 1. In-stream, surface water (SW)/ sediment monitoring locations and the ecotoxicological rating (ETR) procedure.

Station	**Station type**	**Description**
SW-1 Up	Reference	Stone Creek above Ely Creek
SW-1 Dn	Impact/Recovery	Stone Creek below Ely Creek
SW-3	Impact/Recovery	Ely Creek downstream of RT 712; below both SAPS
SW-4	Impact/Recovery	Ely Creek, point nearest mouth of Ely Creek; below both SAPS
SW-6	Impact/Recovery	Bean Creek, above confluence with Ely Creek; below Bean Crk Saps
SW-7	Impact/Recovery	Ely Creek, below confluence with Goose Creek; below Goose Crk SAPS
SW-8	Impact/Recovery	Goose Creek, discharge of pre-remediation acid wetland, below intermittent AMD seep.
SW-9	Impact/Recovery	Bean Creek, head of impact area; below Bean Creek SAPS
SW-11	Impact/Recovery	Ely Creek, upstream of Goose Creek, above SW-11 Seep
SW-11 Seep	Impact/Recovery	Seep below SW-11 flowing into Ely Creek
SW-11 Dn	Impact/Recovery	Ely Creek, below seep at SW-11 Seep but above Goose Crk SAPS discharge[cz1]
SW-13	Impact/Recovery	Ely Creek, impacted area, above all SAPS
SW-14	Reference	Unnamed tributary to Goose Creek, upstream of impact
SW-15	Reference	Ely Creek, upstream of impact area
SW-16	Reference	Goose Creek, upstream of impact area
SW-14/16	Reference	Goose Creek, upstream of impacted area at confluence w/ SW-14
SW-17	Impact/Recovery	Stone Creek, below Ely Creek along RT 713
SW-18	Impact/Recovery	Stone Creek, upstream of Straight Creek confluence

Parameter	ETR procedure and values/concentrations		
	Lowest Score (1)	Highest Score (10)	Value
Taxon richness (x1.25)			12.5
% Ephemeroptera abundance (x1.25)			12.5
Mean pH (x1.25)			12.5
% clam survival (x1.0)			10
% Ceriodaphnia dubia survival (x1.0)			10
Mean conductivity (x1.0)			10
% Daphnia magna survival (x1.0)			10
Asian clam growth (x0.75)[a]			7.5
Iron/sediment (x0.75)			7.5
Aluminum/water (x0.75)			7.5
Total			100

[a] Asian clam growth (mm) replaced *Chironomus tentans* survival from 1997.

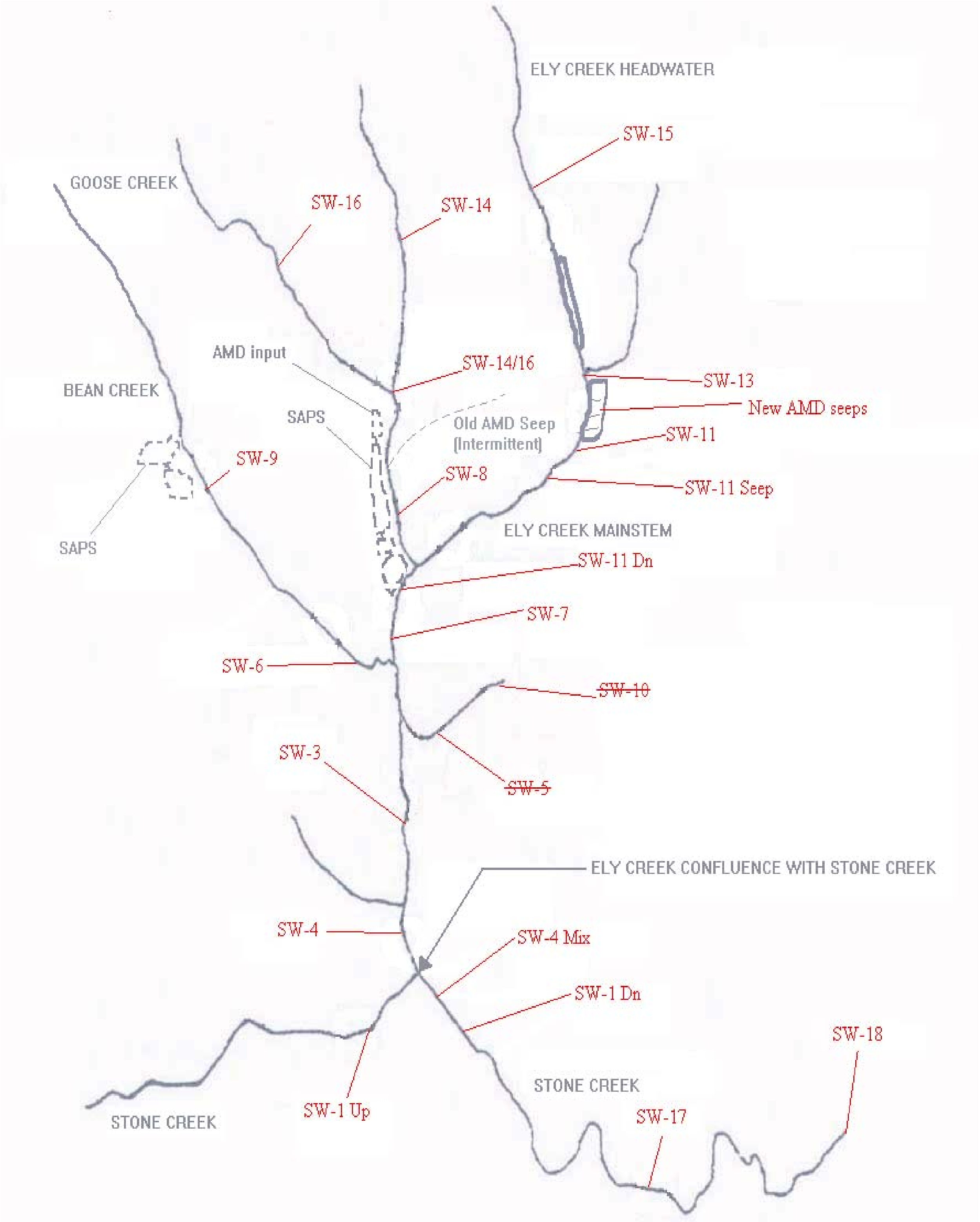

Fig. 1. Diagram of the Ely Creek and Stone Creek subwatersheds with sampling site locations. Strikethrough text represents sites that were sampled in earlier studies (SW-5, SW-10) but not during the current study. [cz2]

Fig. 2. Aerial photograph of SAPS implemented in Ely Creek at SW-8.

2.3. Sediment and Water Column Toxicity Testing and Metals Analysis

Sediment collected from the 18 sampling stations was returned to the laboratory for chronic impairment testing of *Daphnia magna*. Grab samples were collected with a polyurethane dipper, placed in sterile plastic bags and stored on ice at 4°C. Concurrently, a separate set of samples was collected for analysis of selected metals at the Virginia Tech Department of Crop & Soil Environmental Science, Soil Testing Laboratory. Elements analyzed included aluminum (Al), iron (Fe), manganese (Mn) selenium (Se) and strontium (Sr).

Toxicity tests were performed according to Nebeker et al. (1984) and the American Society for Testing and Materials (ASTM, 1995) with slight modifications. Test containers were 1-l beakers with 200 g sediment and 800 ml reference diluent. The *D. magna* test evaluated survival and reproduction (mean number of neonates).Survival and daphnid reproduction in impacted sediments were compared to that in sediments from a laboratory control site. In addition, water column samples were collected from the 20 stations for acute toxicity testing with *Ceriodaphnia dubia*. Neonates <24 hours old were exposed for 48 h to water taken from each station using five replicates per station, with five organisms per replicate.

Differences in mortality and reproduction among treatments in sediment and water column toxicity tests were analyzed at the 95% confidence level using Toxstat® 3.3 (Gulley, 1996). Data were checked for homgeneity of variance using Bartlett's test. If data were normal according to the Shapiro-Wilks test, the Dunnett's test for equal replicate numbers or a *t*-test with Bonferroni's adjustment for uneven replicate numbers was applied. Non-normal data were compared using Steel's Rank Test.

2.4. In situ Toxicity Testing

Asian clams (*Corbicula fluminea*) were placed in 18×36 cm mesh bags (0.5 cm^2 mesh size) for in situ sediment toxicity testing. Five replicate bags were placed in the sediment at each of the 20 sampling stations with five clams per bag. The number of total sampling sites was reduced as the drought conditions worsened. Bags were collected after 62 days, and mortality was recorded. Mortality was based on either the two shell halves being opened, or closed shells opening with a prying tool. Clams were measured, from hinge to the ventral margin, prior to exposure using Fowler Pro-Max electronic digital calipers, measuring to the nearest 0.01mm. Using 18 x 36 cm mesh bags (0.5 cm^2 mesh size), five replicate bags with five clams per bag were secured into the sediment at each site with rebar. Growth was calculated for each bag by comparing mean initial size to mean final size. Differences in survival growth of Asian clams were compared among sites in 2007 using ANOVA and Student's t-test, to test for significant differences at the 95% confidence level using JMP IN® software (Sall et al., 2005).

2.5. Habitat Assessment

A habitat assessment of each station included ten parameters. These were: (1) epifaunal substrate/available cover; (2) embeddedness; (3) velocity/depth; (4) sediment deposition; (5) channel flow status; (6) channel alteration; (7) frequency of riffles; (8) bank stability; (9) bank vegetative stability; and (10) streamside cover. A rating of either 0–10 or 0–20 (depending on the parameter) was developed for each parameter, and the higher the score, the more pristine the station. The habitat assessment approach was the same as that outlined in the US EPA RBPs (Barbour et al., 1999). This assessment was important to determine if shoreline degradation from outside sources (i.e. erosion, misuse of farmland) was instrumental in impairing in-stream habitat or if water-column stress originating from abandoned mined land input was the problem.

2.6. Benthic Macroinvertebrates

Benthic macroinvertebrate surveys were conducted at 12 in-stream stations in the Ely Creek subwatershed and surrounding streams. The sampling was done according to the US EPA

RBPs (Barbour et al., 1999). Riffle, pool and shore-line rooted areas were thoroughly sampled by dip netting. The RBP tier III approach to genus level identification was undertaken. Data were analyzed for total abundance, total richness (i.e. number of different taxa), mayfly (ephemeropteran) abundance and Ephemeroptera–Plecoptera (stonefly)–Trichoptera (caddisfly) (EPT) abundance and richness. The latter group, especially the mayflies, are considered the most sensitive insect orders to environmental perturbations.

2.7. Ecotoxicological Rating [cz3](ETR)

An ETR is a metric that can be used to express the results of an integrative subwatershed assessment, such as this study. An ETR system was developed for Ely Ck in a prior study sponsored by Virginia Department of Mines, Minerals and Energy (Cherry et al. 2001) to rank the Ely Ck sampling stations based on specific bioassessment and water quality parameters influenced by AMD (Table 1). The ETR assigned each station a value on a scale of 1–10 based on the 10 different bioassessment/abiotic parameters, which included: (1) taxon richness; (2) % Ephemeroptera abundance; (3) mean pH; (4) % clam survival; (5) % *Ceriodaphnia dubia* survival; (6) mean conductivity; (7) % *D. magna* survival in sediment tests; (8) Asian clam growth; (9) sediment Fe concentrations; and (10) water column Al concentrations.

Using the weight-of-evidence type approach, a single cumulative value out of 100 points possible became available to characterize the environmental conditions at each station. The higher the point total the less environmentally impacted the station. Reference stations usually have values of 80–90 while AMD sites may range from 5 to 60, depending upon the volume and severity of seepage. A percentile ranking was then developed whereby sites that scored 90% (excellent or “A”), 80% (acceptable or “B”) and 70% (marginal or “C”) would not be considered for future restoration AML/AMD activities. Sites scoring 60 (or "D") and <60% (or "F") were labeled as stressed and severely stressed and would become prime candidates for future restoration activities depending upon the amount of funds available.

3.0 RESULTS AND DISCUSSION

There are 18 tables of data for this mid-2006-2007 study with five tables for water quality and four others for trace metal analysis in water/sediment, seven tables of acute/chronic toxicity data with three test species including two water fleas (*Ceriodaphnia dubia* and *Daphnia magna*) plus the Asian clam (*Corbicula fluminea*) and one table for benthic macroinvertebrate analysis. Then the final table has the compilation of ETR scores generated in 2007 compared to those found in 1997.

3.1 Water Quality

Water quality in Ely creek on 12/12/06 had conductivity ranges from 54 (SW-9) to 683 µS/cm (SW-11 Seep) from the 17 sites analyzed (Table 2). Overall, conductivity was low at the historically impacted site of SW-8 (227 µS/cm) and at the confluence of Ely Ck (423 µS/cm) with Stone Ck. The other important parameter as a marker for AMD impact is pH and it varied from 6.18 (upper reference site, SW-1) in Stone Ck to very neutral ranges of 7.32-7.48 in reference sites (SW-14 and 16) and down Stone CK at sites SW-17 and 18. Site Sw-8 only had pH of 6.99 while the newly found SW-11 Seep had 6.20. All other parameters were in the normal range of general water quality.

Table 2. Water quality data for Ely Creek collected on 12/12/06.

Sample	Temp. (^oC)	Cond. (µS/cm)	DO (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L)
SW-1	24.3	70	11.32	6.18	16	44
SW-3	24.2	474	10.90	6.51	42	180
SW-4	20.0	423	11.47	6.79	52	190
SW-4Mix	21.5	254	11.35	6.94	30	102
SW-6	22.2	440	11.43	6.92	--	--
SW-7	26.0	500	10.73	6.73	--	--
SW-8	24.7	227	11.01	6.99	48	90
SW-9	24.4	54	10.46	6.81	--	--
SW-11	25.2	550	10.45	6.34	--	--
SW-11 Seep	25.1	683	10.44	6.20	14	204
SW-13	22.1	179	9.82	6.59	--	--
SW-14	23.7	107	11.00	7.48	--	--
SW-15	21.8	106	11.38	6.87	52	56
SW-16	25.5	139	11.12	7.34	--	--
SW-14/16	21.2	183	11.60	7.38	40	46
SW-17	20.8	209	12.06	7.32	--	--
SW-18	21.8	197	11.83	7.33	--	--

During 3/29/07, conductivity was even more diluted with a range of 36 µS/cm (SW-9) to 457 µS/cm at the SW-11 Seep (Table 3). The SW-8 site only had 170 µS/cm and the Ely Ck. confluence site (SW-4) had 333 µS/cm. The pH ranged from 6.17 (SW-1 Up) to 7.27 (SW-9) and was 6.71 at the confluence (SW-4). Stone Ck. pH ranged from 6.17-6.87 at sites SW-1 to SW-18. The newly found SW-11 Seep site had a pH reduction to 4.32.

Table 3. Water quality data for Ely Creek collected on 3/29/07.

Sample	Temp. (^oC)	Cond. (µS/cm)	DO (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L)
SW-1 Up	21.2	59	9.69	6.17	20	48
SW-1 Dn	21.0	148	9.57	6.48	30	108
SW-3	21.7	346	9.14	6.43	48	210
SW-4	21.8	333	9.57	6.71	54	208
SW-4Mix	21.6	332	9.59	6.79	32	104
SW-6	21.7	371	9.65	6.85	44	186
SW-7	22.0	349	9.09	6.83	48	176
SW-8	22.8	170	9.71	7.00	56	102
SW-9	21.2	36	9.52	7.27	38	62
SW-11	21.0	331	9.36	6.51	34	196
SW-11 Seep	21.4	457	9.15	4.32	18	220
SW-13	21.1	152	8.75	6.22	42	64
SW-14	22.1	108	9.74	6.50	30	76
SW-15	21.1	84	9.73	6.87	58	60
SW-16	22.2	127	9.69	6.65	52	76
SW-14/16	21.5	117	9.86	6.57	48	74
SW-17	21.1	150	9.84	6.86	26	88
SW-18	22.5	142	9.66	6.87	46	96

On 6/21/07, conductivity increased from the winter/spring sampling levels, ranging from 120 (SW-1 Dn) to 1041 µS/cm (SW-6) in the stream channels (Table 4). Conductivity in Stone Ck below the Ely Creek confluence ranged from 348 to 390 µS/cm, and was 780 µS/cm in Ely Ck just above the confluence with Stone Ck (SW-4). The highest conductivity measured on this date was 1860 µS/cm, at the SW-11 Seep site. Measured pH values in the stream channels, ranged from 6.19 (SW-1 Up) to 7.30 (SW-9); the only pH<6.0 was recorded at the SW-11 Seep (3.04).

Additional water quality measurements were taken in July and September, 2007, at SW-11 Seep and at SW-8, which is influenced by a seep. The SW-11 Seep ranged in conductivity from 1854-2322 µS/cm and SW-8 ranged from 225-2192 µS/cm (Table 5). The lowest values for both sites occurred after a rain event on 7/31/07, and the highest values occurred on 9/18/07, when very little flow was present. The pH ranged for these samples from 3.19 to 4.13 at SW-11 Seep to 5.86 to 6.76 at SW-8.

Table 4. Water quality data for Ely Creek collected on 6/21/07.

Sample	**Temp. (^{o}C)**	**Cond. (µS/cm)**	**DO (mg/L)**	**pH**	**Alkalinity (mg/L)**	**Hardness (mg/L)**
SW-1Up	25.3	120	8.56	6.19	48	184
SW-1Dn	25.1	348	8.54	6.50	46	56
SW-3	25.7	814	8.63	6.44	86	400
SW-4	25.8	780	8.66	6.73	70	354
SW-6	25.7	1041	9.57	6.88	112	500
SW-7	26.0	807	8.17	6.85	84	382
SW-8	26.8	825	8.59	7.05	80	368
SW-9	25.2	200	8.69	7.30	80	90
SW-11 Seep	25.4	1860	8.79	3.04	---	630
SW-11 Dn	25.0	860	8.38	6.27	40	398
SW-13	25.1	436	7.90	6.25	80	244
SW-14/16	25.5	153	8.71	6.59	74	84
SW-17	25.1	350	9.86	6.90	50	170
SW-18	26.5	390	9.70	6.87	78	208

Table 5. Water quality data for Ely Creek collected on 7/3/07, 7/31/07 and 9/18/07.

Sample	Temp. (^oC)	Cond. (µS/cm)	DO (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L)
7/3/07						
SW-11 Seep	24.3	1854	5.68	3.19	---	634
7/31/07						
SW-8	23.7	225	8.92	6.76	88	90
SW-11 Seep	24.0	648	8.41	4.13	34	234
9/18/07						
SW-8	26.1	2192	6.25	5.86	50	1670
SW-11 Seep	26.2	2322	6.19	3.83	12	940

On 1/8/08, conductivity generally declined relative to earlier measurements during Spring/Summer conditions (126 µS/cm at SW-1 Up to 755 µS/cm at SW-7) (Table 6). At the Ely Ck confluence (SW-4) site, conductivity was 686 µS/cm. The SW-11 Seep site had the highest conductivity of 1082 µS/cm which dissipated slightly to 840 µS/cm at the SW-11 Seep Dn sampling location which was ~50m downstream in Ely Ck. Conductivity at SW-8 was only 381 µS/cm, much reduced from the summer drought conditions. The pH ranged from 6.63-6.65 at the SW-11 Seep and SW-8 to 7.44 (SW-1 Up) and 7.79 at SW-17.

Table 6. Water quality data for Ely Creek collected on 1/8/08.

Sample	Temp. (^oC)	Cond. (µS/cm)	DO (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L)
SW-1 Up	24.7	126	10.87	7.44		
SW-1 Dn	27.5	344	10.37	7.21		
SW-3	26.5	777	9.28	6.78		
SW-4	27.5	686	9.56	7.16	60	280
SW-6	26.5	749	9.44	7.45		
SW-7	24.2	755	9.99	6.80		
SW-8	24.2	381	9.60	6.65	58	152
SW-9	23.6	146	9.72	7.20		
SW-11	24.2	739	9.49	6.63		
SW-11 Seep	23.9	1082	9.53	6.57	20	---
SW-13	25.6	736	9.81	6.50		
SW-14	27.0	144	10.01	7.96		
SW-15	24.0	189	10.28	7.38	78	86
SW-16	26.4	175	9.83	7.90		
SW-14/16	24.1	137	10.54	7.54	44	66
SW-17	24.2	363	10.80	7.79		
SW-18	26.3	374	10.46	7.56		

Iron and aluminum in the water column were measured on 7/18/07 at the major sampling sites which had flow (Table 7) and then again at sites SW-8 and SW-11 on 10/17/07 (Table 8). Aluminum was highest 0.139 mg/L in the SW-11 Seep. At the other sites, aluminum ranged from <0.004 mg/L to only 0.139 mg/L. Iron was highest at the SW-11 Seep (9.773 mg/L) followed by 0.320 and 0.302 mg/L at sites SW-7 and SW-9, respectively. All other sites had low iron concentrations that ranged from 0.012-0.132 mg/L. On 10/17/07, aluminum was somewhat elevated (0.508 mg/L) relative to 7/18/08 at SW-11Seep and extremely so for iron at SW-11 Seep (4.050 mg/L). At SW-8, aluminum was low (0.021 mg/L) while iron was elevated (1.432 mg/L).

Table 7. Iron and aluminum water analysis (mg/L) for Ely Creek on 7/18/07.[cz4]

Analyte	Al (mg/L)	Fe (mg/L)
SW- 1 Up	0.004	0.014
SW- 1 Dn	0.021	0.070
SW- 3	0.006	0.132
SW- 6	<0.004	0.006
SW- 13	<0.004	0.012
SW- 7	0.004	0.320
SW- 8	<0.004	0.015
SW- 9	0.025	0.302
SW- 14/16	0.007	0.009
SW- 17	0.010	0.071
SW- 4	<0.004	0.006
SW- 18	<0.004	0.007
SW- 11 Seep	0.139	9.773
SW- 11 Dn	<0.004	0.012

Table 8. Iron and aluminum water analysis (mg/L) for Ely Creek on 10/17/07.

Site	Al (mg/L)	Fe (mg/L)
SW-8	0.021	1.432
SW-11 Seep	0.508	4.050

3.2 Sediment Quality

In the sediment, aluminum was measured twice on 12/22/06 and again on 1/8/08 (Tables 9-10). [cz5]Aluminum was above 10,000 mg/kg at three sites, SW-13 (11,276 mg/kg), SW-7 (12,325 mg/kg) and SW-6 (13,770 mg/kg). Thereafter, aluminum varied from 9,313 mg/kg to 4,282 mg/kg. Iron was highest in sediment at the SW-11 Seep (99,688 mg/kg) and substantially lower (49,247-17,705 mg/kg) at the other sites.

As shown in Table 10 for January 2008, five trace elements were analyzed from the two major AMD impacted sites (SW-11 Seep and SW-8), down the Ely Ck mainstem/confluence (SW-3 and SW-4) and the SW-14/16 reference site. Iron was highest (82,910 mg/kg) at SW-4 followed by SW-11 Seep (42,633 mg/kg) and lowest at SW-14/16 (20,285 mg/kg). Aluminum was fairly consistent at sites SW-4, SW-3 and SW-8 (4,666, 4,225 and 4,124 mg/kg), lowest at SW-11 Seep (2,687 mg/kg) and midway (3,294 mg/kg) at SW-14/16. Manganese was highest (3,863 mg/kg) at SW-3 and much lower (117-580 mg/kg) at the other four sites. Strontium had no specific trend and selenium was below detection limits.

Table 9. Iron and aluminum sediment analysis (mg/kg) for Ely Creek on 12/22/06.

Site	Al (mg/kg)	Fe (mg/kg)[cz6]
SW-1	4996	33129
SW-3	6134	40171
SW-4	6812	28868
SW-4Mix	4282	17705
SW-6	13770	41307
SW-7	12325	37424
SW-8	4492	21161
SW-9	5633	27952
SW-11	8694	49247
SW-11 Seep	6068	99688
SW-13	11276	42889
SW-14	4457	44812
SW-14/16	6711	42835
SW-15	4137	19669
SW-16	9313	39363
SW-17	6461	38017
SW-18	4948	30333

Table 10. Iron, aluminum, manganese, selenium. and strontium sediment analysis (mg/kg) for Ely Creek on 1/8/08.[cz7]

Site	Al (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Se (mg/kg)	Sr (mg/kg)
SW-3	4225.9	35857.6	3863.9	<2.9	10.2
SW-4	4666.6	82910.8	435.7	<2.9	12.4
SW-8	4124.2	8426.3	117.5[cz8]	<2.9	6.2
SW-11Seep	2687.8	42633.9	580.1	<2.9	9
SW-14/16	3294.6	20285.4	298.8	<2.9	5.8

3.3 Toxicity Testing

Toxicity testing with *C. dubia* was conducted using waters from SW-8 and SW-11 Seep site once in July 2007 and twice in September while the *D. magna* sediment toxicity test was run in October (Tables 11-15). The SW-11 Seep was acutely toxic to *C. dubia* with a 48-hr LC50=36.6% (Table 11). When testing the SW-8 site for toxicity using undiluted (100%) water samples, *C. dubia* had 90% survival on 9/14-16/07 and none alive on 9/26-28/07 (Tables 12-13). In the first September 2007 test, conductivity was 228-251 µS/cm after a substantial rain event. Conductivity was much higher, 2,192-2,289 µS/cm, in the sample that caused the 100% kill. Waters from the SW-11 Seep caused 100% mortality in all three tests as conductivity was high for the 7/9-11/07 test (1,859-1,854 µS/cm), low on 9/14-16/07 (629-639 µS/cm) and highest on 9/26-28/07 (2,320-2,589 µS/cm). The pH of the SW-11 Seep samples was low (3.19-3.22, 4.13-5.10 and 3.81-3.91) in all three tests where 100% mortality occurred. In the SW-8 tests, pH was 6.79-7.22 where 90% survival occurred in the 9/14-16/07 test, and reduced slightly to 5.88-6.00 in the 9/26-28/07 test that caused 100% mortality.

Table 11. Survival of *Ceriodaphnia dubia* exposed to SW-11 Seep water from 7/9-11/07.

Concentration (%)	Survival (%)	Conductivity (µS/cm)		pH Range
		START	END	
0	100	270	299	7.54-8.01
6.25	100	375	282	7.38-8.02
12.5	100	476	474	7.26-7.98
25	100	680	684	6.81-7.81
50	5	1028	1020	4.25-4.45
100	0	1859	1854	3.19-3.22

LC50= 36.60% SW-11 Seep water

Table 12. Survival of *Ceriodaphnia dubia* exposed to100% SW-8 and SW-11 Seep water from 9/14-16/07.

Concentration (Site)	Survival (%)	Conductivity (µS/cm)		pH Range
		START	END	
0	100	286	310	7.56-8.01
SW-8	90	228	251	6.79-7.22
SW-11 Seep	0	629	639	4.13-5.10

Table 13. Survival of *Ceriodaphnia dubia* exposed to100% SW-8 and SW-11 Seep water from 9/26-28/07.

Concentration (Site)	Survival (%)	Conductivity (µS/cm)		pH Range
		START	END	
0	100	283	296	7.43-8.07
SW-8	0	2192	2289	5.88-6.00
SW-11 Seep	0	2320	2589	3.81-3.91

The *D. magna* chronic sediment toxicity tests had 80-100% survival for 9 samples and minimal to none alive (20, 7 and 0%) in samples from sites directly downstream of unremediated AMD seeps - SW-8, SW-11 Dn and SW-11 Seep (Table 14). Reproduction for *D. magna* was significantly lowest at the same three sites with high mortality relative to the laboratory control and SW-14/16 reference site (Table 15).

Table 14. *Daphnia magna* survivorship in a 10-day chronic sediment toxicity test run on 10/9-19/07.

SITE		MEAN SURVIVAL	PERCENT SURVIVAL (%)
Control	A	3.0000000	100%
SW-14/16	A	3.0000000	100%
SW-1 Up	A	3.0000000	100%
SW-6	A B	2.8000000	93%
SW-18	A B	2.6000000	87%
SW-7	A B	2.6000000	87%
SW-17	A B	2.6000000	87%
SW-11 Dn	B	2.4000000	80%
SW-4	B	2.4000000	80%
SW-3	B	2.4000000	80%
SW-8	C	0.6000000	20%
SW-11 Dn	C D	0.2000000	7%
SW-11 Seep	D	0.0000000	0

Levels not connected by same letter are significantly different.

Table 15. *Daphnia magna n*eonate reproduction for a 10-day chronic sediment toxicity test on 10/9-19/07.

SITE		MEAN NEONATES
Control	A	77.800000
SW-14/16	A	77.400000
SW-3	A B	74.000000
SW-6	A B	73.400000
SW-1 Up	A B	73.200000
SW-18	A B	66.600000
SW-17	A B	65.600000
SW-1 Dn	A B	64.800000
SW-7	A B	64.600000
SW--4	B	62.200000
SW-11 Dn	C	41.800000
SW-8	C	34.800000
SW-11 Seep	C	31.600000

Levels not connected by same letter are significantly different.

The Asian clam *in situ* test ran for 62 days from June 21- August 18, 2007. Asian clam survivorship was very high (94-100%) at nine sites and dropped to zero at sites impacted by unremediated AMD seeps - SW-11 Seep, SW-11 Dn and SW-8 (Table 16). Hence, the two sites with high *C. dubia* acute and *D. magna* chronic mortality (SW-11 Seep and SW-8) also had zero percent alive for the Asian clam *in situ* test (Table 17). *Corbicula* growth, a more sensitive threshold/ endpoint than survival, was significantly impaired at sites SW-11 Seep, SW-11 Dn and SW-8 relative to other sites. Sites SW-7, SW-3 amd SW-4, sites with the significantly highest growth, are located at the Ely Ck. mainstem below the SAPS installed for AMD remediation by the church, below the Bean Ck confluence and above the confluence with Stone Ck. Growth at reference site SW-14/16 was significantly lower than at sites SW-7, SW-4 and SW-3 due to drought impacts in the upper headwater regions. Because of drought impacts, reference site SW-15 in upper Ely Ck, and sites SW-14 and SW-16, had intermittent flow and could not be evaluated.

Table 16. Ely Creek Asian clam *in situ* survivorship on 6/21/07-8/18/07.

SITE	MEAN SURVIVAL	% SURVIVAL
SW-1 Dn	5.0 a	100
SW-1 Up	5.0 a	100
SW-6	5.0 a	100
SW-14/16	5.0 a	100
SW-4	4.8 a	96
SW-17	4.8 a	96
SW-18	4.8 a	96
SW-3	4.8 a	96
SW-7	4.6 a	94
SW-11 Seep	0 b	0
SW-11 Dn[cz9]	0 b	0
SW-8	0 b	0

Values followed by different letters denotes a significant statistical difference.

Table 17. Ely Creek *in situ* Asian clam growth on 6/21/07-8/18/07.

SITE	MEAN GROWTH (mm)
SW-7	0.7564 a
SW-3	0.7236 a
SW-4	0.7076 a b
SW-17	0.5676 b c
SW-1 Up	0.5492 c d
SW-6	0.5372 c d
SW-1 Dn	0.4792 c d
SW-18	0.4100 d
SW-14/16	0.1436 e
SW-11 Seep	0.0024 e f
SW-11 Dn	0.0008 f
SW-8	0.0000 f

Values followed by different letters denotes a significant statistical difference.

3.4 Benthic Macroinvertebrates and Habitat Assessment

In Table 18, a summary of the benthic macroinvertebrate data for Ely Ck are reported for 12 sampling sites. In the Ely Ck subwatershed, the best reference site was SW-14/16 with 119 abundance and 9.5 different taxa. The other sites ranged with abundance/ richness of 9.0/3.75 (SW-11 Dn) to 38.0/8.75 (SW-4). The few taxa found in SW-11 Dn were environmentally tolerant forms that were not found in the parameters list of Table 18. Mayflies were nearly non-existent even at SW-14/16 which had two organisms. In Stone Ck, the reference site (SW-1 Up) had abundance/richness of 53.75/14.5 and was nearly the same at the downstream sites of SW-17 and SW-18, indicating that the waters entering from Ely Ck were not having a deletrious effect. Some decline in taxa richness (10.25) occurred at SW-1 Dn, the first site below the Ely Ck confluence. Reasons for these dismally low macroinvertebrate numbers can be due to the severe drought conditions that were prevalent in most of 2007 and the presence of unremediated AMD seeps at SW-8 and SW-11 Seep. Sites SW-6 and SW-7, the two sites most directly impacted by the remediation and the SAPS drainages, lacked stoneflies and mayflies. EPT richness and abundance were generally poor throughout the Ely Ck subwatershed, with the only EPT richness > 1.00 being located at the sampling site that is lowest in the subwatershed and closest to Stone Ck, SW-4.

The habitat assessment scores (HAS) in the 12 sampling sites for bugs during unusual drought conditions over the past 2 years had wide ranging scores from 173-31 out of a potential total of 200 (Table 18). The four Stone Ck sites had HAS of 173 to 152 with the highest score furthest downstream and the lowest just below the Ely Ck confluence. Within Ely Ck, HAS ranged from only 131 (SW-14/16) to 31 (SW-8). The Ely Ck reference site had a low HAS due to drought conditions with minimal flow at the sites while the remediated SW-8 site had no riparian vegetation and copious AMD sedimentation into the area. The confluence site of Ely Ck (SW-4) had a HAS of 126 which was quite similar to the reference area of SW-14/16. The new SW-11 Seep site had a score of 68 relative to the next three sites further downstream SW-11 Dn (86), SW-7 (90) and SW-3 (97). The Bean Ck SW-6 site had a score of 122 even though bug parameters there were not better than in the Ely Ck sites.

Table 18. Summary of benthic macroinvertebrate data and habitat assessment scores (HAS) for Ely Creek collected on 7/31/07.

PARAMETER	SW-1 Up	SW-1 Dn	SW-3	SW-4	SW-6	SW-7	SW-8	SW-11 Dn	SW-11Seep	SW-14/16	SW-17	SW-18
Mean Total Abundance	53.75	88.50	21.25	38.00	42.00	14.75	30.50	9.00	9.00	119.00	66.00	64.00
Mean Taxa Richness	14.50	10.25	4.50	8.75	6.00	5.75	5.00	3.75	4.00	9.50	17.75	15.00
Mean Caddisfly Abundance	6.00	4.50	0.25	9.75	6.50	4.50	0.25	0.00	1.50	1.00	6.50	7.50
Mean Stonefly Abundance	2.00	1.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.50	1.50
Mean Mayfly Abundance	6.00	7.75	0.00	0.25	0.00	0.00	0.00	0.00	0.00	1.75	17.50	20.75
Mean Mayfly Richness	2.50	2.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	1.00	3.25	2.75
Mean Percent Mayfly	11.94	9.25	0.00	0.42	0.00	0.00	0.00	0.00	0.00	1.32	24.66	30.16
Mean EPT Abundance	14.00	13.25	0.75	10.00	6.50	4.50	0.25	0.00	1.50	3.00	24.50	29.75
Mean Percent EPT	26.38	15.87	4.69	23.91	17.04	32.78	0.89	0.00	18.75	2.55	35.40	43.95
Mean EPT Richness	4.50	3.75	0.50	1.25	0.50	1.00	0.25	0.00	0.75	2.00	5.25	5.50
Mean Midge/EPT Ratio	1.68	5.49	0.33	1.20	6.91	1.36	1.75	N/A	2.75	21.88	0.25	0.09
HAS	158	152	97	126	122	90	31	86	68	131	164	173

3.5 Comparison of pH and Conductivity between 1995-1997 and 2006-2008

When comparing the potential recovery of acidic pH and high conductivity from AMD in the Ely Ck subwatershed, before versus after remediation, a dramatic increase occurred for pH at formerly AMD-impacted sites along with minor reductions in conductivity for those sites that were formerly most directly impacted by AMD (Table 19). At SW-8, pH increased from 2.73-3.06 in 1997 to 5.86-6.85 in 2007. The pH recovery was even greater in Bean Ck at SW-9 (3.66-4.26 versus 6.81-7.30). At the Ely Ck mainstem near Stone Ck (SW-4), pH changed from 4.71-7.13 to 6.71-7.16. Conductivity declined at SW-8, which formerly received direct AMD discharge but is currently above the SAPS discharge, from 1,600-3,620 µS/cm in 1997 to 170-2,192 µS/cm in 2007. The unusually high conductivity reading of 2,192 µS/cm in 2007 occurred in the summer as the drought caused the slow flowing water in SW-8 to evaporate. The fact that conductivity levels in 2007 at SW-1 Up, which is in Stone Ck above the confluence with Ely Ck, ranged higher than 1997 levels, is direct evidence that measured conductivity differences must be interpreted considering the drier weather conditions of 2007.

Table 19. Conductivity and pH comparisons from 1997 and 2007.

Site	**pH**		**Conductivity (µS/cm)**	
Year	***1997***	***2007***	***1997***	***2007***
SW-1 Up	6.14-6.72	6.17-7.44	40-60	59-126
SW-1 Dn	N/A	6.48-7.21	N/A	148-348
SW-3	4.81-5.96	6.43-6.78	260-560	346-814
SW-4	4.71-7.13	6.71-7.16	240-350	333-780
SW-6	3.82-6.06	6.85-7.45	180-540	371-1041
SW-7	5.20-6.31	6.73-6.85	350-430	349-807
SW-8	2.73-3.06	5.86-6.85	1600-3620	170-2192
SW-9	3.66-4.26	6.81-7.30	200-640	36-200
SW-11	5.00-6.82	6.34-6.63	350-430	331-739
SW-11 Seep	N/A	3.04-6.57	N/A	457-2322
SW-15	6.41-8.02	6.87-7.38	70-90	84-189
SW-14/16	6.36-6.99	6.57-7.54	125-140	117-183

3.6 Comparison of Daphnid and Asian Clam Survivorship between 1997 and 2007

The differences in survivorship of the daphnid (*C. dubia*) and the Asian clam (*C. fluminea*) were even more dramatic between 1997 versus 2007 as survivorship improved at most sampling sites in 2007 (Table 20). For *C. dubia*, 90-100% survivorship occurred at five sites in 1997, three reference sites (SW-1 Up, SW-15, SW-14/16) and the two lower site (SW-17 and SW-18) in Stone Ck beyond the Ely Ck confluence. Data from seven other sites within the Ely Ck subwatershed had 0% alive. In 2007, daphnids had 90-100% survivorship at all sites except two that remain influenced by unremediated AMD seeps, SW-8 (58.3% alive) and the SW-11 Seep (all dead). Although survivorship at SW-8 was low in 2007 compared to all other sites except SW-11, it demonstrated improvement relative to the 0% survival of 1997.

For the Asian clam *in situ* tests carried out within each site for ~ two months, the results were more environmentally sensitive (Table 20). In 1997, only the two reference sites (SW-1 and SW-14/16) had 100% survival, 50 and 75% were alive at SW-6 and SW-7, and five sites (SW-3, SW-4, SW-8, SW-9 and SW-11 Dn) had 0% survival while the upper most reference site SW-15 had 20% alive. Even the two sites in Stone Ck furthest below the Ely Ck confluence (SW-17 and SW-18) only had 15 and 30% alive in 1997. In 2007, however, clam survival resulted in 94-100% alive at all but three sites directly impacted by unremediated AMD seeps - SW-8, SW-11 Seep and SW-11 Dn - where survival was 0%.

Table 20. Percent *Ceriodaphnia dubia* and *Corbicula fluminea* (Asian clams) survival at 100% surface water comparisons from 1997 and 2007.

Site	**Percent Survival (%)**			
	C. dubia		***C. fluminea***	
Year	***1997***	***2007***	***1997***	***2007***
SW-1 Up	100	100	100	100
SW-1 Dn	N/A	95	N/A	100
SW-3	0	90	0	96
SW-4	0	95	0	96
SW-6	0	95	50	100
SW-7	0	95	75	94
SW-8	0	58.3*	0	0
SW-9	0	95	0	---
SW-11	0	90	0	0
SW-11 Seep	N/A	0*	N/A	0
SW-15	20	90	20	---
SW-14/16	100	100	100	100
SW-17	90	95	15	96
SW-18	100	90	30	96

* Denotes mean survival for three tests conducted.
N/A means that the data was not collected because the site was not part of the 1997 study.
--- means that the data could not be generated because of no flow or dry conditions.

3.7 Ecotoxicological Rating

For the 12 sites studied in 2006-2007, ETR scores were calculated as described in the Cherry et al (2001) publication and then compared to the conditions prior to AMD reclamation (Table 21). In 2006-2007, only four sites, SW14/16, SW-8, SW-11 Dn and SW-11 Seep, had “failing” ETRs (scores <60) that were 58.0, 33.9, 30.0 and 33.3, respectively. The upstream reference site (SW-14/16) in Bean Ck had a low ETR of 58.0 indicating stress at this site due to the drought conditions in 2006-2007. Water flow above this site at the two converging tributary sites of SW-14 and SW-16 was so limited that the combined site had to be used, although its flow was minimal in the riffle area as well. The other three failing sites are impacted by unremediated AMD seeps.

Four sites in the lower portion of the Ely Ck subwatershed that were deemed AMD impacted in 1997 (SW-4, SW-6, SW-7 and SW-3) and Stone Ck directly below the Ely Ck confluence (SW-1 Dn,) had stressed 2006-2007 ETRs that ranged from 69.6 to 65.3. Three sites (SW-17, SW-1 Up, SW-18) had ETRs of 77.8-74.7 which were in the marginal or "C" grade. All three of these sites were in Stone Ck which had consistently greater flow throughout 2006-2007 than did Ely Ck. The two sites (SW-17 and SW-18) downstream of the Ely Ck confluence had ETRs of 77.8 and 74.7 which were in the marginal grade.

The highest ETR measured in 2006-2007 was 77.8, in Stone Ck. We interpret the fact that all measured ETRs were within the "marginal" or lower range to indicate the biota were stressed by the drought.

A more enlightening picture of potential recovery trends in Ely Ck can be seen when comparing the ETRs developed in 1997 to ETRs for the same sites sampled in 2007 (Table 21). Eleven of the 12 sites could be compared directly to those evaluated in 2007; SW-1 Dn was added into the 2007 study to address potential immediate impacts of the Ely Ck confluence just beyond the mixing zone in Stone Ck. In 1997, eight of the 11 sites studied 7 had scores of "F" (<60) or severely stressed; the only sites having ETRs > 60 were two in Stone Ck (SW-17, and SW-1 up which is upstream of the Ely Ck confluence) and SW 14/16, which was selected as a reference site above the AMD impacts. In the 2007 study, only four sites failed (ETR<60) (SW-14/16, SW-8, SW-11 Dn and SW-11 Seep) and one of these (SW-8, which formerly received a direct and constant AMD from the old "Wetland") had an ETR of 33.9 in 2006-2007 compared to 5.2 in 1997. The other two low failing sites in 1997, SW-11 Dn and SW-11 Seep, had ETRs that were (37.5-35.8) slightly higher than those observed in 2007 (30.0-23.3).

The recovering sites of Ely Ck (SW-4, SW-6, SW-7 and SW-3 with ETRs of 69.6, 67.2, 67.4 and 65.3 in 2007), all showed substantial improvement relative to the ETRs in 1997 of 36.0, 50.5, 42.5, and 51.5, respectively (Table 21). Site SW-4 at the confluence of Ely Ck into Stone

Ck had ETRs that nearly doubled between 1997 (36.0) and 2007 (69.6). The two sites in the Ely Ck mainstem above SW-4 at SW-3 and SW-7 had ETRs that were 15 to 25 points higher in 2007. In Bean Ck where one major AMD seep was remediated, the recovery was quite evident at SW-6 as well. The ETRs at SW-6 between 2007 versus 1997 were 67.2 to 50.5 with a ~15 point improvement. The only site that had the reverse or a lower ETR in 2007 versus 1997 was the upstream reference site (SW-14/16) in Goose Ck. The ETRs were 58.0 in 2007 versus 81.0 in 1997 which indicated how severe the drought was in the Ely Ck subwatershed in 2006-2007.

In the three Stone Ck sites, SW-18 had recovered by nearly 17 points in 2007 since 1997 (Table 21). The other downstream site below the Ely Creek Confluence (SW-17) had a 12-point increase from 1997 (65.0) to 2007 (77.8). In the reference site (SW-1 Up) of Stone Ck above the Ely Ck confluence, the ETR was lower in 2007 (77.2) than in 1997 (89.2) which may have been influenced by the overall drought conditions in Stone Ck. Overall, the ETR scores indicate some noticeable improvement in the AMD impacted sites of 1997 after reclamation.

Table 21. Comparison of ecotoxicological ratings (ETRs) at sites in the Ely Creek/ Stone Creek subwatersheds between periods of severe Acid Mine Drainage impacts in 1997 relative to current conditions in 2007 four years after reclamation. The ETRs can grade as excellent or "A" (90-100), acceptable or "B" (80-89), marginal or "C" (70-79), stressed or "D" (60-69) and severely stressed/failing or "F" (<60).

2007					*1997*			
Rank	**Station**	**Description**	**ETR Score**	**Grade**	**Rank**	**Station**	**ETR[b] Score**	**Grade**
1	SW-17	Recovered	77.8	C (marginal)	3	SW-17	65.0	D
2	SW-1 Up	Reference	77.2	C "	1	SW-1 Up	89.2	B
3	SW-18	Recovered	74.7	C "	4	SW-18	57.8	F
4	SW-1 Dn	Recovering	69.6	D (stressed)	--	SW-1 Dn	--[a]	--[a]
5	SW-4	Recovering	69.6	D "	10	SW-4	36.0	F
6	SW-6	Recovering	67.2	D "	6	SW-6	50.5	F
7	SW-7	Recovering	67.4	D "	7	SW-7	42.5	F
8	SW-3	Recovering	65.3	D "	5	SW-3	51.5	F
9	SW-14/16	Reference	58.0	F (failing)[c]	2	SW-14/16	81.0	B
10	SW-8	AMD impact	33.9	F "	11	SW-8	5.2	F
11	SW-11 Dn	AMD impact	30.0	F "	8	(SW-11) SW-11 DN	37.5	F
12	SW-11 Seep	AMD impact	23.3	F "	9	(SW-12) SW-11 Seep	35.8	F

[a] Site not sampled in 1997.
[b] Generated by Cherry et al (2001)
c Site stressed due to acknowledgments

3.8 Photographic Analysis of Selected Mainstem/ Seep Sites

In the upper-most area of Ely Ck is the reference site, SW-15, that has a number of features for a pristine area except that the drought inhibited the quality of benthic macroinvertebrates found when it became intermittent during sampling this Spring 2007 (Fig. 3). Proceeding down Ely Ck is SW-13 which is now a pond like, backed-up area from the new beaver dams below it (Fig. 4). The beaver pond area just below has three AMD seeps that enter it from the outer perimeter of the area close to the county road (Fig. 5 A, B). Further downstream is the new SW-11 Seep discharge into Ely Ck and as the mainstem proceeds downstream toward Goose Ck (Fig. 6 A,B). The orange iron precipitate is quite apparent in this part of the upper mainstem of Ely Ck.

In Goose Ck, the reference sites SW-14 and SW-16 became intermittent during the bug sampling effort as these two small tributaries merge and then becomes SW-14/16 (Fig. 7 A,B). The Successive Alkalinity Producing System (SAPS) in the remediated area is shown in Fig. 8 A as it is along the right side of the county road by the SW-8 site, and the wetland area in the SAPS parallel to SW-8 is found in Fig. 8 B. The “notorious” SW-8 site of the 1990’s is shown in Fig. 9 A as it flows parallel to the county road on the left with the massive remediated area on the right. The close-up view of SW-8 shows the prevalent nature of the orange, iron precipitate in Goose Ck (Fig. 9 B). The current seep into SW-8 emerges from an old AMD seep in the highwall area parallel to the right side of the county road (facing upstream) that flows under the road through a culvert into Goose Ck (Fig. 10 A). This old AMD seep was not addressed in the remediation efforts probably due to the massive discharges of the two old seeps that emerged from the wetlands. In Fig. 10 B the Goose Ck confluence into Ely Ck is seen. In Fig. 11, Ely Ck flows beyond the Goose Ck confluence (A) at the SW-11 Dn site and the SAPS discharge point is also seen (B). Another view of Ely Ck parallel to the SAPS discharge area is shown in Fig. 12 A, B with Rt 765 parallel along the left side. Beyond this point, the SW-7 site is shown at various angles above and below the church (Fig. 13 A, B). At this point, the Bean Ck confluence enters the Ely Ck mainstem. The other major remediation effort is located just above SW-9 in Bean Ck (Fig. 14 A), and as it enters Ely Ck one can notice the change in water clarity into the orange/ iron influenced area from the SW-8 site upstream (Fig. 14 B).

Below the Bean Ck confluence into Ely Ck is site SW-3 adjacent to Rt 765 (Fig. 15 A, B) with rip-rap reinforcement along the edge of the mainstem. Just prior to Ely Ck entering Stone Ck an upstream view is presented in Fig. 16. At the Ely Ck confluence with Stone Ck, one can see the difference in water quality/ color from Ely Ck (Fig. 17) relative to the more pristine area upstream in Stone Ck. (Fig. 18). Another view of the Ely Ck confluence into Stone Ck is shown clearly in Fig. 19 relative to the SW-1 Dn site below the mixing zone area in Stone Ck. The next to last sampling site (SW-17) downstream in Stone Ck is shown in Fig. 20. Then another mile thereafter is SW-18 further downstream in Stone Ck (Fig. 21).

Fig. 3. Upstream view of SW-15 the most upstream site in the Ely Creek mainstem.

Fig. 4. Beaver pond back up to SW-13 in the Ely Ck mainstem.

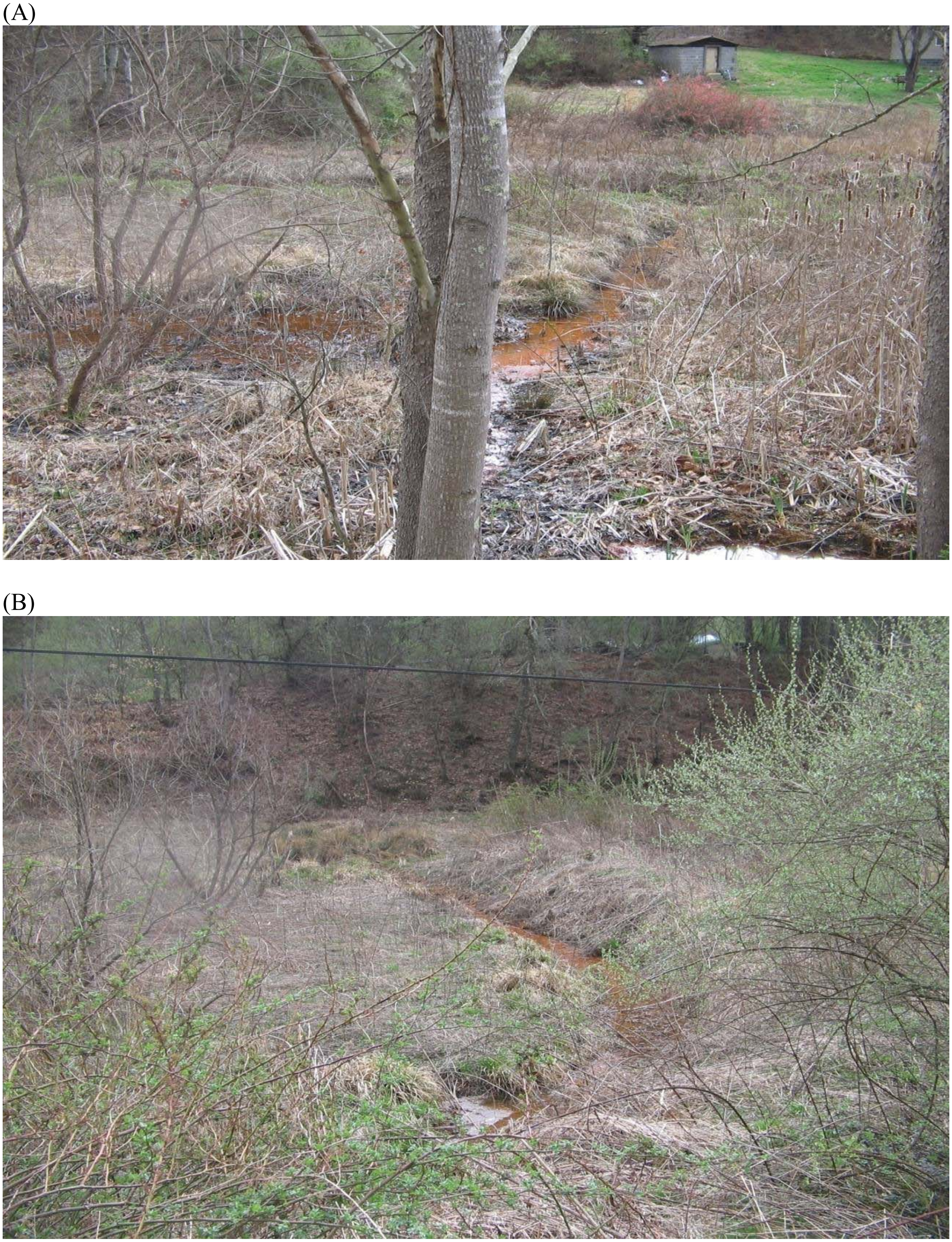

Fig. 5. Upper (A) and lower (B) beaver dam seeps in the new beaver pond.

Fig. 6. (A) SW-11 Seep discharge into Ely Creek and (B) SW-11 Seep downstream view.

Fig. 7. (A) SW-14 and SW-16 merging view and (B) SW-14/16 combined further downstream.

(A)

(B)

Fig. 8. (A) Discharge point to SAPS at SW-8 site and (B) wetland in SAPS at SW-8 site.

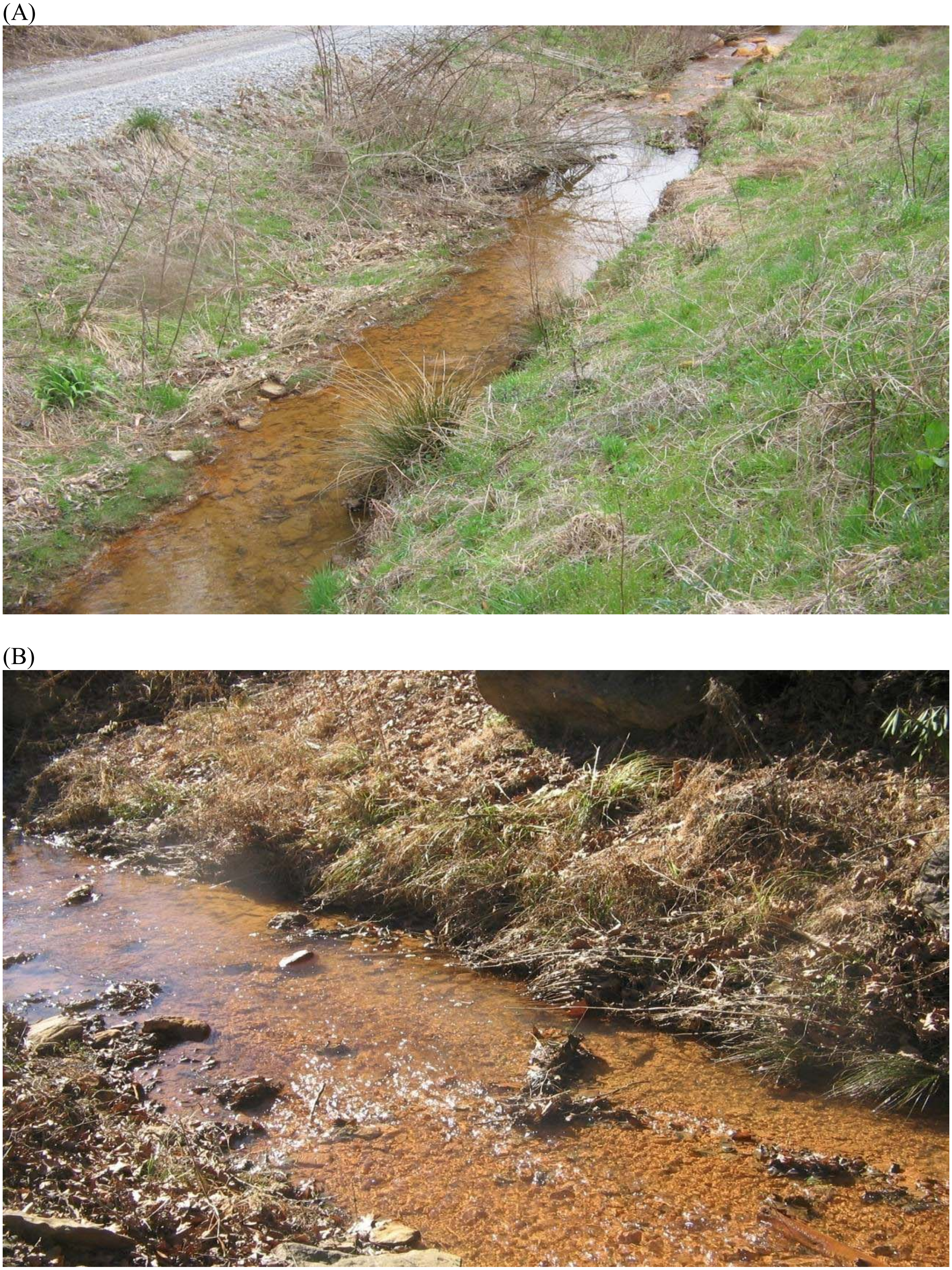

Fig. 9. (A) Downstream view of SW-8 and (B) close up of SW-8 water and sediment.

Fig. 10. (A) Upstream view of intermittent seep at SW-8 and (B) Goose Ck confluence into Ely Ck.

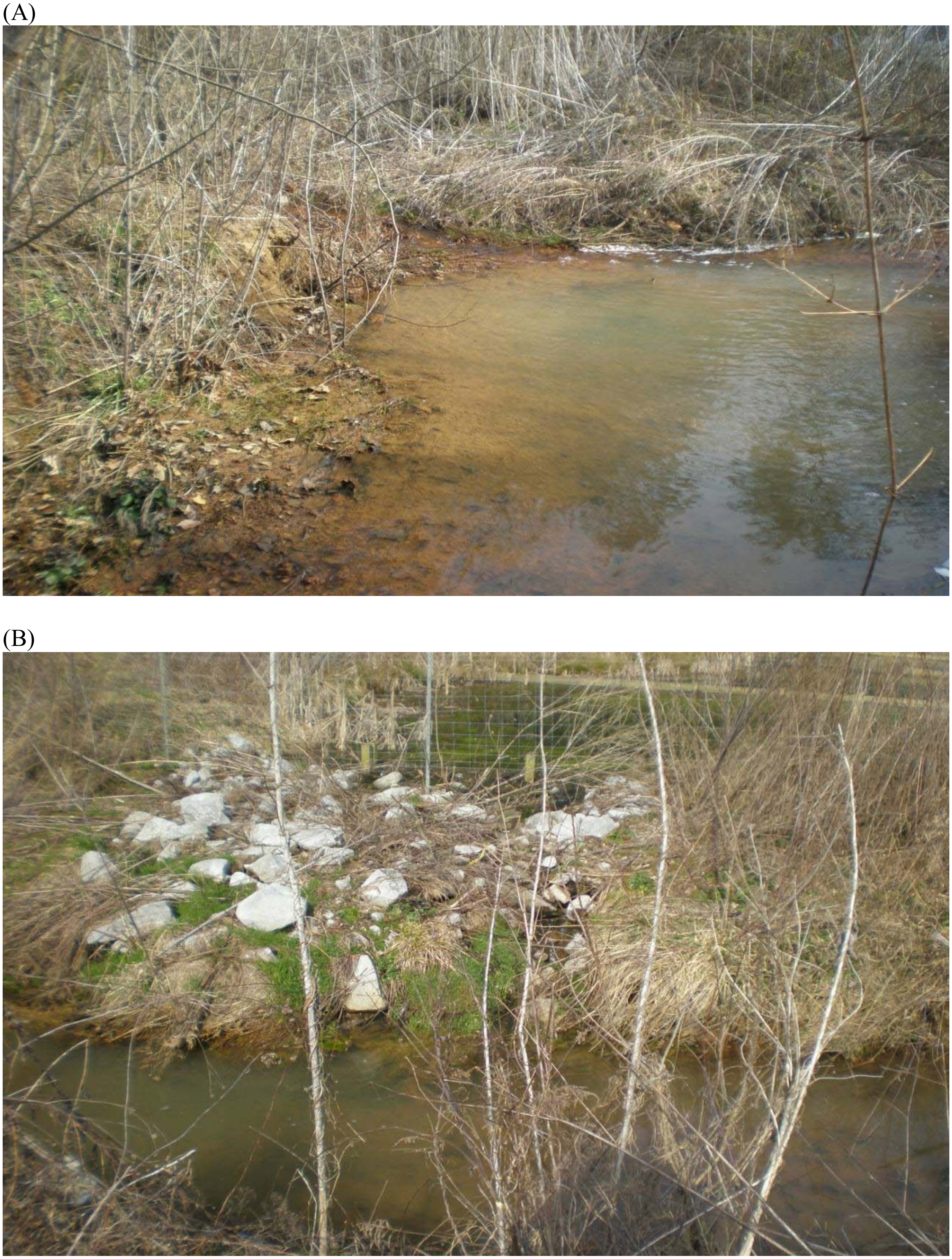

Fig. 11. (A) Goose Ck confluence view from Ely Ck and (B) SAPS discharge at old SW-8 site.

Fig. 12. (A) SW-11 Dn in Ely Ck mainstem and (B) SW-11 Dn just after SAPS discharge.[cz10]

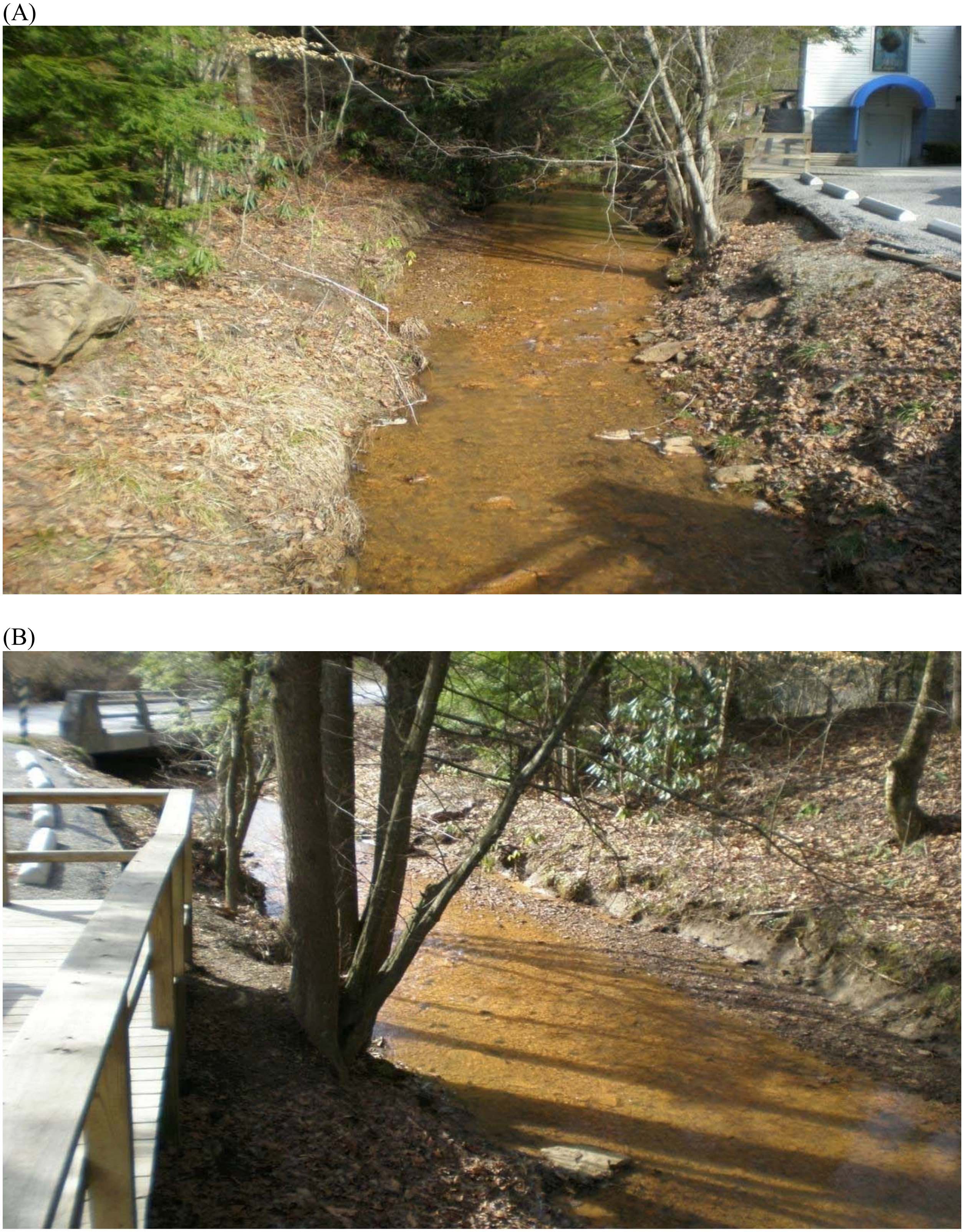

Fig. 13. (A) SW-7 upstream view at the church and (B) downstream view at the church.

(A)

(B)

Fig. 14. SW-9 site in Bean Ck with downstream view and part of SAPS and (B) Bean Ck confluence with Ely Ck.

(A)

(B)

Fig. 15. (A) Downstream and (B) Upstream view at SW-3 in the Ely Ck mainstem.

Fig. 16. Upstream view at SW-4 just before Ely Ck confluence with Stone Ck.

Fig. 17. View of Ely Creek and Stone Ck confluence from SW-4 in Ely Ck.


Fig. 18. Upstream view of Stone Ck reference site SW-1Up.


Fig. 19. Ely Ck confluence with Stone Ck and first downstream site in Stone Ck at SW-1Dn.

Fig. 20. Next downstream site in Stone Ck at SW-17.

Fig. 21. Upstream view of lowest Stone Ck site at SW-18.

4.0 SUMMARY AND CONCLUSIONS

In reference to the three impacted AMD sites in 2007 that had failing or severely stressed ETRs of 30.8-41.4, the following conclusions can be drawn here by D.S. Cherry after studying this subwatershed over the past 12 years. Prior to remediation, the SW-8 or "Wetlands" site was the most severely impacted site with the lowest ETR score relative to many other AMD impacted sites in Black Ck, Puckett's Ck, etc. Although the two major AMD seeps were successfully remediated in 2003 near the Wetlands area, the current AMD impact in Goose Ck is not due to runoff from the adjacent holding ponds in the SAPS. The AMD source is from the mid-upper ridge area located on the opposite side of the county road that parallels Goose Ck. It releases AMD intermittently which flows through a pipe or culvert under the road and discharges into Goose Ck. This site was presumably overlooked for reclamation due to its intermittently pulsed discharge.

The site SW-11 Seep was a minor one in 1997 and still appears to be so in 2007 except that the seep has been focused at one discharge point in the upper Ely Ck mainstem above the Goose Ck confluence and it flows continuously. Somehow over the past decade, this subtle AMD discharge area has become focused into one discharge point since 2006-2007 and several years before. Site SW-11 Dn receives the input of the SW-11 Seep discharge and the SW-8 influence from Goose Ck and, hence, receives AMD impact from two stream inputs. In Bean Ck, the new SAPS is working very well and no other nearby AMD seeps are discharging out of it. Again, the overall area of the Ely Ck subwatershed has been vastly improved due to remediation efforts to counteract the three main AMD point discharges that occurred in 1997 but other old seeps are still prevalent.

However, habitat assessment scores or HAS indicated that benthic macroinvertebrates/ bugs cannot relocate very well at these Goose Ck/ Ely Ck sites due to limited riparian vegetation, high sediment deposition, trace metal influence and other impacts from the unremediated AMD site discharges. Perhaps during years of higher subwatershed stream flows, the results obtained in 2006- January 2008 could have been better than those found in this study, but they are not at this time. Unfortunately, a small subwatershed such as Ely Ck suffers from unusual drought conditions just like the receiving N.F. Powell River watershed located just below the Stone Ck confluence into this river where the severe loss of native mussels has become a major center of controversy over the current decade.

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Section B.
Ecotoxicological Recovery Status of the Black Creek Subwatershed After Acid Mine Drainage Remediation

by

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EXECUTIVE SUMMARY

A 1.5 year research project (October 2006- March 2008) was undertaken to evaluate abandoned mined land discharges (AMD) or seeps in the Black Creek subwatershed, Wise Co., VA, complimented with laboratory toxicity studies at Virginia Tech, Blacksburg, VA. The focus was upon the remediation efforts that were completed by a coal mining company engaged in active mining and by activities conducted through Virginia Department of Mines, Minerals and Energy's Abandoned Mine Land Reclamation Program. A previous study identifyied AMD seeps, assessed water quality and aquatic life in Black Creek and tributaries, and conducted laboratory and in-situ bioassays to determine toxicity of water and sediments to test organisms to assess subwatershed condition (Cherry et al 1997). The current study applies similar methods as a means of comparing current to pre-remediation conditions and applies an Ecotoxicological Rating (ETR) to assess subwatershed condition.

Twenty seven sampling sites were evaluated in the Black Ck subwatershed during 1995-1997 and 2006-2008, including 11 mainstem sites and the recent active and intermittent seeps. The five sites in the upper mainstem and four sites in the lower mainstem were studied during both periods. In the lower area, one site was discontinued (LBC-2, which became submerged after the 1990's by the combining of the two beaver ponds into one pond) and another (LBC-5) was added in 2006-2008. The LBC-5 site was just below a new dual settling pond system located on both sides of the mainstem by coal mining activities being conducted in the lower portion of the subwatershed.

Of the six AMD sites studied in the upper subwatershed in the 1990's, one of these (UD-4) was remediated and therefore was not monitored during the current study. Several additional sampling sites were added in the 2000's (UD-5a, UD-5b, UD-6a and UD-6b) to evaluate AMD dissipation from the seep origins. In the lower subwatershed, most of the former seeps were removed during the active mining process; one major seep, LD-1, continues to flow but emits water that is not a major environmental concern.

Sampling sites for which the 2006-2008 study revealed environmental concern included three seeps in the upper subwatershed (UD-2a, UD-3 and UD-5 including auxiliary sampling sites UD-5a and UD-5b) plus the two lowest mainstem sites (LBC-5 and LBC-6) located below the new settling ponds near the confluence with the Powell River. Acidic pH was a major concern at these flowing seep sites with ranges of 3.76-7.07 (UD-2a), 3.86-6.01 (UD-3) and 3.34-4.97 at UD-5. Concentrations of dissolved aluminum were very high during some sampling events at the seeps, with maximum values of 22.145 mg/L at UD-2a, 12.784 mg/L atUD-3, and 39.181 mg/L at UD-5. These levels are far in excess of the environmentally safe levels, as aluminum is considered hazardous to aquatic life at 0.087 mg/L, the USEPA Water Quality Criteria (2002) limit. There were no overt water quality issues at the two new settling ponds located around this LBC-5 site. Reasons for 100% mortality in the *in situ* Asian clam tests at LBC-5/ LBC-6 are unknown but warrant further investigation.

The benthic macroinvertebrate (bug) data were considered to be inconclusive because the numbers were depressed due to low flow/ drought conditions over the year. Habitat Assessment Scores (HAS) confirmed this result as the HAS at the pristine reference site (UBC-1) had the lowest score (138) relative to all other mainstem sites (140-156) due to limited flow that reduced the presence of riffles and increased standing pools. The sites further down the mainstem were augmented with flow from other tributary inputs that increased the habitat parameters for velocity/ depth, channel flow status and frequency of riffles, but the bug data still remained poor at all mainstem sites and the reference.

The ETR's obtained from the 2006-2008 study were disappointing in the mainstem sites with the highest value only 77.0 ("C" rating) at the reference site (UBC-1) to 42.8/ 42.5 ("F" rating) at the two lowest sites, LBC-5/ LBC-6. Primary factors causing the depressed ETR score at the reference site were low values for the bug taxa richness and % mayfly abundance parameters of the ETR due to drought impacts. In the two lowest mainstem sites, LBC-5/ LBC-6 received the lowest ETR scores of all mainstem sites not only due to limited bugs but more so to the newly found toxicity for *Daphnia magna* laboratory chronic and Asian clam *in situ* test results. Hence, the dismal ETR's at the two lowest mainstem sites were also toxicity oriented. The three lowest seep sites in the upper mainstem, UD-1, UD-2 and UD-5, which had continuous flow regimes and varying riffle and pool-like habitat for bug studies, had the lowest ETR's, 56.3, 38.5 and 12.1, respectively. Overall, these ETRs do not indicate any major degree of restoration in the ten sites evaluated as the other mainstem sites had values ranging from 63.9 (LBC-3) to 58.2 (LBC-4); however, for mainstem stations other than LBC-4 and LBC-5, the primary factors responsible for the low ETRs were the drought-impacted benthic macroinvertebrate metrics and high iron concentrations in the sediments.

A portfolio of photographs was included in this report so that the reader could view the general condition of the AMD seeps and selected mainstem sites as they occur today since 1995-1997. The UD-1 seep has been shown to be vastly improved now as no orange/ iron discharge is entering Black Creek Lake. The UD-2 site still has the typical orange/ iron discharge through it while UD-3 is ~3 times more expanded in size now with a gray/ aluminum coloration. Site UD-4 was removed during the active mining process by covering the seeping highwall with ~20m of soil which brought this new wall of grass and vegetation to the edge of the current UD-5 seep. Site UD-5 is still very multicolored from orange to gray in the pool-like origin area before it discharges into its tributary adjacent to the road leaving the Black Creek Recreational Lodge area. The UD-6 deep mine hole site in the 1990's has had the open entrance closed by ~80% in the 2000's and it has a new settling pond adjacent to it. The UD-7 intermittent seep has not been a major area of environmental concern due to its partial flow status. Other sites are also shown from LD-1, the Beaver Pond area at the upper (LBC-1) and lower (LBC-3) ends plus the new LD-3 Seep flowing into LBC-3. Other photographs further downstream show the current width of the channel and riffle flow at LBC-4/ LBC-5 which is ~6m wide relative to the riffle area at UBC-1 (~ 1m wide). Pictures of the two new settling ponds (LD-5 and LD-5a) adjacent to the mainstem at LBC-5 are included to show a new effluent release from the wall of the primary settling pond LD-5a directly into the creek that was discovered on 3/3/08.

The most recent results from the 3/3/08 reconnaissance trip indicated that a new source of toxicity may have been pinpointed in the lower Black Ck mainstem sites at LBC-5 and LBC-6 located on each side of RT 58 before the creek confluence into the upper Powell River and downstream of the discharges from settling ponds created by new mining operations. Mortality was 100% for the Asian clams that were placed in the stream at this point, and most of the clams similarly placed and maintained at other mainstem sites survived and grew. The source of this toxicity is unknown. Given that the sites are located downstream of the settling ponds, it is possible that the primary settling pond discharge is responsible although there is no other indication of negative impacts by the secondary pond. The larger primary pond has not been evaluated for water quality/toxicity concerns because the new discharge from it into LBC-5 was discovered in the last month of this study. Another possible source is the intermittent high-iron seep that has been reported to occur in the vicinity of the settling pond discharge points by mining company personnel, but which we did not observe. Another possibility is runoff from the vehicle “turnaround” that is located near LBC-5 and upstream of LBC-6, which drains into a culvert that leads directly to the creek near LBC-5; the discharge point of this culvert can be seen in the photograph of Figure 15(b). According to mining company personnel, this turnaround is heavily used by coal trucks accessing the coal-handling facility located across Rte 58 and on the other side of the Powell River. The turnaround is paved and there is no sump-pond to hold runoff from the pavement, so oils, grease, coal particles, and other substances falling onto the turnaround from the trucks would be washed directly into the culvert and creek by rain events.

This potential site of toxic releases at the Black Ck confluence is noteworthy due to the high level of concerns associated with the loss of mussel assemblages in the Powell River further downstream toward the Tennessee border.

1.0 INTRODUCTION

The Black Creek subwatershed in Wise County, VA was found to be the most heavily impacted basin from acid mine drainage (AMD) in the Powell River watershed in a studies from 1995-1997 by Cherry et al (1995, 1997). Then in 2006-2008, more comprehensive studies were then conducted at 20 sampling sites in the subwatershed using an intensive series of water quality (pH, conductivity, aluminum concentrations), iron in sediment, toxicity testing (water column, sediments), *in situ* Asian clam toxicity tests and benthic macroinvertebrate assemblages to evaluate the overall current condition. Thereafter, an ecotoxicological rating (ETR) procedure was developed to evaluate the major stream sampling sites that synthesized the ten abiotic/ biotic laboratory and field testing parameters into a single composite value up to 100 points with the higher the ETR, the better the condition (Cherry et al 2001). An excellent rating was 90-100 points, followed by good/acceptable (80-89), fair/marginal (70-79), stressed (60-69) to failing/ severely stressed (≤59). These data were used to assess the current conditions in Black Ck since active coal mining activities were completed in the upper half of the subwatershed in 2005. Then coal mining activities eventually ended in the lower half of the subwatershed with Phase I remediation completed in 2006. Several of these testing parameters were then compared to the conditions that occurred in 1995-1997 to determine the general condition of Black Ck and if the remediation efforts in the lower half were successful relative to ecological recovery occurring there.

Twelve active AMD sites in the Black Ck subwatershed were studied in 1995-1997 - seven in the upper creek mainstem area and five in the lower region. After remediation, seven were still flowing and five had been removed. The acidic pH historically in three of the upper seeps had ranges of 3.40-4.40 (UD-2), 3.20-5.25 (UD-3) and 3.10-6.46 (UD-7) while the most environmentally severe seep (UD-5) ranged from pH 2.75-3.60 in 1995-1997. These four AMD-influenced seeps are still actively flowing in 2007-2008. Conductivity ranged from 1,706-2,040 µS/cm in UD-5 to 929-1,980 µS/cm in the other three major seeps in the upper region of Black Ck. None of the five AMD seeps in the lower portion of the subwatershed had any historical pH/ conductivity values of environmental concern. In the mainstem sites in 1995-1997, pH was 6.69-7.43 at the reference area to 6.10-7.93 at all other sites except UBC-4 (4.70-6.30).

Conductivity in the seep sites currently flowing ranged widely from 974-1,980 µS/cm at UD-7 to 1,706-2,040 µS/cm (UD-5) in 1995-1997. Conductivity was lowest at UD-3 (929-1088 µS/cm) and higher at UD-2 (1,530-1,864 µS/cm). In the mainstem sites conductivity was highest (1,244-1,376 µS/cm) at UBC-3, UBC-4 and LBC-1, the sites that were most directly influenced by the actively flowing seeps that remain in the upper subwatershed, and declined further downstream at LBC-2 to LBC-5. At the reference site, UBC-1, conductivity was only 196-518 µS/cm.

For the active seeps, in 1995-1997, site UD-5 had the highest concentrations of aluminum (31.7 mg/L) and iron (13.6 mg/L) in 1995 followed by UD-2 for aluminum (12.7 mg/L) (Yeager 1995). In 1995-1997, mainstem sites UBC-3 and UBC-4 also were impacted from these seeps with high aluminum (2.12-6.54 mg/L) and iron (1.31-1.87 mg/L). In the upstream reference site (UBC-1), aluminum (0.250-0.486 mg/L) [cz11]and iron (0.237-0.390 mg/L) concentrations were substantially lower. Iron in sediment was highest at UD-4 seep (176,000 mg/kg) followed by 19,600 mg/kg at UD-5 compared to 9,500 mg/kg at UBC-1 throughout 1995-1997.

The purpose of this report is to address the success of the reclamation efforts and potential ecotoxicological recovery in the Black Ck subwatershed. The reclamation efforts in

Black Ck were completed by the Red River Coal Co. located nearby in Dorchester, VA., working in close cooperation with Virginia Department of Mines, Minerals and Energy.

2.0 MATERIALS AND METHODS

2.1. Study Sites

Twenty-two sampling sites were selected in Black Ck, 14 in the upper area above White Oak Rd (Rt 618) and eight in the lower section (Fig. 1). The reference site (UBC-1) was above Black Creek Lake and a total of five sites were found in the upper mainstem. Five seeps (UD-1, UD-2, UD-3, UD-5 and UD-7) were still actively flowing in the upper region and a new one (UD-6c) was found on 3/4/08. Two seeps, UD-4 from a high wall drainage and UD-6, a deep mine seep, were remediated during the active coal mining activity. Two new holding ponds were created, one which received the discharge from UD-5 and the other for the remediated deep mine (old UD-6). In addition, a second larger holding pond adjacent to LD-5 in the lower mainstem was studied on 3/4/08.

In the lower section, five mainstem sites were sampled along with three seeps (LD-1, LD-5, and a new seep designated as LD-3 Seep) while the other three seep sites (LD-2, LD-3 and LD-4) had been remediated. Two of these active seeps, LD-1 and LD-3 Seep, were flowing intermittently in 2007 due to the drought and neither had overt pH/ conductivity data of environmental concern. The remediated LD-5 site had become a holding pond that was thought to have received a discharge from an adjacent holding pond (LD-5a) that was formed from the recently ended coal mining activities there. One mainstem site, LBC-2, was no longer available as it was part of the creek mainstem that separated the two beaver pond areas in the 1990s. Eventually, these two ponds merged into one larger beaver pond now and completely submerged

LBC-2. A new lower site, LBC-5, was added into the current sampling format due to the discharge from the new holding pond (LD-5) site and because there was a new discharge type pipe that ended in the stream at this site. Then LBC-6 became the last mainstem site found beyond the US 58 bridge and the confluence with the Powell River.

Table 1. In-stream, surface water/sediment monitoring locations and the ecotoxicological rating (ETR) procedure.

Station	**Station type**	**Description**
UBC-1	Mainstem reference site	Above Black Creek Lake (BCL) in forested area
UD-1	First upstream seep	Discharges into BCL
UBC-2	Second mainstem site	Just below BCL
UBC-3	Third mainstem site	Above UD-2a confluence
UD-2	Second upstream seep	Flows under road below BCL
UD-2a	Continuation of UD-2	Tributary through marshland below UD-2
UBC-4	Fourth mainstem site	Below UD-2a confluence
UD-3	Gray pool-like seep	~1.3 km along road below BCL from UBC-1
UD-4	Fourth seep	~1.8 km along high wall below BCL from UBC-1
UD-5	Continuation of UD-4	Small pool along road that flows south
UD-5a	Continuation of UD-5	~Halfway between UD-5 and UD-5b
UD-5b	New settling pond of UD-5	Adjacent to remediated UD-6
UD-6	Sixth seep (remediated)	Remediated deep mine discharge ~1.9 km below UBC-1
UD-6a	New settling pond	New settling pond for UD-6
UD-6b	Discharge from pond	Discharges under road into mainstem
UD-6c	New seep just at base of UD-6a	adjacent to the bottom of the holding pond along the road
UBC-5	Fifth mainstem site	At road to Paramount Coal Co.
UD-7	Last upper seep	~2.0 km south of UBC-1
LBC-1	First lower mainstem site	Just after mainstem passes under Oak Gap Rd (OGR)
LD-1	First lower seep	Flow under VA 618 below OGR
LBC-2	Second lower mainstem site	Once midway in beaver dam pond, now submerged
LBC-3	Third lower mainstem site	Below discharge of main beaver dam pond
LD-2	Second lower seep (remediated)	Once midway between beaver dam pond
LD-3 Seep	New seep by LBC-3	Intermittent new seep in LBC mainstem
LD-3	Remediated wetland seep	Runoff from wetland at old Betty B mine
LD-4	Remediated mine seep	Drainage from deep mine
LBC-4	Fourth lower mainstem site	Above LD-5 pond
LD-5	New settling pond	Just above US 58
LD-5a	New settling pond/wetland area	Adjacent to LD-5
LBC-5	New lower mainstem site	Just above US 58 and below LD-5 discharge
LBC-6	Last mainstem site	Just below US 58 at Powell River

Parameter	ETR procedure and values/concentrations		
	Lowest Score (1)	Highest Score (10)	Value
Taxon richness (x1.25)			12.5
% Ephemeroptera abundance (x1.25)			12.5
Mean pH (x1.25)			12.5
% clam survival (x1.0)			10
% Ceriodaphnia dubia survival (x1.0)			10
Mean conductivity (x1.0)			10
% Daphnia magna survival (x1.0)			10
Asian clam growth (x0.75)[a]			7.5
Iron/sediment (x0.75)			7.5
Aluminum/water (x0.75)			7.5
Total			100

[a] Asian clam growth (mm) replaced *Chironomus tentans* survival from 1997.

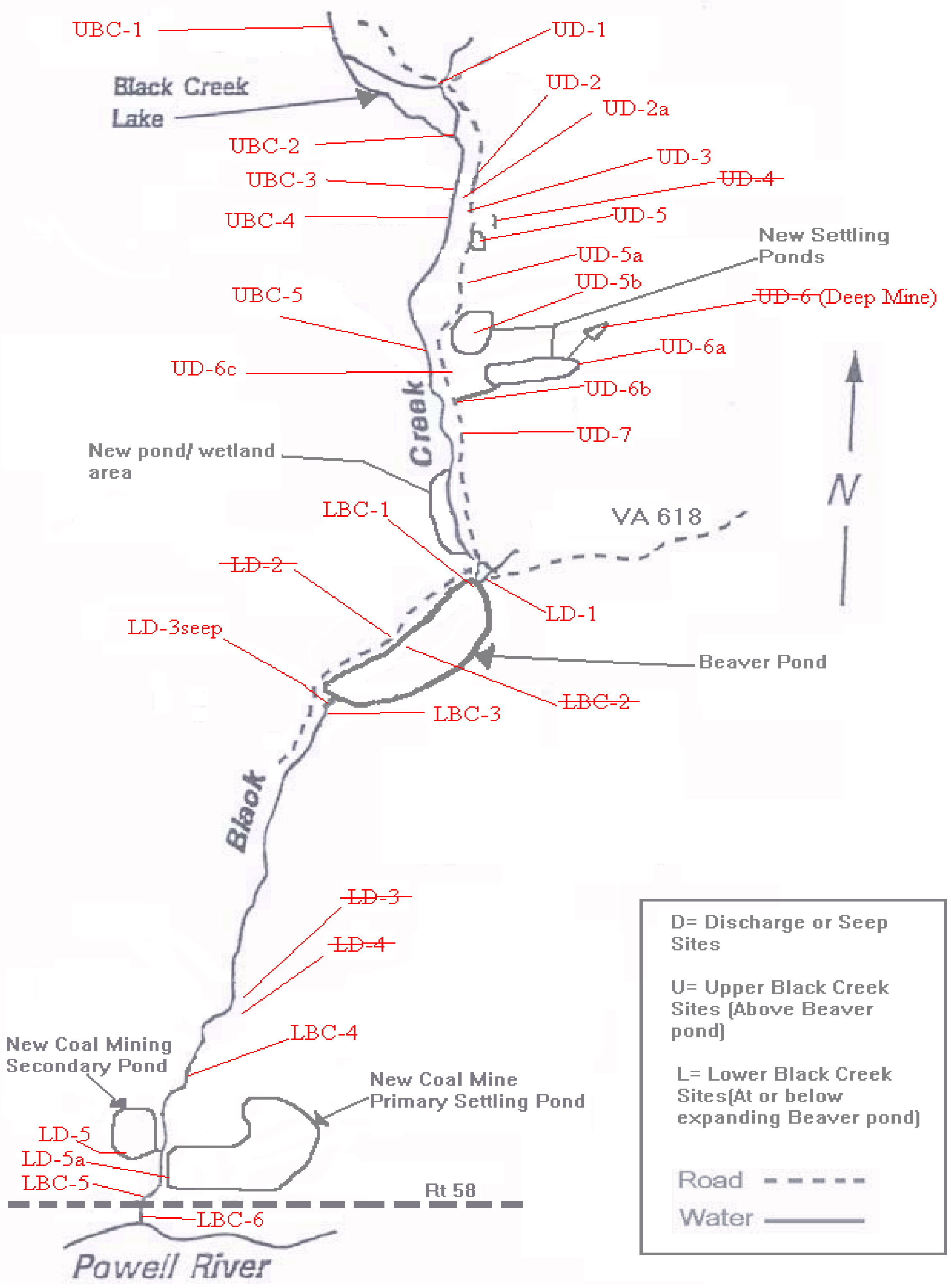

Fig. 1. Sampling sites in the Black Creek subwatershed. Strikethrough represents former AMD seeps that have been remediated (UD-6, LD-2, LD-3, LD-4) and a former sampling site that has become submerged due to Beaver activity (LBC-2).

2.2. Water Quality[cz12]

Water chemistry was conducted in the laboratory on grab samples collected from the 21 sites in Black Ck. Unfiltered water samples were collected in acid washed one-liter polyethylene bottles and transported to the lab on ice where they were refrigerated at 4 C until analyzed (less than 24 hours after collection). The pH was measured using an Accumet BASIC AB15 (Fisher Scientific) bench meter. An YSI 55 (YSI Inc., Yellow Springs OH) DO meter was used to measure DO, and an YSI 30 (YSI Inc., Yellow Springs OH) conductivity meter was used to measure the conductivity. Alkalinity and hardness were measured by titration as described in APHA et al., (1995).

2.3. Sediment and Water Column Toxicity Testing and Metals Analysis

Sediment collected from 10 sampling stations was returned to the laboratory for chronic impairment testing of *Daphnia magna*. Overall, 15-18 sites were analyzed for trace metal content in the water and sediment. Grab samples were collected with a polyurethane dipper, placed in sterile plastic bags and stored on ice at 4°C. Concurrently, a separate set of samples was collected for analysis of selected metals at the Virginia Tech Department of Crop & Soil Environmental Science, Soil Testing Laboratory. Elements analyzed included aluminum (Al), iron (Fe), manganese (Mn) selenium (Se) and strontium (Sr).

Toxicity tests were performed according to Nebeker et al. (1984) and the American Society for Testing and Materials (ASTM, 1995) with slight modifications. Test containers were 1-l beakers with 200 g sediment and 800 ml reference dilutent. The *D. magna* test evaluated survival and reproduction (mean number of neonates). Survival and daphnid reproduction in impacted sediments were compared to that in sediments from a laboratory control site. In addition, water column samples were collected from 21 stations for acute toxicity testing with *Ceriodaphnia dubia*. Neonates <24 hours old were exposed for 48 h to water taken from each station using five replicates per station, with five organisms per replicate.

Differences in mortality and reproduction among treatments in sediment and water column toxicity tests were analyzed at the 95% confidence level using Toxstat® 3.3 (Gulley, 1996). Data were checked for homogeneity of variance using Bartlett's test. If data were normal according to the Shapiro-Wilks test, the Dunnett's test for equal replicate numbers or a *t*-test with Bonferroni's adjustment for uneven replicate numbers was applied. Non-normal data were compared using Steel's Rank Test.

2.4. *In situ* Toxicity Testing

Asian clams (*Corbicula fluminea*) were placed in 18×36 cm mesh bags (0.5 cm^2 mesh size) for *in situ* sediment toxicity testing at 12 sites. Five replicate bags were placed in the sediment at each of the 12 sampling stations with five clams per bag. The number of total sampling sites was reduced from the initial desired number of 20 as the drought conditions worsened so that tests run would still have flowing water over the clam bags. Bags were collected after 62 days, and mortality was recorded. Mortality was based on either the two shell halves being opened, or closed shells opening with a prying tool. Clams were measured, from hinge to the ventral margin, prior to exposure using Fowler Pro-Max electronic digital calipers, measuring to the nearest 0.01mm. Growth was calculated for each bag by comparing mean initial size to mean final size. Differences in survival growth of Asian clams were compared among sites in 2007 using ANOVA and Student’s t-test, to test for significant differences at the 95% confidence level using JMP IN® software (Sall et al., 2005).

2.5. Habitat Assessment

A habitat assessment of each stream station included ten parameters. These were: (1) epifaunal substrate/available cover; (2) embeddedness; (3) velocity/depth; (4) sediment deposition; (5) channel flow status; (6) channel alteration; (7) frequency of riffles; (8) bank stability; (9) bank vegetative stability, and (10) streamside cover. A rating of either 0–10 or 0–20 (depending on the parameter) was developed for each parameter, and the higher the score, the more pristine the station. The habitat assessment approach was the same as that outlined in the US EPA RBPs (Barbour et al., 1999). This assessment was important to determine if shoreline degradation from outside sources (i.e. erosion, land misuse) was instrumental in impairing in-stream habitat, if water-column stress originating from abandoned mined land input was the problem, or if the drought was a contributing factor.

2.6. Benthic Macroinvertebrates

Benthic macroinvertebrate surveys were conducted at 10 in-stream stations in the Black Ck subwatershed and surrounding seeps. The sampling was done according to the US EPA RBPs (Barbour et al., 1999). Riffle, pool and shore-line rooted areas were thoroughly sampled by dip netting. The RBP tier III approach to genus level identification was undertaken. Data were analyzed for total abundance, total richness (i.e. number of different taxa), mayfly (ephemeropteran) abundance and Ephemeroptera–Plecoptera (stonefly)–Trichoptera (caddisfly) (EPT) abundance and richness. The latter group, especially the mayflies, are considered the most sensitive insect groups to environmental perturbations.

2.7. Ecotoxicological Rating

An ETR system was developed to rank the Black Ck sampling stations based on specific bioassessment and water quality parameters influenced by AMD (Table 1). The ETR assigned each station a value on a scale of 1–10 based on the 10 different bioassessment/abiotic parameters, which included: (1) taxon richness; (2) % Ephemeroptera abundance; (3) mean pH; (4) % clam survival; (5) % *Ceriodaphnia dubia* survival; (6) mean conductivity; (7) % *D. magna* survival in sediment tests; (8) Asian clam growth; (9) sediment Fe concentrations; and (10) water column Al concentrations. A more specific review of the ETR was published for these sites by Cherry et al (2001).

Using the weight-of-evidence type approach, a single cumulative value out of 100 points possible became available to characterize the environmental conditions at each station. The higher the point total the less environmentally impacted the station. Reference stations usually have values of 80–90 while AMD sites may range from 5 to 60, depending upon the volume and severity of seepage. A percentile ranking was then developed whereby sites that scored 90% (excellent or “A”), 80% (acceptable or “B”) and 70% (marginal or “C”) would not be considered for future restoration AML/AMD activities. Sites scoring 60 (or “D”) and <60% (or “F”) were labeled as stressed and severely stressed and would become prime candidates for future restoration activities depending upon the amount of funds available.

3.0 RESULTS AND DISCUSSION

There are 22 tables of data for this latter-2006-2008 study (not including the initial table in the Materials and Methods section) with five tables for water quality and five others for trace metal analysis in water/sediment, nine tables of acute/chronic toxicity data with three test species including two water fleas (*Ceriodaphnia dubia* and *Daphnia magna*) plus the Asian clam

(*Corbicula fluminea*), one table for benthic macroinvertebrate analysis and habitat assessment and one for pH and conductivity comparisons between 1995-1997 versus 2006-2008. Then the final table has the compilation of ETR scores generated in 2007.

3.1 Water Quality

Water quality in Black Ck on 12/7/06 had conductivity ranges from 225-2,722 µS/cm from the 18 sites analyzed (Table 2). Overall, conductivity was lowest at the reference site (229 µS/cm) compared to the UD-5 seep (2,373 µS/cm) and its flow down that tributary (2,602-2,722 µS/cm) at UD-5a and UD-5b. Conductivity was second highest at the UD-2/UD-2a seep sites (1,788-1,687 µS/cm) and UD-7 (1,717µS/cm). In the mainstem conductivity was highest at UBC-5 (1,479 µS/cm), LBC-1 (1,315 µS/cm), and LBC-5 (1,349 µS/cm). The new holding pond at LD-5 only had conductivity of 705 µS/cm. The other important parameter as a marker for AMD is pH and it varied from 7.21 (upper reference site, UBC-1) in Black Ck to very neutral ranges of 7.51-8.07 in the other mainstem sites throughout the creek. Site UD-5 had the lowest pH of 4.71 and it increased to 7.02-6.90 at the next two sites below (UD-5a and UD-5b). All other parameters were in the normal range of general water quality, especially dissolved oxygen which ranged from 9.02-10.59 mg/L.

Table 2. Water quality data for Black Creek on 12/7/06.

Sample	**Temp. (^oC)**	**Cond. (µS/cm)**	**DO (mg/L)**	**pH**	**Alkalinity (mg/L)**	**Hardness (mg/L)**
UBC-1	23.0	229	10.55	7.21	58	102
UD-1	25.8	1197	10.32	7.96	158	602
UBC-2	27.9	700	9.75	8.07	---	---
UD-2	25.0	1788	9.53	6.73	---	---
UD-2a	23.5	1687	9.53	6.71	---	---
UBC-3	21.8	1003	10.06	7.63	---	---
UBC-4	27.8	1148	9.65	7.51	---	---
UBC-5	28.2	1479	9.66	7.65	196	686
UD-5	23.5	2373	9.00	4.71	36	924
UD-5a	28.9	2602	10.35	7.02	50	810
UD-5b	29.0	2722	9.55	6.90	---	---
UD-7	25.8	1717	9.64	7.59	---	---
LBC-1	24.2	1315	10.56	7.85	190	570
LD-1	27.6	1279	10.42	7.76	---	---
LBC-3	29.0	1210	10.59	7.90	---	---
LBC-4	29.4	1250	10.14	7.99	---	---
LD-5	29.7	705	9.02	7.99	154	312
LBC-5	26.0	1349	10.09	7.91	210	590

During 3/29/07, conductivity was similar to that which occurred on 12/7/06 (Table 3). Sites UD-5, UD-5a and UD-5 b had conductivity values of 1,866, 2,258 and 2,169 µS/cm with UD-3 being 2,295 µS/cm. The UD-2/UD-2a sites dropped slightly to 1,470-1,490 µS/cm relative to the December sampling effort. The reference site, UBC-1, was lowest at 191 µS/cm. The pH again was lowest at UD-5 (4.78) and second lowest at UD-3 (6.01). Thereafter, pH varied from 7.05 (UBC-1) to 8.08 (LD-5). Dissolved oxygen ranged from 8.40 (UD-5) to 10.12 mg/L (LD-1) at the seep sites to 9.53 mg/L and higher at the mainstem sites.

Table 3. Water quality data for Black Creek on 3/29/07.

Sample	Temp. (^oC)	Cond. (µS/cm)	DO (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L)
UBC-1	23.8	191	9.77	7.05	52	96
UD-1	21.0	951	9.76	7.81	138	570
UBC-2	23.2	522	9.72	7.95	82	286
UD-2	23.7	1470	9.11	7.14	94	1016
UD-2a	23.8	1490	9.03	7.07	84	926
UBC-3	23.9	852	9.29	7.25	126	500
UD-3	22.1	2295	9.18	6.01	68	1064
UBC-4	24.8	956	9.27	7.15	128	570
UBC-5	22.7	1180	9.63	7.44	196	698
UD-5	24.4	1866	8.40	4.78	42	702
UD-5a	21.2	2258	9.92	7.21	102	854
UD-5b	22.4	2169	9.73	7.32	61	970
UD-6a	21.1	1852	9.93	7.40	188	716
UD-6b	23.7	1393	9.35	7.82	300	710
UD-7	23.4	1499	9.83	7.77	72	584
LBC-1	22.5	1116	9.54	7.77	228	598
LD-1	21.0	1098	10.12	7.81	158	626
LBC-3	22.9	990	9.98	7.87	148	570
LD-3 Seep	20.1	988	9.79	7.91	132	586
LBC-4	23.5	1000	9.53	7.89	128	592
LD-5	20.4	711	9.55	8.08	172	328
LBC-5	20.7	1110	9.62	8.06	222	620

On 6/27/07, conductivity was higher than on the two previous sampling dates with 304 µS/cm at UBC-1 and 1,026 (UBC-3) to 1,350 µS/cm (UBC-5) at the other mainstem sites (Table 4). Conductivity was highest at the UD-5a (2,420 µS/cm) and UD-5 to UD-5b (2,318-2,340 µS/cm) as well as at UD-3 (2,387 µS/cm). The UD-2/UD-2a sites were 1,614-1,659 µS/cm and lowest (691 µS/cm) at LD-5. The pH was lowest again at UD-5 (4.55) and then at UD-3 (4.65). The UD-2a site was third lowest at 6.76 followed by 7.06 at UD-2. All other seeps and mainstem sites ranged from 7.22 (UBC-4) to 8.37 (LD-5). Dissolved oxygen was lowest (6.84 mg/L) at UD-3 and then increased to 7.56-9.10 mg/L at all other sites.

Table 4. Water quality data for Black Creek on 6/27/07.

Sample	Temp. (°C)	Cond. (µS/cm)	DO (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L)
UBC-1	24.8	304	8.57	7.27	64	142
UD-1	20.0	1058	9.10	7.89	146	644
UD-2	18.1	1614	8.61	7.06	80	1018
UD-2a	24.2	1659	8.30	6.76	92	1010
UBC-3	18.9	1026	8.20	7.63	148	582
UD-3	17.2	2387	6.84	4.65	44	2060
UBC-4	23.4	1106	7.56	7.22	130	590
UBC-5	20.8	1350	8.74	7.82	206	706
UD-5	19.6	2318	7.78	4.55	24	1650
UD-5a	22.0	2420	8.87	7.26	100	860
UD-5b	18.7	2340	8.64	7.66	186	710
LBC-4	21.9	1258	8.61	8.12	130	600
LBC-5	21.6	1243	8.67	8.08	240	650
LD-5	21.1	691	8.71	8.37	142	386
LBC-6	19.3	1254	8.28	8.20	190	770

Water quality on 7/12/07, 8/7/07 and 9/18/07 focused on a few selected seep sites (Table 5). Conductivity ranged from 2,347-2,609 µS/cm at UD-5 and was also high at UD-3 (2,371 µS/cm). The UD-2 site was lowest (1,820-1,876 µS/cm) while the next site below it (UD-2a) was substantially higher (2,468-2,541 µS/cm). The pH was consistently lowest at UD-5 (3.34, 4.20 and 5.64) and also low at UD-2a (3.76-4.28). Dissolved oxygen was high overall in all sites ranging from 6.51 mg/L (UD-2a) to 8.71 mg/L at UD-5.

Table 5. Water quality data for Black Creek on 7/12/07, 8/7/07, and 9/18/07.

Sample	Temp. (^oC)	Cond. (µS/cm)	DO (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L)
7/12/07						
UD-3	25.0	2371	7.83	4.58	46	2250
UD-5	25.4	2347	7.20	4.20	40	2220
8/7/07						
UD-2	24.4	1820	8.12	6.40	80	---
UD-2a	25.3	2468	6.51	4.28	36	60
UD-5	22.7	2570	8.71	5.64	40	---
UD-5a	25.3	2583	7.82	6.69	82	672
9/18/07						
UD-2	21.6	1876	8.27	6.31	92	1430
UD-2a	25.4	2541	7.21	3.76	30	1490
UD-5	25.6	2609	7.52	3.34	26	1500

On 1/8/08, 21 sites were sampled for water quality (Table 6). Conductivity was highest in the three UD-5/UD-5a/UD-5b sites (2,604-2,673 µS/cm) and second highest in UD-3 (2,290 µS/cm). The UD-2/UD-2a sites were third highest (1,924-1,826 µS/cm). The new seep site in the lower mainstem (LD-5) had the lowest conductivity (712 µS/cm) for any seep site and was slightly more than twice as high as the reference mainstem site (313 µS/cm). The other mainstem sites were high other than UBC-2 (727 µS/cm) which receives the outfall from Black Creek Lake. These mainstem sites had conductivity of 1,595-1,633 µS/cm at UBC-3/UBC-4 and 1,511-1,513 µS/cm at UBC-5 and LBC-1. Sites LBC-3/ LBC-4 had values of 1,380/1,338 µS/cm while they were elevated at LBC-5/LBC-6 to 1,591/1,509 µS/cm. For the first time, pH was lowest at UD-3 (3.86) and then second at UD-5 (4.97). The pH below the UD-5 seep origin increased to 6.15/6.14 at sites UD-5a/ UD-5b. The pH at seeps UD-2/UD-2a were 6.87/6.39. Thereafter, pH was 7.24 at the reference site (UBC-1) and higher at the other seep and mainstem sites. Dissolved oxygen was somewhat low (7.77 mg/L) at site LD-5 relative to all other seep/ mainstem sites which ranged from 8.75-10.75 mg/L except for site UD-3 (6.91 mg/L).

Table 6. Water quality data for Black Creek on 1/8/08.

Sample	Temp. (°C)	Cond. (µS/cm)	DO (mg/L)	pH	Alkalinity (mg/L)	Hardness (mg/L)
UBC-1	27.8	313	8.75	7.24	58	132
UD-1	25.7	1206	9.39	7.93		
UBC-2	25.2	727	9.63	7.56		
UD-2	26.0	1924	8.51	6.87	66	---
UD-2a	27.0	1826	8.98	6.39	54	---
UD-3	28.4	2290	6.91	3.86	20	---
UBC-3	27.7	1595	8.56	6.97		
UBC-4	26.0	1633	8.54	7.28		
UBC-5	22.8	1511	9.50	7.50		
UD-5	24.1	2611	8.68	4.97		
UD-5a	22.7	2673	9.09	6.15		
UD-5b	24.6	2604	10.02	6.14		
UD-6b	23.6	1321	9.21	6.78		
UD-7	22.5	1871	9.14	7.29		
LBC-1	23.9	1513	10.75	6.94		
LD-1	24.2	1219	9.66	6.68		
LBC-3	26.5	1380	10.70	7.61		
LBC-4	25.6	1338	9.97	7.90	156	616
LD-5	26.4	712	7.77	7.74		
LBC-5	25.2	1591	9.61	7.65	268	728
LBC-6	27.3	1509	9.59	7.52	250	530

Iron and aluminum were measured in the water samples at 15 sites in the summer of 2007 (Table 7) and at three specific seep sites on 10/17/07 (Table 8). On 7/18/07, aluminum was highest at UD-3 (12.784 mg/L) and UD-5 (10.678 mg/L) and dropped to a low level (0.139 mg/L) at LD-5 and then to more minute ranges (<0.004-0.075 mg/L) at all other seep sites. Aluminum in mainstem sites was also low at 0.004 mg/L (UBC-1) to 0.057 mg/L (UBC-5) and down to <0.004 mg/L at UBC-3/ LBC-4/ LBC-5. Iron concentrations in water (Table 7) on 7/18/07 were all low ranging from <0.002 to 0.082 mg/L. On 10/17/07, aluminum was highly elevated in seeps UD-5 (39.181 mg/L) and UD-2a (22.145 mg/L) while it was non-detectable (<0.004 mg/L) at UD-2 (Table 8). Iron was elevated to 2.817 mg/L at UD-5 and then declined to 0.200 and 0.036 mg/L at UD-2a and UD-2, respectively.

Table 7. Aluminum and iron water analysis (mg/L) for Black Creek 7/18/07.

Site	Al	Fe
UBC-1	0.004	0.003
UD-1	<0.004	0.008
UD-2	<0.004	0.014
UD-2a	0.027	0.010
UBC-3	<0.004	0.047
UD-3	12.784	0.077
UBC-4	0.057	0.082
UBC-5	0.014	0.062
UD-5	10.678	0.045
UD-5a	0.075	0.006
UD-5b	0.012	<0.002
LBC-4	<0.004	0.057
LD-5	0.139	0.073
LBC-5	<0.004	0.039
LBC-6	0.011	0.037

Table 8. Aluminum and iron water analysis (mg/L) for Black Creek 10/17/07.

Site	Al	Fe
UD-2	<0.004	0.036
UD-2a	22.145	0.200
UD-5	39.181	2.817

3.2 Sediment Quality

Sediment aluminum and iron were measured at 18 sites on 12/22/06 (Table 9) and at four sites on 10/17/07 (Table 10). Aluminum was highest at UD-5 (110,836 mg/kg), UD-5a (107,093 mg/kg) and UD-5b (88,453 mg/kg), and then intermediate at LBC-1 (17,043 mg/kg) to 11,977 mg/kg at UD-2. Aluminum sediment concentrations then declined to 8.806 mg/kg (UBC-2) to 4,841 mg/kg at the remaining sites and was lowest (3,557 mg/kg) in the UBC-1 reference site. Iron in sediment was highest at UD-5b (106,029 mg/kg) followed by UD-5 (67,706 mg/kg) and LD-1 (50,483 mg/kg). Thereafter, iron concentrations at the remaining sites declined from 49,353 mg/kg (LBC-3) to 9,917 mg/kg at the reference site, UBC-1. On 10/17/07, aluminum in the three lower mainstem sites was quite similar in the sediment (Table 10). For example, aluminum was 6,022, 5,640 and 3,469 mg/kg at LBC-5/LBC-4/LBC-6 relative to the upper reference site (5,116 mg/kg). Iron concentrations were lowest in mainstem site (16,916 mg/kg, comparable to 17,950 mg/kg at the reference site) and somewhat higher at the two other mainstem sites sampled on that date (26,806 and 38,066 mg/kg at LBC-4 and LBC-5, respectively). On 1/8/08, aluminum and iron in sediment were measured at six sites (Table 11). Aluminum was highest (20,130 mg/kg) at UD-3 followed by 8,500 mg/kg at UD-2. The reference site had 2,419 mg/kg aluminum while the three lower mainstem sites ranged from 1,581-2,996 mg/kg. Iron in sediment on 1/8/08 was highest at UD-3 (55,606 mg/kg) followed by UD-2 (33,262 mg/kg) and then declined to 9,159-12,266 mg/kg at the three lower mainstem sites. In the reference site, iron was 9,773 mg/kg. Manganese was also high at UD-2 and UD-3 (8736 and 6972 mg/kg), respectively, and at LBC-5 (7637 mg/kg), relative to the reference site (540 mg/kg).

Table 9. Aluminum and iron sediment analysis (mg/kg) for Black Creek on 12/22/06.

Site	Al (mg/kg)	Fe (mg/kg)
UBC-1	3557	9917
UD-1	6672	35058
UBC-2	8806	35175
UD-2	11977	41990
UD-2a	8929	15286
UBC-3	4841	20073
UBC-4	5066	16270
UBC-5	16563	35255
UD-5	110836	67706
UD-5a	107093	45841
UD-5b	88453	106029
UD-7	6268	12734
LBC-1	17043	30812
LD-1	8643	50483
LBC-3	16436	49353
LBC-4	8030	35605
LD-5	6936	18735
LBC-5	5107	29243

Table 10. Aluminum and iron sediment analysis (mg/kg) for Black Creek on 10/17/07.

Site	Al (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Se (mg/kg)	Sr (mg/kg)
UBC-1	5116	17590	923	<2.7	8.9
LBC-4	5640	26806	4095	<2.7	15.1
LBC-5	6022	38066	3480	<2.7	29.8
LBC-6	3469	16916	3703	<2.7	114.3

Table 11. Aluminum and iron sediment analysis (mg/kg) for Black Creek on 1/8/08.

Site	Al (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Se (mg/kg)	Sr (mg/kg)
UBC-1	2419	9773	540	<2.9	3.4
UD-2	8500	33262	8736	<2.9	23.4
UD-3	20130	55606	6972	<2.9	31.2
LBC-4	2241	9159	1290	<2.9	6.6
LBC-6	1581	7934	3328	<2.9	30.4
LBC-5	2996	12266	7637	<2.9	31.7

3.3 Toxicity Testing

3.3.1 Water Column

Toxicity testing for *C. dubia* was conducted at 21 sites from the 1/8/08 sampling effort whereby the 100% sample was used with no dilution (Table 12). Survival of *C. dubia* was 90-95% in the mainstem sites and 100% in the upper reference site (UBC-1). Four seep sites in the upper area of Black Ck had 0% alive (UD-3, UD-5, UD-5a and UD-5b). Site flow was enhanced in the upper seep sites, relative to the summer and fall sampling, due to more consistent precipitation. Conductivity was 312-317 µS/cm at UBC-1 and 741-746 µS/cm at UBC-2. All other mainstem sites had conductivity that ranged from 1,321-1,491 µS/cm at LBC-4 to 1,671-1,700 µS/cm at UBC-4. Of the four toxic seep sites, conductivity was lowest at UD-3 (2,282-2,296 µS/cm) and highest at UD-5a (2,678-2,844 µS/cm). The pH in the mainstem sites was in the neutral range from 6.94-7.03 (UBC-4) to 6.96-8.33 (LBC-1). At the four toxic seeps, pH was lowest at UD-3 (4.04-4.06) followed by 5.00-5.04 at UD-5, to 6.14-7.83 at UD-5a/UD-5b. Of all the sites tested, pH was lowest for the toxic sites.

Table 12. Survival of *Ceriodaphnia dubia* to 100% Black Creek water samples collected on 1/8/08.

SITE	**Percent Survival (%)**	**Conductivity (µS/cm) ***	**pH** [cz13]*****
UBC-1	100	312-317	7.39-7.48
UD-1	90	1154-1168	8.04-8.17
UBC-2	90	741-746	7.87-7.97
UD-2	85	1895-1899	6.90-6.93
UD-2a	85	1894-1896	7.03-7.12
UD-3	0	2282-2296	4.04-4.06
UBC-3	95	1552-1561	7.03-7.91
UBC-4	90	1671-1700	6.94-7.03
UBC-5	80	1517-1617	7.47-8.41
UD-5	0	2616-2684	5.00-5.04
UD-5a	0	2678-2844	6.14-7.71
UD-5b	0	2612-2718	6.16-7.83
UD-6b	85	1319-1355	6.81-7.04
UD-7	80	1872-1958	7.31-8.06
LBC-1	90	1510-1579	6.96-8.33
LD-1	90	1219-1316	6.72-8.43
LBC-3	95	1386-1486	7.63-8.41
LBC-4	95	1321-1491	7.94-8.52
LD-5	90	715-815	7.81-8.61
LBC-5	95	1592-1602	7.72-8.22
LBC-6	90	1521-1622	7.62-8.22

* Ranges are from the beginning to end of the test.

Acute toxicity testing with *C. dubia* was conducted using water from the UD-3 and UD-5 seep sites with several dilutions in complete acute toxicity testing format. Both samples were acutely toxic to *C. dubia* with 48-hr LC50 values of 31.86 and 24.15 %, respectively, from UD-3 and UD-5 water (Tables 13-14). Hence, the UD-5 seep was slightly more toxic than UD-3 with a lower LC50 value. The pH in the 100% concentration of the samples was 4.43-4.38 (UD-5) and 4.90-5.01 (UD-3) in the 48-hr exposures. However, at the 50% dilution, pH was substantially lower in UD-5 (4.93-4.93) then is UD-3 water (6.22-7.03) which resulted in 100%

mortality in the UD-5 water and 75% dead in the UD-3 exposure. Conductivity was also high (>2,000 µS/cm) at the 100% concentration for both tests.

Table 13. Survival of *Ceriodaphnia dubia* exposed to UD-3 water from 7/17-19/07.

Concentration (%)	**Survival (%)**	**Conductivity (µS/cm)**		**pH Range**
		START	**END**	
0	100	299	358	7.66-8.20
6.25	100	504	531	7.39-8.11
12.5	90	674	712	7.24-7.97
25	70	984	1054	6.91-7.63
50	25	1527	1716	6.22-7.03
100	0	2526	2726	4.90-5.01

48-hr LC50= 31.86% in UD-3 water.

Table 14. Survival of *Ceriodaphnia dubia* exposed to UD-5 water from 7/19-21/07.

Concentration (%)	**Survival (%)**	**Conductivity (µS/cm)**		**pH Range**
		START	**END**	
0	100	290	314	7.46-8.00
6.25	100	464	474	7.22-7.98
12.5	95	627	631	7.10-7.92
25	50	934	985	6.64-7.74
50	0	1479	1590	4.93-4.93
100	0	2506	2802	4.43-4.38

48-hr LC50= 24.15% in UD-5 water.

On 9/14-16/07, samples of 100% concentration were tested from seep sites, UD-2, UD-2a, UD-5 and UD-5b (Table 15). Seeps UD-2a and UD-5 had 100% mortality while UD-2 and UD-5b had 90 and 50 % alive, respectively. The pH was lowest in the UD-2a (4.17-4.38) and UD-5 (5.59-6.12) exposures. The pH in the UD-2/UD-5b tests ranged from 6.37-6.89. A second test was conducted in 9/26-28/07 with seeps UD-2, UD-2a and UD-5 (Table 16). Mortality was

100% in seeps UD-2a and UD-5 as pH ranged from 3.33-4.06. The UD-2 seep with a pH of 6.33-7.64 had 75% alive.

Table 15. Survival of *Ceriodaphnia dubia* exposed to 100% concentration of water from UD-2, UD-2a, UD-5, and UD-5b from 9/14-16/07.

Concentration (Site)	**Survival (%)**	**Conductivity (µS/cm)**		**pH Range**
		START	**END**	
0	100	286	310	7.56-8.01
UD-2	90	1821	1822	6.37-6.82
UD-2a	0	2420	2507	4.17-4.38
UD-5	0	2658	2690	5.59-6.12
UD-5b	50	2633	2701	6.69-6.89

Table 16. Survival of *Ceriodaphnia dubia* exposed to 100% concentration of water from UD-2, UD-2a, UD-5 from 9/26-28/07.

Concentration (Site)	**Survival (%)**	**Conductivity (µS/cm)**		**pH Range**
		START	**END**	
0	100	283	296	7.43-8.07
UD-2	75	1878	1841	6.33-7.64
UD-2a	0	2540	2565	3.77-4.06
UD-5	0	2611	2634	3.33-3.40

3.3.2 Sediment tests with Daphnia magna

Sediment toxicity tests were conducted with *D. magna* at ten sites and survival was lowest (13%) at UD-5 followed by 53% alive at LBC-5 (Table 17). Survival was only 60% at UD-1, LBC-6 and UD-2. Even though daphnid survival was 80-93% at UBC-4 and LBC-4, reproductive impairment was significant relative to the laboratory control and UBC-1 reference site (Table 18). Survival was 100% at UBC-1, UBC-3 and LBC-3 but reproduction was significantly impaired at sites LBC-3 and UBC-3. It should be understood that chronic toxicity tests have two endpoints and usually reproductive impairment is more sensitive than survivorship. Hence, only one site, the UBC-1 reference passed this test and actually had a number of mean neonates or babies (81.0) greater than the laboratory control (75.8).

Table 17. *Daphnia magna* survivorship in a 10-day chronic sediment toxicity test run on 10/19-29/07.

SITE					Mean Survival	% Survival
Control	A				3.0000000	100
LBC-3	A				3.0000000	100
UBC-3	A				3.0000000	100
UBC-1	A				3.0000000	100
UBC-4	A				2.8000000	93
LBC-4	A	B			2.4000000	80
UD-1		B	C		2.0000000	66
LBC-6		B	C		1.8000000	60
UD-2		B	C		1.8000000	60
LBC-5			C		1.6000000	53
UD-5				D	0.4000000	13

Levels not connected by same letter are significantly different.

Table 18. *Daphnia magna n*eonate reproduction for a 10-day chronic sediment toxicity test on 10/19-29/07.

SITE						Mean Neonates
UBC-1	A					81.000000
Control	A					75.800000
LBC-3		B				55.200000
LBC-4		B				51.600000
UD-1		B				49.000000
LBC-6		B	C			46.600000
UBC-3		B	C	D		45.200000
UBC-4		B	C	D		43.800000
UD-2			C	D		31.800000
LBC-5				D		29.000000
UD-5					E	7.400000

Levels not connected by same letter are significantly different.

3.3.3 Asian clam in situ

The Asian clam *in situ* test is also known to be a sensitive bioassay for two reasons, (1) it has a sensitive growth impairment threshold besides survivorship and (2) it runs for two months or longer. Four sites, UD-2a and UD-5b seeps plus the two lowest mainstem sites, LBC-5 and LBC-6, had 100% clam mortality (Table 19). Site UD-5 only had 28 % survivorship followed by UBC-5 with 60%. The six remaining sites had 88-100% alive. Clam growth was highest at UBC-1 and LBC-3 and then significantly lower at all other sites (Table 20). Hence, six mainstem sites had significantly impaired clam growth besides the four seep sites, UD-1, UD-2a, UD-5 and UD-5b.

The environmental sensitivity of Asian clams has been documented by studies conducted by D.S. Cherry in this arena. D.S. Cherry started the use of these studies in the 1980's and thereafter (Graney et al. 1983, 1984; Farris et al. 1988, 1989, 1994; Belanger et al. 1990, and Soucek et al. 2000, 2001). Recently, Hull et al (2002, 2004 and 2006) indicated that these studies can be more sensitive endpoints than benthic macroinvertebrate community richness and *in situ* mussel (*Villosa iris*) tests at distinguishing biotic impairment from coal-burning power plant effluents in the Clinch River, VA. Two recent papers for the outstanding success of Asian clam *in situ* studies (Cherry 1996, Cherry and Soucek 2006) provide the best defense in a general overview of clam studies with a 3-yr study conducted concerning potential biocidal impacts in the Ohio River from a nuclear power plant effluent in Pennsylvania. Nearly a dozen other types of toxicity test organism responses plus bug surveys in the Ohio River could not match the sensitivity of the Asian clam *in situ* studies (Cherry and Soucek 2006). The "secret" in developing sensitive, Asian clam *in situ* tests is to obtain the clams from a pristine area in the

Clinch River, VA where they have not developed a tolerance to environmental discharges before placing them in the streams being evaluated for ecotoxicological influences.

Table 19. Black Creek Asian clam *in situ* survivorship.

Site		**Mean Survivorship**	**% Survival**
UBC-4	A	5.00	100
LBC-4	A	5.00	100
UBC-3	A	5.00	100
UBC-1	A	5.00	100
UD-1	A	4.60	92
LBC-3	A	4.40	88
UBC-5	B	3.00	60
UD-5	C	1.40	28
LBC-5	D	0	0
LBC-6	D	0	0
UD-2a	D	0	0
UD-5b	D	0	0

Levels not connected by same letter are significantly different.

Table 20. Black Creek Asian clam *in situ* growth.

Site		**Mean Growth**
UBC-1	A	0.483
LBC-3	A B	0.358
UBC-5	B C	0.288
UBC-4	C D	0.167
UBC-3	D	0.135
LBC-4	D E	0.116
UD-1	D E	0.090
UD-5	D E	0.039
LBC-5	E	0
LBC-6	E	0
UD-2a	E	0
UD-5b	E	0

Levels not connected by same letter are significantly different.

3.4 Benthic Macroinvertebrates and Habitat Assessment

The benthic macroinvertebrate (bug) data are summarized from ten sites, seven mainstem sites and three seeps, UD-1, UD-2 and UD-5 (Table 21). Other seeps and a few mainstem sites were not included because the habitat was too limiting (e.g., intermittent flow or no riffle/pool areas). Bug abundance was depressed or very low (≤ 18.5) at all sites except LBC-3 (73.0), where caddisflies were abundant and comprised 78% of total abundance. Taxa richness was highest (7.25) at UBC-1 and similar at LBC-5 (6.75), UBC-3 (6.5) and LBC-3 (6.5). Mayfly taxa were zero at all sites except UBC-1 which had two organisms. Stonefly abundance was zero at all sites except UBC-1 which had one. Caddisflies were highest at LBC-3 (57.25) followed by 10.25 to 1.0 at the other sites. The severe drought conditions in winter 2006 as well as in spring 2007 when sampling occurred caused the bug data to be so severely low. Also, the consistently continuous flow of seeps UD-2, UD-3 and UD-5, and downstream movement of metals released from these seeps (see Soucek et al. 2002) could have adversely affected the benthic macroinvertebrates in some mainstem sites. It is also possible that consistently high total dissolved solids (TDS) are affecting some benthic macroinvertebrate species, as conductivity values at mainstem sites below UBC-2 are consistently within the 1000 – 1500 µS/cm range, indicating TDS levels on the order of 700 – 1000 mg/L.

The habitat assessment scores (HAS) in Black Ck ranged from 156 at LBC-3 to a low of 138 (UBC-1) in the mainstem sites and 120-126 in the three seep sites (Table 21). Low water flow reduced the HAS in the reference site as some riffles were dry and flow across the stream did not reach bank to bank. Riparian vegetation width was limited in the lower three mainstem sites as the width of the remediated vegetative zone was usually 2-10 meters. However, creek flow was much more robust here from other apparent tributary inputs which did not hinder the habitat scoring as it did in the reference site at UBC-1. The three seep sites had limited flow as well in their tributaries that are generally quite narrow. Hence, the limited HAS were much less than the maximum of 200 points due to the drought conditions as was reflected by the poor bug data as well.

Table 21. Summary of benthic macroinvertebrate data for Black Creek and habitat assessment scores (HAS) collected on 7/12/07.[cz14]

	UBC-1	**UD-1**	**UD-2**	**UBC-3**	**UD-5**	**UBC-4**	**LBC-3**	**LBC-4**	**LBC-5**	**LBC-6**
Mean Total Abundance	18.00	18.00	3.00	18.50	4.75	8.25	73.00	16.75	17.25	18.00
Mean Taxa Richness	7.25	5.75	2.75	6.50	1.75	4.00	6.50	5.50	6.75	4.75
Mean Caddisfly Abundance	3.25	10.25	0.75	4.50	1.00	3.50	57.25	7.75	1.50	6.25
Mean Stonefly Abundance	1.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean Mayfly Abundance	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean Mayfly Richness	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean Percent Mayfly	4.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean EPT Abundance	5.00	10.25	0.75	4.50	1.00	3.50	57.25	7.75	1.50	6.25
Mean Percent EPT	28.18	44.82	33.33	28.61	11.11	34.09	80.40	32.22	10.84	40.98
Mean EPT Richness	2.25	1.00	0.50	1.50	0.25	1.00	1.00	1.00	1.00	1.00
Mean Midge/EPT Ratio	0.31	0.92	0.00	2.20	0.25	0.66	0.10	1.00	4.50	0.16
HAS	138	126	124	140	120	144	156	143	140	149

3.5 Comparison of pH and conductivity between 1995-1997 and 2006-2008

During the studies in Black Ck in 1995-1997 and again in 2006-2008, 27 sampling sites had data for water pH and conductivity (Table 22). However, several sites analyzed in the 1990's were not done so in the 2000's because some sites disappeared during mining/ reclamation activities and others emerged thereafter.

The pH was relatively stable in the mainstem sites between >6.0 and <9.0 but several AMD sites that were acidic in the 1990's continued to be so ~ten years later. For example, UD-1 remained stable in pH during both decades. Sites UD-2 and UD-2a were different in that acidic pH in the 1990's (3.40-4.40) was replaced with pH of 7.81-7.96 (Table 22). However, as UD-2 emerged further down into the marsh near its confluence with the mainstem, this site (UD-2a) had acidic pH of 3.62-4.12 in the 1990's versus 3.76-7.07 thereafter.

AMD site UD-3 was prevalent and highly acidic during both sampling periods with pH measurements commonly at <4 levels. The highwall seep (UD-4) was very acidic in the 1990's; its removal in the 2000's can be expected to have improved water quality. The most acutely toxic seep (UD-5) in the 1990's was also very toxic in the next decade as pH ranged from 2.75-3.60 and 3.34-4.97, respectively (Table 22).

The deep mine AMD site (UD-6) was actively remediated with a holding pond complex and pH was neutral in the 1990's and still remained so from the holding pond in the 2000's (Table 22). Site UD-7 was acidic (pH=3.10-6.46) in the 1990's but not so in the next decade (7.29-7.77). The first seep in the lower subwatershed (LD-1) was always in the natural pH range although it was intermittent in flow in 2007 due to the drought conditions in general. The LD-2 seep was remediated because no traces of its flow could be found in the 2000's even though pH was neutral. A new seep (LD-3 Seep) was found after the end of the combined beaver pond area but its pH in the intermittent flow this past year was also neutral. The last seep (LD-5) in the lower region of Black Ck was now replaced by a new coal mine secondary holding pond and pH discharges there were neutral (7.74-8.37).

Conductivity data indicated that the upstream reference site, UBC-1, located above Black Creek Lake, had the lowest measurements by far (196-518 µS/cm in the 1990's versus 191-313 µS/cm in the 2000's) in any mainstem site and certainly so in all AMD sites (Table 22). In general, conductivity tended to increase in the mainstem sites at UBC-2 and thereafter in the 2006-2008 data. For example, site UBC-4 had 650-1,341 µS/cm in the 1990's versus 956-1,633 µS/cm in the 2000's. Site UBC-5 had conductivity increase from 615-647 µS/cm (1990's) to 1,180-1,511 µS/cm (2000's). At the lowest site, the Black Ck confluence with the Powell River, conductivity increased from 770-957 µS/cm in the 1990's to 1,214-1,509 µS/cm, thereafter. The major acutely toxic seeps (eg., UD-3 and UD-5) also had similar increases in conductivity between both sampling intervals. At UD-3, conductivity increased from 929-1,088 µS/cm to 2,290-2,387 µS/cm between the 1990's versus 2000's. At UD-5, it increased from 1,706-2,040 µS/cm to 1,866-2,611 µS/cm. We interpret these conductivity increases to have occurred as a result of the dry conditions in 2006-2007.

Table 22. Comparison of pH and conductivity data at 27 sites between 1995-1997 and 2006-2008 before and after coal mining/ restoration activities in the Black Creek subwatershed.

Site	**pH**		**Conductivity (µS/cm)**	
	1995-1997	***2006-2008***	***1995-1997***	***2006-2008***
UBC-1	6.69-7.43	7.05-7.27	196-518	191-313
UD-1	6.15-7.58	7.81-7.96	809-889	951-1206
UBC-2	7.14-7.28	7.56-8.07	369-392	522-727
UBC-3	6.43-7.03	6.97-7.63	561-1244	852-1595
UD-2	3.40-4.40	6.31-7.14	1530-1864	1470-1924
UD-2a	3.62-4.12	3.76-7.07	1087-1795	1659-2541
UBC-4	4.70-6.30	7.15-7.51	650-1341	956-1633
UD-3	3.20-5.25	3.86-6.01	929-1088	2290-2387
UD-4	3.10-5.84	---	1048-1938	---
UD-5	2.75-3.60	3.34-4.97	1706-2040	1866-2611
UD-5a	---	6.15-7.26	---	2258-2673
UD-5b	---	6.14-7.66	---	2169-2722
UD-6	6.21-7.67	---	827-865	---
UD-6a	---	7.40	---	1852
UD-6b	---	6.78-7.82	---	1321-1393
UBC-5	6.13-6.74	7.44-7.82	615-647	1180-1511
UD-7	3.10-6.46	7.29-7.77	974-1980	1499-1871
LBC-1	6.10-7.81	6.94-7.85	738-1367	1116-1513
LD-1	7.11-7.81	6.68-7.85	829-1376	1098-1279
LBC-2	6.54-7.33	---	802-820	---
LBC-3	7.48	7.61-7.90	791	990-1380
LD-2	6.05-7.10	---	881-930	---
LBC-3seep	---	7.91	---	988
LBC-4	7.68	7.89-8.12	794	1000-1338
LD-5	---	7.74-8.37	---	691-711
LBC-5	---	7.65-8.08	---	1110-1591
LBC-6	7.37-7.93	7.52-8.20	770-957	1254-1509

3.6 Ecotoxicological Rating

For the ten sampling sites studied in 2006-2008, ETR scores were calculated from the Ely Ck ETR study by Cherry et al. (2001). Unfortunately, there were various ETR parameters from the Ely Creek study that prevented a generation of ETR comparisons in Black Ck between 1995-1997 versus 2006-2008. These problems included no Asian clam data in the Black Ck 1995-1997 study as well as no *Ceriodaphnia dubia* acute toxicity testing results. Other problems included impacts to the lower sampling sites in Black Ck after mining activities resumed in the latter 1990's so that direct comparisons could not be made to 2006-2008, in some cases because various seep and mainstem sites were removed. Therefore, ETRs can be generated now using the scoring metrics from Ely Ck because the current study generated a data base to allow their calculation. The primary focus of the 1995-1997 Black Ck study was to find and characterize all the AMD seeps influencing the Black Ck mainstem, so ETR's were not generated for Black Ck in the earlier study.

The ETRs generated from the 2006-2008 study in Black Ck had scores ranging from 77.0 which were marginal ("C" rating at the upstream reference site (UBC-1) to as low as 12.1 at UD-5 (Table 23). Also, three sites (UBC-3, UBC-4 and LBC-3) had stressful designations in the "D" range from 63.9-62.7. After that, six of the ten other sites had failing scores from 58.2 (LBC-4) to 12.1 (UD-5). Also, the two lowest mainstem sites (LBC-5 and LBC-6) had considerably lower ETR failing scores (42.8-42.5) than the other mainstem sites above them (eg., 62.7-62.9 at UBC-3 /UBC-4 and 63.9-58.2 at LBC-3/LBC-4). Hence, instead of predicting that the still current AMD seeps in the upper Black Ck subwatershed could be diminished by dilution downstream in the lower mainstem, the opposite is the result at sites LBC-5/LBC-6! There appears to be some form of subtle, long-term type of toxicity influence occurring there from the 100% die-off in the *in situ* Asian clam tests because all other upper mainstem sites had 60-100% survival.

Table 23. Calculation of the Ecotoxicological Rating in ten sampling sites in Black Creek for 2006-2008 using the parameter format for Ely Creek data generated in the 1990's.

Parameter	**UBC-1**	**UD-1**	**UBC-3**	**UD-2**	**UBC-4**	**UD-5**	**LBC-3**	**LBC-4**	**LBC-5**	**LBC-6**
Taxa Richness (12.5)	5.5	4.0	4.5	2.0	4.5	1.0	4.5	3.0	3.5	3.0
% Eph. Abund (12.5)	0.5	0	0	0	0	0	0	0	0	0
Mean pH (12.5)	12.5	12.5	12.5	12.5	12.5	5.5	12.5	12.5	12.5	12.5
% Clam Surv. (10)	10	9.2	10	0	10	2.8	8.8	10	0	0
% Cerio. Surv. (10)	10	10	10	8.0	10	0	10	10	10	10
Mean Cond. (10)	10	5.0	6.0	2.5	5.5	1.0	5.0	5.0	4.0	4.0
% Daphnia Surv. (10)	10	6.6	10	6.0	9.3	1.3	10	8.0	5.3	6.0
Clam Growth (7.5)	7.5	1.5	2.2	0	2.6	0.5	5.6	2.2	0	0
Iron/Sediment (7.5)	3.5	0	0	0	1.0	0	0	0	0	0
Aluminum/ Water (7.5)	7.5	7.5	7.5	7.5	7.5	0	7.5	7.5	7.5	7.0
(100) TOTAL	**77.0**	**56.3**	**62.7**	**38.5**	**62.9**	**12.1**	**63.9**	**58.2**	**42.8**	**42.5**

3.7 Photographic Analysis of Selected Mainstem/ Seep Sites

A photograph with the widest point of the UBC-1 reference site just before the confluence into Black Creek Lake (BCL) indicated that the riparian vegetative zone is natural and ≥ 18m wide on each side of the mainstem (Fig. 2). However, water flow in the channel was limited and the average width of the riffles were ~1m wide due to the effects of prolonged drought condition.

The UD-1 discharge into BCL was clear without any orange coloration of the iron precipitate that was highly prevalent in the photograph found in the Cherry et al (1997) report (Fig. 3). Flow was quite strong into the lake due to runoff from a recent rain event. The seep is fed from two separate tributaries with heavy vegetation that combine on the opposite side of the road into a culvert that discharges into the lake.

The UD-2 discharge is still very orange tainted in color relative to that seen in the 1990's (Fig. 4). Today, there is a weir after the culvert so that flow measurements can be accessed easily.

The UD-3 seep was clearly prevalent in the 1990's with a distinctly silver-gray coloration of high aluminum contamination. The only difference today is that the surface area of the seep is much longer and wider with the same distinct contamination (Fig. 5). Actually, after the recent rain event the day before the current photograph was taken, the seep overflowed its perimeter and descended down the slanted landscape into the creek mainstem in an intermittent fashion due to recent rainfall.

The pool-like area of the UD-5 seep was full, enlarged and flowing well downstream and had the typical mixture coloration of orange and gray trace metal combination (Fig. 6). The old source of this seep (UD-4) is now fully covered by ~ 15m of soil over the highwall which is filled with grass and other small vegetation close to the perimeter of the UD-5 pool.

The UD-6 deep mine hole is now ~ 80% filled with soil and the discharge is not readily prevalent and probably is in a trench deeper underground (Fig. 7). However, the eroded landscape outside of the mine entrance has been repaired along with a new settling pond that receives the UD-6 discharge. One new orange precipitate site (UD-6c) was found adjacent to the bottom of the holding pond along the road that leaves the lodge and was brilliantly shining as well in the sun (Fig. 8). This newest seep area was just recently discovered on 3/3/08 after the recent heavy rain event.

At the junction of the Black Ck mainstem by VA 618, the area appears to have been impounded to form a slow flowing expanded pool which should be the new created wetland area (Fig. 9). On the opposite side begins LBC-1 that enters the single, large beaver pond (Fig. 10).

Entering under the road is LD-1, a small tributary that parallels VA 618 and at times was intermittent in flow (Fig. 11).

Below the beaver pond area where the mainstem emerges again is LBC-3 and a newly found intermittent seep (LD-3 Seep) with a colorful orange precipitate (Fig. 12). Toward the end of Black Ck is LBC-4 with some remediated vegetation on both sides of the mainstem that covers 2-5 m of width. The quite active flow here (Fig. 13) from other tributaries that feed the creek resulted in riffle areas being ~6m wide or ~6X wider than that found at the upper reference site, UBC-1 (Fig. 2).

Before the next to last mainstem site near Rt 58 is a series of two new holding ponds developed sometime in the 2000's during the active coal mining activity that occurred there. The secondary pond on the right side of the mainstem (LD-5) discharges directly into Black Ck above LBC-5 (Fig. 14). Its discharges have been studied for water chemistry changes throughout 2007 and partly into 2008. A new intermittent seep has been discovered by coal company personnel to occur in the upper LBC-5 area of Black Ck (Fig. 15) although D.S. Cherry has not seen any evidence of this seep since studying the lower mainstem from latter 2006 to early 2008. Hence, its specific source of discharge/ impact ramifications are unknown for this report.

Near the LBC-5 site is a coal truck turnaround adjacent to Rt 58 where coal trucks turn and enter the coal processing facility on the other side of the Powell River (Fig. 16). An entry from this area for runoff water is shown in Fig. 17 as the culvert pipe under the turnaround area enters the upper LBC-5 site (Fig. 18). Another view of this culvert discharge area is shown in Fig. 19. The concern here is that significant (40-47%) to 100% mortality occurred at this mainstem site to chronic *D. magna* sediment laboratory and Asian clam *in situ* tests, respectively in 2007.

On 3/3/08, we discovered an unusual feature occurring at the LBC-5 site in that water was cascading through and down the outer perimeter wall of the primary settling pond into the creek. A view of this outer wall from within the pond is shown in a combined picture effect in Fig. 20. The perimeter wall along the upper right side is >1 meter above the pond water surface while the lower, left side end has the perimeter wall descending to the pond surface as one approaches Rt 58 on the opposite side. At this far left end is where the new, cascading discharge was found at LBC-5. From the opposite side of the primary pond, one can see the descending landscape along the pond where little natural vegetation has developed since remediation was completed ~4 years ago (Fig. 21 A,B). During a rain event, water must flow rapidly down the denuded surrounding landscape and be deposited into this pond.

A close up of the far right hand pond wall shows an overflow pipe located at/above the pond uppermost surface that was designed to discharge water into the LD-5 secondary pond located on the opposite side of the creek (Fig. 22 A). Then, again at the far left end of the descending pond perimeter wall near Rt 58, a close-up is shown. The pond upper water surface is nearly equal to or overflowing at the top of the perimeter wall (Fig. 22 B). This is the point within the pond where the cascading discharge is found flowing into the upper LBC-5 site. A partial view of the coal truck turnaround circle near the coal preparation plant adjacent to RT 58 and the Powell River can also be seen relative to the full view in Fig. 16 and in Fig. 22 A. After the recent rain event just before the 3/3/08 trip, this pond was ~ full and discharging directly into the Black Ck LBC-5 site through a system of heavy rocks, forming a spillway ~20 m long / ~5m high, with sand/ soil immersed between the rocks which allowed a substantial discharge through this pond edge perimeter to cascade directly down the slope into the Black Ck mainstem just above the LBC-5 site (Fig. 23).

Unfortunately, this newly found observation that results with intermittent slugs of its primary settling pond water after a rain event to flow directly into the mainstem occurred at the end of this study. The last sampling site, LBC-6, is located a very short distance on the other side of Rt 58 that confluences into the Powell River and also had the same new overt toxicity to *D. magna* and Asian clam *in situ* tests (Fig. 24).

The final point of concern here is that there is a recently found toxicity situation occurring in Black Ck just prior to the confluence with the Powell River at sites LBC-5 and LBC-6 at the end of this March 2008 study and reasons why need to be determined hereafter. This type of gradual or pulsed toxicity from two chronic, toxicity test results obtained at different times, have pinpointed a newly released toxicant(s) into the upper Powell River where there is no space to develop additional wetland areas to mediate the toxicity that occurs in the lower mainstem sites just ~30 meters from the river confluence.


Fig. 2. Upstream mainstem site UBC-1 with riparian vegetation.


Fig. 3. Upstream seep UD-1 discharge into Black Creek Lake.

(A)

(B)

Fig. 4. (A) UD-2 seep origin and (B) UD-2 discharge with weir.

Fig. 5. (A) Left side of UD-3 and (B) right side of UD-3.

Fig. 6. (A) UD-5 looking downstream and (B) UD-5 upstream.

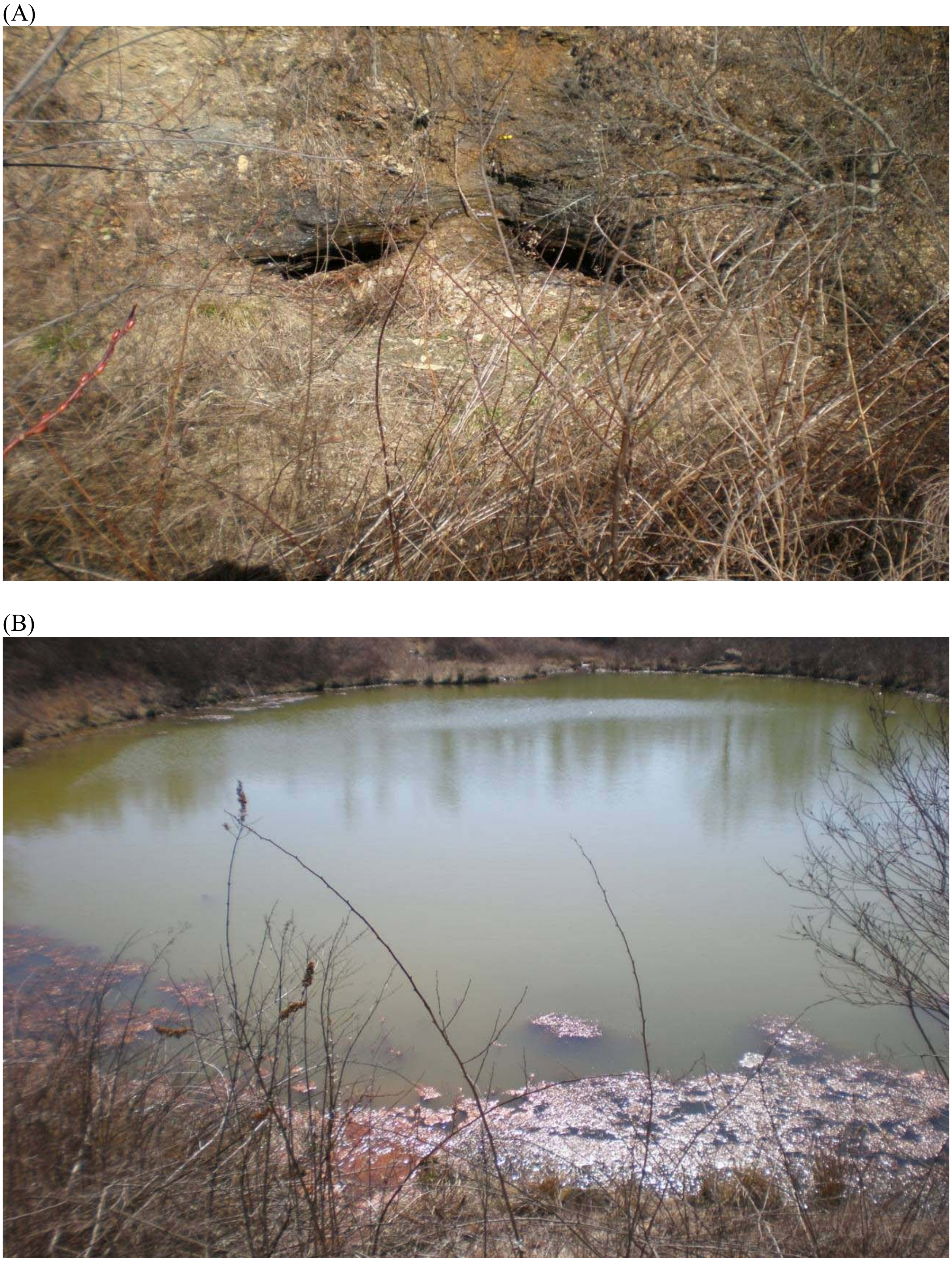

Fig. 7. (A) UD-6 deep mine site remediated and (B) UD-6a holding pond for UD-6 discharge.

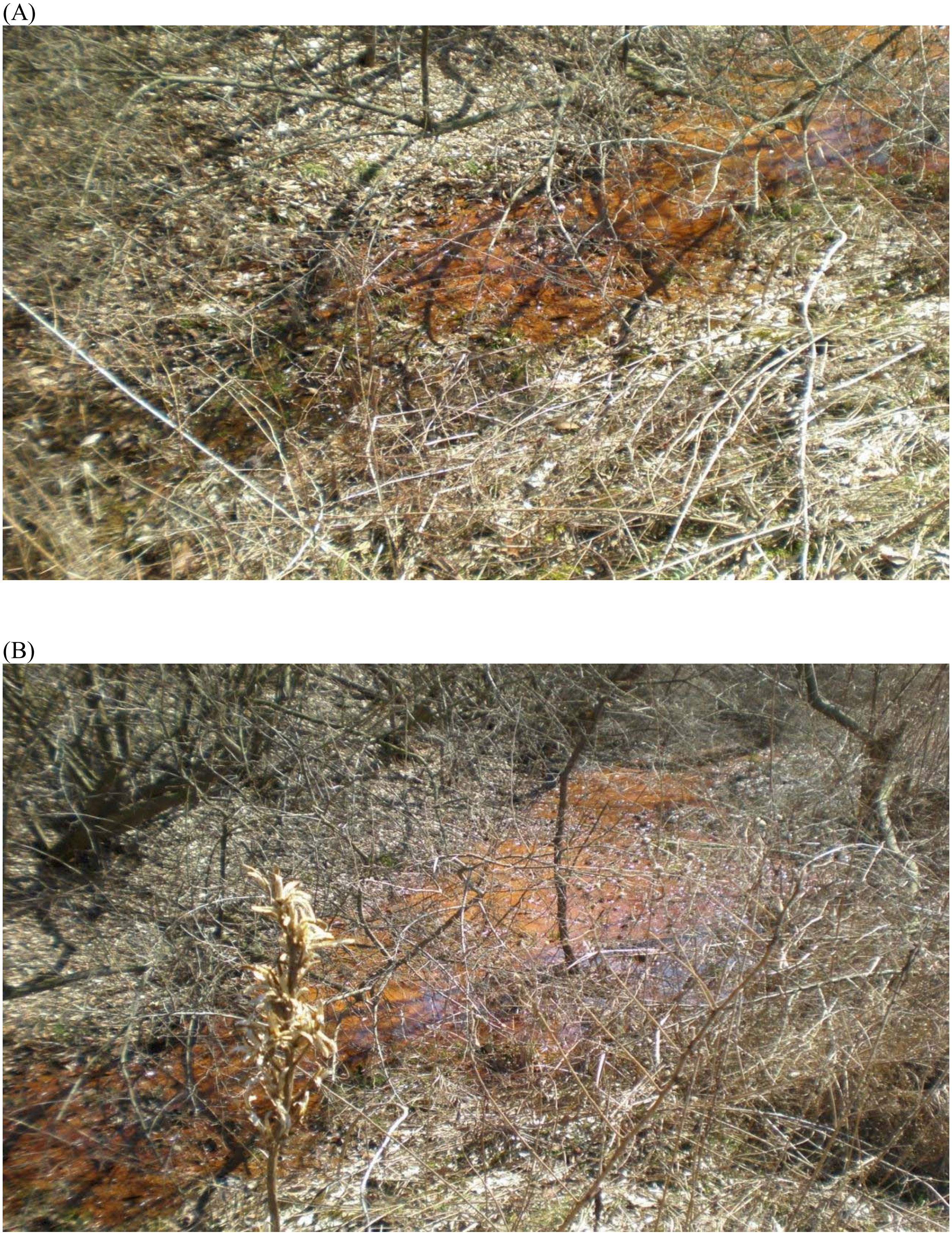

Fig. 8. (A) Start of new seep at UD-6c and (B) downstream view of UD-6c.

Fig. 9. (A) Downstream view at a new settling pond or wetland area before LBC-1 and (B) culvert pipe that leads to LBC-1.

Fig. 10. (A) Downstream view of LBC-1 toward LBC-3 and (B) upstream toward LBC-1 from end of pond just before LBC-3.

Fig. 11. Upstream look at LD-1.

Fig. 12. New seep LD-3 Seep at LBC-3.

Fig. 13. (A) Upstream view at LBC-4 and (B) downstream at LBC-4.

Fig. 14. (A) View of LD-5 from upstream end and (B) of LD-5 from downstream end of the secondary pond.


Fig. 15. Intermittent AMD seep above LBC-5 (photograph provided by coal company personnel)


Fig. 16. Coal truck turnaround at bottom of Black Creek subwatershed.

Fig. 17. Entry to culvert in center of vehicle turnaround near LBC-5. (Photograph provided by coal company personnel).

Fig. 18. Culvert that discharges runoff from vehicle turnaround to Black Creek near LBC-5 (photograph provided by coal company personnel).

Fig. 19. View looking from road at LBC-5 at culvert pipe.

Fig. 20. Combined pictures of LD-5a, so that the entire pond wall closest to Black Ck can be seen.

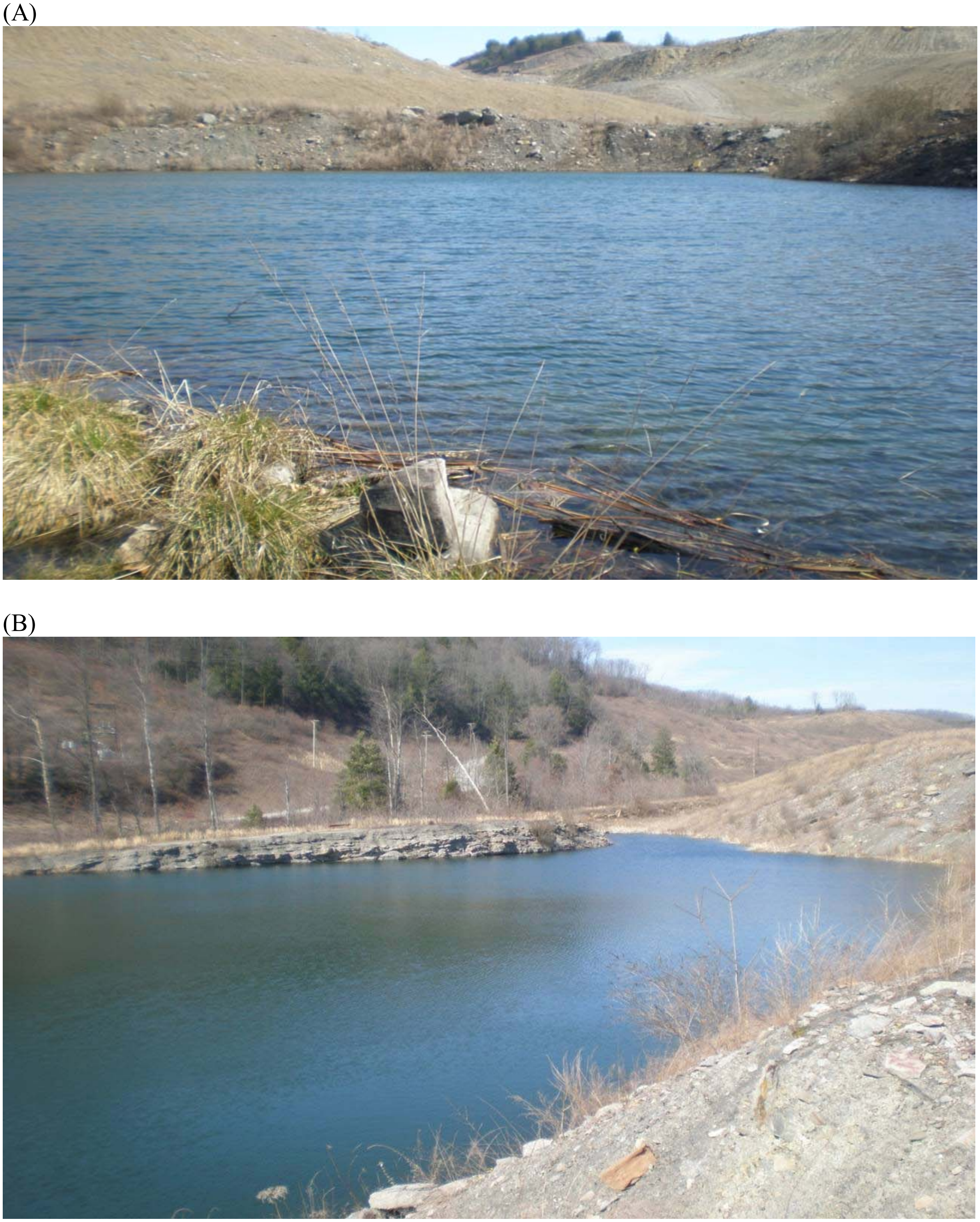

Fig. 21. (A)&(B) Views of LD-5a showing the barren landscape surrounding the settling pond.

Fig. 22. (A) Discharge point of LD-5a and (B) view from other side of pond toward discharge.

Fig. 23. View from LD-5a discharge to creek just above LBC-5.

Fig. 24. LBC-6 at the confluence with the Powell River.

4.0 SUMMARY AND CONCLUSIONS

Of the 12 active AMD seeps found in the Black Ck subwatershed in 1995-1997, seven are still discharging into the creek mainstem. These active seep sites include UD-1, UD-2, UD-3, UD-5, UD-7, LD-1 and LD-5 with one new one (LD-3 Seep) found in the 2006-2008 study at LBC-3. Certain seep sites are of no ecotoxicological concern and include UD-1, LD-1, LD-3 Seep and LD-5. Some seep sites were totally remediated from the past active mining operations and include UD-4, UD-6, LD-2, LD-3, LD-4 and LD-5; of these, UD-4 was of the greatest concern because of measured pHs ranging from 3.1 to 5.8 . However, four of the remaining seeps are of ecotoxicological concern due to acute toxicity to laboratory test organisms and include UD-2, UD-3, UD-5 and UD-7. One AMD seep, UD-5, was considered to be the most consistently toxic seep (e.g., always acutely toxic) in the 1990's and still continues to be so in the current 2006-2008 study. The UD-5 AMD source was the highwall seep site (UD-4) that was in close proximity in the 1990's, but that highwall was covered in the active mining operations in the 2000's. Still, the UD-5 seep continues to discharge with acidic pH, measured at 3.3–5.0 during the current study, slightly improved from its former levels (2.7 – 3.6) but still of significant ecotoxicological concern. The very actively flowing seep from the deep mine site (UD-6) in the 1990's was remediated with its discharge flowing into a new settling pond that had no appreciable acute toxicity (UD-6b) and environmentally acceptable pH/ conductivity values. However, a new intermittent seep (UD-6c), first found in March of 2008 along the lower edge of the new settling pond, has released an acutely toxic amount of orange-tainted water along the lower intermittent stream bed area.

In the ten mainstem sites studied in 2006-2008, no acute toxicity was found to laboratory test organisms. However, chronic toxicity (40-47% survival) occurred from the sediments to

Daphnia magna at sites LBC-5 and LBC-6. In the more sensitive Asian clam *in situ* testing, clam mortality was 100% at these two lowest sampling sites in the Black Ck mainstem. Reasons for this newly-found toxicity at sites LBC-5 and LBC-6 are unknown when 100% survival occurred for Asian clams and 80% were alive for *D. magna* at site UBC-4, just above the newly developed holding ponds in the lower mainstem. However, we found a new intermittent discharge occurring from the wall of the primary settling pond adjacent to the mainstem as effluent was cascading down from between large boulders located in the upper third of the pond wall. This discharge apparently occurs after a rain event nearly fills the pond close to full pool and the overflow pipe from this pond to the secondary pond, unfortunately, is located at the top of the pond wall at the opposite higher end. The source of this toxicity is unknown. Given that the mainstem sites are located downstream of the settling ponds, it is possible that the primary settling pond discharge is responsible via either toxic content or hydrologic effects, although we have had no time to test for possible negative impacts from this pond other than finding its new discharge into the creek. The new discharge from this larger primary pond was only discovered during the last month of this study, so potential toxicity issues from the cascading discharge into the LBC-5 site have not been studied due to the fact that this study has ended.

Another possible source is the intermittent high-iron seep that has been reported to occur in the vicinity of the settling pond discharge points by mining company personnel (see Fig. 15), but which we did not observe. Another possibility is runoff from the vehicle “turnaround” that is located near LBC-5 and upstream of LBC-6, which drains into a culvert that leads directly to the creek near LBC-5 (see Figs. 16 and 17). According to mining company personnel, this turnaround is heavily used by coal trucks accessing the coal-handling facility located across Rte 58 and on the other side of the Powell River. The turnaround is paved and there is no sump-pond to hold runoff from the pavement, so oils, grease, coal particles, and other substances falling onto the turnaround surface from the trucks could be washed directly into the culvert and creek by rain events. More research needs to be conducted on the acute and chronic toxic ramifications of these effluents in 2008 if possible.

Hence, it appears that the remediation efforts had positive environmental input upon the UD-6 seep but not so in the continuous acutely toxic seep of UD-5 originating from the previous UD-4 seep. The newly found chronic toxicity at the two lowest mainstem sites continues to be a mystery and concern to these researchers. The benthic macroinvertebrate (bug) data sampled in July 2007 indicated that the active drought conditions in the subwatershed had a negative role in comparing the composition of the residing bugs in the mainstem sites from the upper reference site (UBC-1) to the lowest ones (LBC-5 and LBC-6) because all bug parameters were extremely limited in numbers. Unfortunately, the reference site had the severest impact from the drought while the two lowest sites had more positive/ active water flow from other tributary sources downstream in the subwatershed. In fact, the severe low flow at LBC-1 caused its low HAS (138) to be the lowest score of all mainstem sites. If creek water flow had been normal, it would have had the highest score. Therefore, the bug data are inconclusive in comparing differences between the uppermost sampling site in Black Ck to the two lowest ones. The UBC-1 site should have had a HAS of >170 points due to its pristine conditions but because the site was barely flowing during bug sampling, bug assemblages were naturally eradicated.

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