

**An Ecotoxicological Assessment of a Treated Coal-
mining Effluent in the Leading Creek Watershed, Meigs
County, Ohio**

by

Alan J. Kennedy

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Biology

APPROVED:

Donald S. Cherry
Carl E. Zipper
Rebecca J. Currie
E. Fred Benfield

December 2002
Blacksburg, Virginia

KEYWORDS: mining effluent, conductivity, ecotoxicology, coal mine, total dissolved solids

An Ecotoxicological Assessment of a Treated Coal-mining Effluent in the Leading Creek Watershed, Meigs County, Ohio

Alan J. Kennedy

(ABSTRACT)

The majority of research studying the ecological impacts of the coal mining industry on freshwater systems has focused on abandoned-mined land, and the associated acid drainage and metals toxicity. Treated discharges from active mining and preparation facilities, however, can also impair lotic ecosystems through total dissolved solids (TDS) toxicity, caused primarily by the reagents used for pH modifications and the oxidation of reduced sulfur. Such impairment was best detected through application of (1) benthic macroinvertebrate surveys using metrics of biotic impairment such as relative Ephemeroptera abundance and Ephemeroptera-Plecoptera-Trichoptera (EPT) minus the tolerant caddisfly family Hydropsychidae (2) *in situ* growth of *Corbicula fluminea* during 96-d exposure and (3) laboratory testing using *Ceriodaphnia dubia*. Traditional metrics such as total taxa richness, EPT, diversity and biotic indices were not sensitive to elevated TDS levels.

Further study using strength of evidence, regression analysis and manipulation of laboratory formulated media, indicated that the mine effluent was the primary causal agent of the observed biotic impairment, and its toxicity could be attributed to $\text{Na}^+/\text{SO}_4^{2-}$ dominated TDS, which is significantly ameliorated by water hardness. Finally, although testing with lentic cladocerans, such as *Ceriodaphnia*, is consistent, cost-effective and sensitive to TDS related toxicity, the ecological relevance and protective capability of such testing is questionable when assessing contaminant effects on sensitive macroinvertebrates indigenous to lotic systems. A more ecologically relevant laboratory

bioassay using the mayfly, *Isonychia bicolor*, in simulated lotic microcosms provided more sensitive endpoints than *Ceriodaphnia* and *Pimephales promelas*. Although the heartiness of *Isonychia* in laboratory conditions is poorly understood relative to standardized test organisms, these results, along with potential toxic impacts from numerous $\text{Na}^+/\text{SO}_4^{2-}$ dominated wastewaters discharging into freshwater systems, may have important implications to future national pollution discharge and elimination system (NPDES) permit testing. Currently, however, strong recommendations can only be made using *Ceriodaphnia* endpoints. Potential acute toxicity to aquatic organisms in high hardness solution (≥ 790 mg/L as CaCO_3) is possible where $\text{Na}^+/\text{SO}_4^{2-}$ dominated TDS levels exceed ~ 7000 $\mu\text{S}/\text{cm}$ (5167 mg/L), with potential chronic toxicity occurring at ~ 3200 $\mu\text{S}/\text{cm}$ (2342 mg/L). These endpoints were significantly reduced in solutions of lower hardness (88 mg/L as CaCO_3), with acute and chronic toxicity occurring at 5100 $\mu\text{S}/\text{cm}$ (3754 mg/L) and ~ 2100 $\mu\text{S}/\text{cm}$ (1523 mg/L), respectively. Point source discharges causing instream TDS concentrations to exceed these levels risk impairment to aquatic life.

AKNOWLEDGMENTS

I thank my major advisor and chairman, Dr. Donald S. Cherry, whose knowledge of the field and olfaction for acquiring, developing and funding cutting-edge research provided me with a project that was not only original, stimulating and pressing, but also publishable. His mission and undying motivation as a research-warrior have gained my respect and driven me to achieve. I also thank the rest of my committee, Dr. Carl E. Zipper, Dr. Rebecca Currie and Dr. E. F. Benfield for the significant contributions they made to my development as a researcher.

I also thank the members of the Cherry Lab, Matt Hull, Dave Soucek, Chad Merricks, Brian Denson, Patrick Barry, Jenny Uerz and Travis Schmidt, for their perseverance during sometimes rigorous, field-sampling excursions. It was indeed a pleasure to work in a lab in which its members got along and were genuinely interested in helping to make everyone's research product better. Never grow up kids!

Certainly I cannot forget my parents, David and Roberta, whose contributions would dwarf the length of this thesis if described in full. Thank you for your love, support, patience and restraint. Thank you for the opportunities you made possible for me. Speaking of patience, I must thank Christina for hers. How annoying my sedentary existence in Blacksburg must have been. Thank you for all the intrastate driving you endured so that we could see each other on a semi-regular basis. You've definitely made my time here happier...look forward to being repaid.

TABLE OF CONTENTS

(ABSTRACT)	i
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	vii
LIST OF FIGURES	viii
INTRODUCTION & BACKGROUND	1
References.....	3
1 Field and Laboratory Assessment of a Coal Processing Effluent in the Leading Creek Watershed, Meigs Co., Ohio	6
1.1 Introduction.....	7
1.2 Materials and Methods.....	9
1.2.1 Study Sites	9
1.2.2 Water Chemistry Analysis	10
1.2.3 Dissolved Ion and Solids Analysis.....	10
1.2.4 Benthic Macroinvertebrate Sampling.....	11
1.2.5 In Situ Toxicity Testing	11
1.2.6 Water Column Toxicity Testing.....	12
1.2.7 Statistical Analysis.....	12
1.3 Results.....	13
1.3.1 Physicochemical Parameters	13
1.3.2 Benthic Macroinvertebrates	14
1.3.3 In Situ Toxicity Testing	15
1.3.4 Correlation analysis.....	15
1.3.5 Water Column Toxicity Testing.....	15
1.4 Discussion.....	16
1.5 Conclusions.....	19
1.6 References.....	20
2 Evaluation of Ecologically Relevant Bioassays for a Lotic System impacted by a Coal-mining effluent, using <i>Isonychia bicolor</i> [Walker]	32
2.1 Introduction.....	33
2.2 Methods	35
2.2.1 Study sites	35
2.2.2 Water chemistry analyses.....	36
2.2.3 Dissolved ion analysis.....	36
2.2.4 Simulated coal-mining effluent.....	37
2.2.5 Test organisms	37
2.2.6 Ceriodaphnia toxicity tests.....	38
2.2.7 <i>Isonychia</i> toxicity tests.....	38
2.2.8 Instream Ephemeroptera Distribution	40
2.2.9 Statistical analysis.....	40
2.2.10 Quality assurance – quality control (QA-QC).....	41
2.3 Results.....	41
2.3.1 Ceriodaphnia toxicity tests.....	41
2.3.2 <i>Isonychia</i> toxicity tests.....	42
2.3.3 Instream Ephemeroptera distribution.....	43
2.3.4 Quality assurance – quality control (QA-QC).....	43
2.4 Discussion.....	43
2.5 References.....	48
3 Stressor Identification and Evaluation of the Relative Ionic Contribution of a Coal-mine effluent to Biotic Impairment	63

3.1	INTRODUCTION	64
3.2	MATERIALS AND METHODS	67
3.2.1	Study sites	67
3.2.2	Physicochemical parameters	67
3.2.3	Dissolved ion analysis	68
3.2.4	Habitat assessment	68
3.2.5	Sediment toxicity tests	68
3.2.6	Establishing causal relationships	69
3.2.7	Laboratory formulated effluents	69
3.2.8	Water hardness study	70
3.2.9	Water column toxicity testing	71
3.2.10	Statistical analysis	71
3.3	RESULTS	72
3.3.1	Physicochemical parameters	72
3.3.2	Biotic response	73
3.3.3	Habitat assessment	73
3.3.4	Sediment toxicity tests	74
3.3.5	Causality considerations	74
3.3.6	Laboratory formulated solutions	74
3.3.7	Water hardness study	75
3.3.8	Water column toxicity testing	76
3.4	DISCUSSION	77
3.5	LITERATURE CITED	83
4	The Influence of Total Dissolved Solids on Aquatic Life	98
4.1	INDRODUCTION	99
4.2	BACKGROUND	101
4.2.1	Source of TDS levels	101
4.2.2	Mechanism of TDS toxicity	102
4.3	METHODS	103
4.3.1	Biotic response	103
4.3.2	Toxicity endpoints	103
4.4	RESULTS & DISCUSSION	104
4.4.1	Field response	104
4.4.2	Laboratory bioassay response	104
4.4.3	Field-lab comparisons	106
4.4.4	Implications to NPDES permit testing	108
4.5	References	109
	Curriculum Vita	118

LIST OF TABLES

Table 1.1: Mean physicochemical parameters for study sites, with ranges and standard deviations indicated.....	25
Table 1.2: Dissolved ion concentrations in the mining effluent in samples taken November 24, 2000 and October 12, 2001. US EPA acute and chronic water quality criteria (WQC) were included, where available.....	26
Table 1.3: Benthic macroinvertebrate indices from Fall 2000 (a) and Spring 2001 (b). One standard deviation from each mean is indicated and sites with the same letter designation were not statistically significant from one another. The dashed lines indicate where the coal-processing effluent enters each system.....	27
Table 1.4: Relative abundance of indigenous Ephemeroptera and 36-d <i>in situ</i> <i>Corbicula</i> growth during July 2001 for selected conductivity categories (a). Laboratory conducted chronic toxicity tests for <i>Ceriodaphnia</i> (b) and <i>Pimephales</i> (c) in field-collected Meigs County Mine #31 effluent. Asterisks denote a significant difference from the reference or control.....	28
Table 2.1. Comparison of mean water physicochemical parameters and total ions in the coal-mining effluent (sampled November 24, 2000), the simulated effluent and the diluent.....	55
Table 2.2. Collection, acclimation and laboratory testing conditions (given as ranges) for <i>Isonychia bicolor</i> tests.....	56
Table 2.3. Comparison of NOAEC and LOAEC endpoints for 7-d mean <i>Ceriodaphnia dubia</i> survival and reproduction in the coal-mining effluent and the simulated effluent. Tests were analyzed individually and asterisks denote a significant difference from the control.....	57
Table 2.4. Mean <i>Isonychia bicolor</i> survival and four molting endpoint variations for test II (a), test III (b) and test IV (c), conducted at $20.0 \pm 2.0^{\circ}\text{C}$	58
Table 2.5. Mean Relative Ephemeroptera abundance (%E) for categorized Leading Creek Watershed sites during Fall 2000 and Spring 2001. Asterisks denote a significant difference from the reference group.....	59
Table 3.1: Causal considerations for strength of evidence analysis from EPA 822-B-00-025 applied to the Leading Creek Watershed.....	89
Table 3.2: Matrices and associated hardness series and specific conductivity (SC) levels used in the hardness study to generate the Na_2SO_4 -hardness model (a), and mean 48-h LC_{50} values for the mine effluent, simulated effluent and mock solutions, with 95% confidence intervals provided in parentheses, and the predicted LC_{50} generated by the Na_2SO_4 -hardness model.....	90
Table 3.3: Physiochemical parameters for Leading Creek Watershed sites and the Spearman correlation (r) between these parameters and metrics of biotic impairment from Kennedy <i>et al.</i> (in press). Bold r -values represent relationships significant at the $\alpha \leq 0.05$ level.....	91
Table 3.4: Ion concentrations (a) and mean reproduction for 7-d <i>Ceriodaphnia</i> tests (b) for testing media. Each test was analyzed separately and boldface treatments with asterisks indicate a significant difference ($\alpha < 0.05$) from the control.....	92
Table 4.1: Acute testing endpoints in high salinity solution. Table includes test organism, testing temperature (Temp), water hardness testing duration, testing endpoints in the original units reported by the researchers and endpoints converted into conductivity. All endpoints were based on survivorship of the test organism.....	114
Table 4.2: Chronic testing endpoints in high salinity solution. Table includes test organism, testing temperature (Temp), water hardness testing duration, testing endpoints in the original units reported by the researchers and endpoints converted into conductivity. The following abbreviations were used: Survival (S), Reproduction (R), Growth (G) and Survival to Hatching (SH).....	116

LIST OF FIGURES

Fig. 1.1. Leading Creek Watershed study sites (not to scale).....	29
Fig. 1.2. Mean specific conductivity ($n \geq 4$), total dissolved solids ($n = 3$) and mean 48-h <i>Ceriodaphnia</i> survivorship ($n = 2 - 7$) for all Leading Creek Watershed sites. Spikes at OR1 and PR3 correspond to the mining activity. The ranges for SC and TDS data points, excluded for clarity, are provided in Table 1.1.	30
Fig. 1.3. <i>Corbicula fluminea</i> growth at study sites for the 98-day survey (a) and mean percentage of total collectors-filterers (b). Sites sharing the same letter designation were not significantly different from one another and bars represent one standard deviation from the mean.....	31
Fig. 2.1. Simulated Lotic Microcosm (SLM) used for toxicity testing with <i>Isonychia bicolor</i>	60
Fig. 2.2. Seven-day survivorship for <i>Isonychia bicolor</i> at 12°C for test I (a), and at 20°C for test II (b), test III (c) and test IV (d). Dashed lines indicate a significant difference from the control and inverse correlations between survival and SC are indicated in black.....	61
Fig. 2.3. Mean relative Ephemeroptera abundance (%E) at Leading Creek Watershed study sites for Fall 2000 (shaded bars) and Spring 2001 (white bars) data, ranked by specific conductivity.	62
Fig. 3.1. Leading Creek Watershed study sites (not to scale).....	93
Fig. 3.2. Summary of potential stressors in the Leading Creek Watershed that confound establishment of a causal relationship between the observed biotic impairment and total dissolved solids (TDS) toxicity. Small arrows denote connections between potential causes of impairment (within the boxes), dashed arrows pointing to macroinvertebrate impairment indicate null hypotheses deemed as unlikely causal agents and the large solid arrow indicates the most plausible cause of impairment.	94
Fig. 3.3. Comparison between metrics of biotic impairment (columns) from Leading Creek Watershed sites and specific conductivity (line) for benthic macroinvertebrate surveys (a) and <i>in situ</i> <i>Corbicula fluminea</i> growth (b). Data are from Kennedy <i>et al.</i> (in press) and bars represent one standard deviation from the mean.	95
Fig. 3.4. Logarithmic correlation of water hardness and 48-hour LC_{50} values ($\mu\text{S}/\text{cm}$) for <i>Ceriodaphnia dubia</i> . Filled diamonds represent data points derived by solutions in a series of set water hardness and specific conductivity adjusted to $\sim 8900 \mu\text{S}/\text{cm}$ with Na_2SO_4 (Matrix I) while open squares represent data points derived using solutions of the same set hardness series but each with 6 g/L Na_2SO_4 (Matrix II).	96
Fig. 3.5. 7-d <i>Ceriodaphnia dubia</i> mean reproduction in the mine effluent, simulated effluent mock solution I and mock solution II. Equations for each linear regression are provided in the legend.	97

INTRODUCTION & BACKGROUND

The coal industry potentially impacts aquatic biota in a variety of ways, including acidification, metal loading and fly ash disposal (*e.g.*, Roback and Richardson, 1969; Kelly, 1988; Cherry *et al.*, 1979; Specht *et al.*, 1984). The metals (*e.g.*, Al, Cu, Fe, Zn) associated with these heavily studied stressors have water quality criteria (WQC) to protect aquatic life. WQC are acute and chronic safe levels for toxicants based on the thresholds of selected test organisms that are recommended by the U.S. Environmental Protection Agency (U.S. EPA), based on carefully reviewed studies. To date, WQC do not exist for total dissolved solids (TDS) in the U.S. EPA National Recommended Water Quality Criteria--Correction document (U.S. EPA, 1999). TDS levels can be elevated by sodium (Na^+) and sulfate (SO_4^{2-}) concentrations in treated wastewater discharges from active coal mines, which can create adverse conditions for some sensitive macroinvertebrates.

Coal formation is a natural process beginning with compaction of organic matter into peat (Mitchell, 1950). Peat is further compacted to lignite, the lowest ranking of coal. As pressure and heat persist, sub-bituminous coal, bituminous coal and anthracite form in succession. The composition of coal is primarily dependent on the geological configuration of the land; therefore, the sulfur content of coal cannot be predicted by rank. The majority of mined coal is bituminous coal, as is the case at Meigs County Mine #31, located in southeastern Ohio. This 15,000 by 1,100 ft long-wall mine (Crowell, 1997) discharges its waste into Parker Run (PR), a tributary of the Leading Creek Mainstem (LCM), following transport through a settling pond. The coal seams of Appalachia are characterized by relatively high sulfur content (0.2-10%; Train, 1976).

Dissolved solids concentrations become especially high in treated discharges from underground mines, such as Mine #31 (3.4% sulfur), relative to western seams (e.g., 0.4% sulfur) (E.I.A.). The state of Ohio ranks 15th in the nation in coal production (E.I.A.), and Meigs ranks 9th among all Ohio counties (Crowell, 1997).

Meigs County Mine #31 delivers its prepared coal via a conveyor belt to the General James M. Gavin Plant at Cheshire, OH. Title IV of the Clean Air Act Amendments (1990) required coal-fired power plants to install scrubbers, a post-combustion cleaning technology, to decrease global SO₂ emissions. However, pre-combustion technologies such as coal-washing can reduce pyritic sulfur by 30 – 50% and coal ash by ~60%, ultimately reducing up to 50% of SO₂ emissions (Crowell, 1997). Coal preparation is a three-step process for acquisition of uniform coal size, moisture removal, decreased ash content, increased caloric value and decreased sulfur content (Mitchell, 1950). The first step is crushing and sizing which elicits maximum caloric recovery. Step two is the hydraulic separation and cleaning stage with the objective of removing metals, a process that often requires pH control with sodium hydroxide (NaOH) or quick lime (CaO). As a guideline to remove toxic acid-soluble metals, manganese (Mn) concentrations are reduced below 2.0 mg/L (pH ~ 9.50), due to the tenacity of Mn in aqueous solution relative to other metals (Zipper, 2000). This practice is intended to assure that more toxic metals with WQC are not discharged at high dissolved, and thus bioavailable, concentrations. Therefore, the source of the high TDS levels in the Mine #31 discharge is likely from SO₄²⁻, the oxidized form of sulfur, and Na⁺, from the NaOH used to increase the pH. These first two preparation stages produce the greatest amount of suspended and dissolved solids and require use of a settling pond

prior to waste discharge to the receiving system. The third step is the dense medium separation stage, which results in further reduction in ash and sulfur (Train, 1976).

This thesis was delineated into four chapters to clearly compartmentalize its objectives. All chapters are closely related, but each can be read as a separate entity. Chapter One, “Field and Laboratory Assessment of a Coal-processing Effluent in the Leading Creek Watershed, Meigs Co., Ohio,” established that the effluent was toxic to aquatic organisms. Chapter One is published in the Archives of Environmental Contamination and Toxicology. With the adverse effects on aquatic assemblages established, the second chapter, “Evaluation of Ecologically Relevant Bioassays for a Lotic System impacted by a Coal-mining effluent, using *Isonychia bicolor* [Walker],” aimed to evaluate whether the sole use of standardized test organisms, such as *C. dubia*, was adequately protective of the most sensitive benthic assemblages, using a mayfly in specially designed simulated lotic microcosms for the laboratory. Chapter Two was submitted to Environmental Monitoring and Assessment. Chapter 3, “Stressor Identification and Evaluation of Relative Ionic Contribution of a Coal-mining Effluent to Biotic Impairment,” was geared toward eliminating all other potential stressors in the LCW from confounding the linkage between mining related TDS and the biotic impairment observed in the previous two chapters. Chapter Three is to be submitted to Environmental Toxicology and Chemistry. Finally, Chapter Four “The Effects of Total Dissolved Solids on Aquatic Life” summarized the most important findings of the previous three chapters, including the integration of an extensive literature review.

References

Cherry D.S., Larrick S.R., Gunhrie, R.K, Davis, E.M., Sherberger F.F. 1979. Recovery of

- invertebrate and vertebrate populations in a coal ash stressed drainage system.
Can J Fish Aquat Sci 36:1086-1096.
- Clean Air Act. 1990. Public Law 101-549. U.S.C. 7641 et seq.
- Crowell, D.L. 1997. History of Coal mining in Ohio: Ohio Division of Geological
Survey: Geofacts 14.
- Crowell, D.L. 1997. Coal Mining and Reclamation: Ohio Division of Geological
Survey: Geofacts 15.
- Energy Information Administration, 100 Independence Ave., Washington, D.C. 20585.
- Kelly M. 1988. *Mining in the Freshwater Environment*. Elsevier Applied Science,
London, UK.
- Mitchell, David R. 1950. Coal Preparation. The American Institute of Mining and
Metallurgical Engineers, New York.
- Roback S.S., Richardson J.W. (1969) The effects of acid mine drainage on aquatic
insects. Proc Acad Nat Sci Phila 121:81-107
- Specht W.L., Cherry D.S., Lechleiter R.A., Cairns J.C. 1984. Structural, functional,
and recovery responses of stream invertebrates to fly ash effluent. Can J Fish
Aquat Sci 41:884-896.
- Train, Russell. 1976. Development document for interim final effluent limitations
guidelines and new source performance standards for the coal mining point source
category. US Environmental Protection Agency, Washington D.C. 20460.
- U.S. Environmental Protection Agency. 1999. National recommended water quality
criteria – correction. Office of Water 4304.

Zipper C.E. 2000. Coal mine reclamation, acid mine drainage and the Clean Water Act.
p. 169-191, in: R. Barnhisel, W. Daniels and R. Darmody (eds). Reclamation of
Drastically Disturbed Lands. American Society of Agronomy. Madison WI.
Monograph 41.

**1 Field and Laboratory Assessment of a Coal Processing Effluent in the Leading
Creek Watershed, Meigs Co., Ohio**

Abstract. The US Environmental Protection Agency has not recommended water quality criteria (WQC) to protect aquatic life from elevated sodium and sulfate concentrations, such as those associated with the coal-processing effluent of Meigs County Mine #31. This discharge, received by a tributary of the Leading Creek Watershed (SE Ohio), had a mean specific conductivity (SC) of 8,109 (7,750-8,750) $\mu\text{S}/\text{cm}$ and total metal concentrations below acute WQC. The mean 48-h LC_{50} for *Ceriodaphnia dubia* in the effluent was $6,713 \pm 99 \mu\text{S}/\text{cm}$, and mean 48-h survival was 44% for study sites downstream of the effluent. The best indicators of impairment used in this study were *Ceriodaphnia* fecundity, *in situ* *Corbicula fluminea* growth, EPT minus Hydropsychidae (richness and relative abundance) and relative Ephemeroptera abundance. Mayflies, reduced by more than 99% below the effluent, were absent from all but the furthest downstream study site. SC was strongly correlated with *Corbicula* growth ($r = -0.9755$, $p = 0.0009$) and EPT minus Hydropsychidae richness ($r = -0.8756$, $p < 0.0001$), suggesting the effluent was primarily responsible for biotic impairment. Our results indicated that SC levels, a measure of dissolved solids, in the Leading Creek Watershed that exceeded $\sim 3,700 \mu\text{S}/\text{cm}$ impaired sensitive aquatic fauna.

1.1 Introduction

The coal mining industry potentially impacts aquatic biota in a variety of ways, including acidification, metal loading and fly ash disposal. The abundance of studies assessing the ecological impacts of abandoned mined land (*e.g.*, Roback and Richardson 1969, Armitage 1980, Kelly 1988) and fly ash (*e.g.*, Cherry *et al.* 1979, Specht *et al.* 1984) are indicative of the contaminants with water quality criteria (WQC) protecting aquatic life, established by the US Environmental Protection Agency (EPA). Fewer

researchers (e.g., Radford and Graveland 1978, Latimer 1999) have assessed the impacts of coal-processing effluents and no WQC (US EPA 1999) exist for the elevated Na and SO₄ concentrations associated with these discharges.

Coal seams in Meigs County, Ohio are high in total sulfur (2.51%) relative to seams the western United States (Mitchell 1950). Title IV of the Clean Air sets regulatory limits on the aerial emissions of coal-fired power plants in terms of total atmospheric loading of SO₂ (CAA90). Consequently coal is washed to reduce sulfur content, decrease ash and increase caloric value (Train 1976), ultimately resulting in a waste discharge that can compromise freshwater stream quality (this study).

The Leading Creek Watershed (LCW), located in Meigs County, is impacted by erosion, sedimentation, agricultural practices, flooding, poor drainage, acid mine drainage (AMD) and coal mining activity (Currie 1999). Recently, high specific conductivity (SC) levels associated with the coal-processing effluent from Meigs County Mine #31, an underground longwall mine and preparation facility, warranted further research on the adverse impacts to community structure (Cherry *et al.* 1999). Following neutralization in a settling pond (e.g., NaOH, CaO), the effluent discharges into Parker Run (PR), a tributary of the Leading Creek Mainstem (LCM). Although the effluent rarely surpassed 6000 µS/cm during sampling from 1995 to 1999 (Currie 1999, Latimer 1999), contemporary data revealed SC levels in PR routinely exceeded 8000 µS/cm.

The purpose of this study was to conduct a preliminary bioassessment of this poorly understood stressor, allowing for more comprehensive research in the future. More specifically, our objectives were to quantify macroinvertebrate impairment in PR and LCM below the coal-processing discharge and to determine the role of total

dissolved solids (TDS) concentrations, measured as SC, on biotic impairment.

Examination of a system at only one organization level (Clements 2000) and with only one test organism (Pontasch *et al.* 1989) restricts the strength of association between stressors and impacts. Thus, three levels of biological organization were utilized to gauge the influence of the effluent on aquatic biota. Individual responses were measured *in vitro* for *Ceriodaphnia dubia* and *Pimephales promelas* and *in situ* for *Corbicula fluminea*, and aquatic macroinvertebrate assemblages were sampled to determine population (*e.g.*, relative mayfly abundance) and community (*e.g.*, richness) responses. Subsequently, we established which taxa were most sensitive to derive appropriate metrics for assessing coal-processing effluent toxicity in the LCW, with the possibility of extrapolation to similar systems.

1.2 Materials and Methods

1.2.1 Study Sites

Fourteen study sites were selected to assess the potential ecological impact of the coal-processing effluent (Fig. 1.1). The tributaries, Ogden Run (OR) and Parker Run (PR), received mining effluents and were the primary sources of highly conductive waters entering LCM. Of the 14 sites selected, five were considered reference stations (LCRS1, LCRS2, LCRS3, PR1, LPR1) and eight served to assess impact (OR1, LCS4, PR2, PR4, PR5, LCS6, LCS7, LCS9) caused by the effluent raceway (PR3), immediately received by PR. Sites were not selected below LCS9 due to backflow from the Ohio River.

PR is an 8.33 km, third order stream with a 4,715-acre drainage basin (Cherry *et al.* 1999). PR1 was the reference, while PR2, located just upstream of the effluent, was periodically exposed to run-off from settling ponds and backflow of the effluent (O EPA

1994). Little Parker Run 1 (LPR1), added for the 2001 sampling season, flowed into the south bank of PR between PR4 and PR5. Study sites were grouped into 1000 $\mu\text{S}/\text{cm}$ intervals and July 2001 data were categorically analyzed to suggest tolerance thresholds for *in situ* *Corbicula* growth and relative instream Ephemeroptera abundance. Sampling excursions followed a semi-monthly regime (8-26-00, 9-15-00, 10-14-00, 11-24-00, 3-15-01, 5-31-01, 7-5-01, 8-7-01, 9-6-01, 10-12-01).

1.2.2 Water Chemistry Analysis

A Yellow Springs (RDP, Dayton, OH, USA) model 54A meter was used to measure dissolved oxygen (DO_2). SC was determined in the field with an Orion[®] model 122 meter. The pH was determined in the laboratory within 24-h of collection with an Accumet[®] (Fisher Scientific, Pittsburgh, PA, USA) meter equipped with an Accumet[®] gel-filled combination electrode (accuracy ± 0.05 pH @ 25°C). Alkalinity and hardness (mg/L as CaCO_3) were determined by titration (APHA 1995).

1.2.3 Dissolved Ion and Solids Analysis

Samples were filtered (Whatman 934-AH) and submitted to the Virginia Tech Department of Environmental Engineering to determine anion concentrations. Cations were analyzed by inductively coupled plasma (ICP) spectrometry at the Virginia Tech Soil Testing Laboratory (APHA 1995). Ions analyzed were Al, Cl, Cu, Fe, Mg, Mn, Na, SO_4 and Zn based on Latimer (1999). Additional analyses for Ca, Cr, NO_3 , P and K were conducted. TDS and total suspended solids were determined as described in APHA (1995), with slight modifications. Samples were heated in a Fisher Isotemp oven[®] (100 series model 1266) at 80°C, to prevent boiling over, prior to the increase to 180°C.

1.2.4 Benthic Macroinvertebrate Sampling

Four replicate qualitative samples were collected with 800 μm D-frame nets (Wildco 425-D10) during two field excursions from 12-LCW sites on October 14, 2000 and from 14-LCW sites on July 7, 2001. Collection of each replicate involved thorough five-minute sampling of all available habitat types (Barber *et al.* 1999). Samples were preserved in $\sim 70\%$ ethanol, processed in the lab and identified to the lowest practical taxonomic level using standard keys (Merritt and Cummins 1996, Pennak 1989).

The surveys were analyzed separately to avoid comparisons between different seasons and systems. Indices used were abundance, richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) metrics, relative Ephemeroptera abundance (%E), the Shannon-Weiner diversity index, the Hilsenhoff biotic index (HBI) and percent collectors-filterers (%CF), described in Barber *et al.* (1999). EPT minus Hydropsychidae (EPT-H) richness and %EPT-H were also applied because they provided an improved depiction of the impact. Percent reductions in abundance were calculated by subtracting the quotient of the mean of all impacted sites by the mean of all reference sites from 100%.

1.2.5 In Situ Toxicity Testing

Two *in situ* toxicity tests were conducted using *Corbicula*. Organisms were collected with clam rakes from the New River near Ripplemead, VA, and kept in a Living Stream[®] (Frigid Units, Toledo, Ohio). Individuals between 9.0 and 12.0 mm were selected for testing. For each LCW site, 5 replicates, each consisting of 5 uniquely marked clams in mesh bags with $\sim 5 \text{ mm}^2$ openings, were secured with rebar and cobble in riffle areas. Clams were retrieved simultaneously from sites and returned to the Ecosystem Simulation Laboratory (ESL) for survival and growth analysis after 29 days (September 2000) and 98-days (May 31 to September 6, 2001), and checked monthly (7-

6-02, 8-8-01, 9-6-01) for survival, growth and debris accumulation. Organisms were considered dead if valves were gaping or could be easily teased apart. Data obtained for PR1 were excluded due to low DO₂ conditions (3.78 mg/L) and clams at LCS9 were not recovered. Although virtually stagnant by month 3, LPR1 was added as a reference site to mitigate the loss of PR1. Categorized clam data were taken from the first 36-days of the 98-d test to relate more closely to the 2001 benthic survey in terms of temporal scale and ambient conditions.

1.2.6 Water Column Toxicity Testing

Acute toxicity of site water using <1-d old *C. dubia*, cultured at according to US EPA (1993), was determined by 48-h tests, conducted as described in US EPA (1993) at 25±1°C using 50 mL beakers as test chambers. Moderately hard, reconstituted (EPA¹⁰⁰) water was used as the diluent and control (US EPA 1993). The accuracy of the 0.5 dilution series used in Fall 2000 was inadequate to assess the acute toxicity of the mining effluent. Thus, 0.25 and 0.15 serial dilutions were used for testing effluent samples collected in 2001 to increase precision.

Static, daily renewal, chronic water column toxicity tests, conducted according to US EPA (1994) at 25±1°C using 50 mL beakers as test chambers, involved a 0.25 serial dilution with EPA¹⁰⁰ water used as the diluent and control. *Ceriodaphnia* (<1-d old) were fed daily with 0.36 mL of 1:1 *Selenastrum capricornutum* and YCT (yeast, cerophyll and trout chow) per 30 mL of test solution. *Pimephales* (<1-d old) obtained from Aquatox, Inc. (Hot Springs, Arkansas) were fed 3-4 drops of *Artemia* twice daily.

1.2.7 Statistical Analysis

Tests were analyzed with TOXSTAT[®] (Gulley 1996), applying appropriate parametric (Dunnett's Test, T-test with Bonferroni Adjustment) or nonparametric

(Steel's-Many One Rank Test, Wilcoxon Rank Sum with Bonferroni Adjustment) analyses according to US EPA (1994), to determine no observable adverse effects concentrations (NOAEC), lowest observable adverse effects concentrations (LOAEC), lethal concentration (LC) and inhibition concentration (IC) values (US EPA 1994). Macroinvertebrate indices and clam endpoints were analyzed using the Statistical Analysis System (Statistical Analysis Institute, Inc. 1996). Data were tested for normality with the Shapiro-Wilks Test ($\alpha=0.05$), non-normal data were transformed to satisfy the assumptions of normality and Tukey's HSD test determined differences between sites. Correlation analyses and comparisons of normalized data using Tukey-Kramer HSD were conducted using JMP IN[®] (Sall and Lehman 1996). Mean SC used in correlations was based on 2001 data and taken over 3 months ($n = 3$) during macroinvertebrate surveys and 4 months ($n = 4$) during the *Corbicula* test. All significant relationships were determined at the $\alpha = 0.05$ level.

1.3 Results

1.3.1 Physicochemical Parameters

The mean SC of PR3 was 8,109 (7,750-8,750) $\mu\text{S}/\text{cm}$ (Table 1.1), elevated primarily by SO_4 and Na ions (Table 1.2). During low flow conditions when the effluent contributed >90% of the flow at PR4, SC, TDS and six ions (SO_4 , Na, Cl, Ca, Mg, K) at impacted sites closely reflected levels measured at PR3, but were gradually diluted downstream (Fig. 1.2). The concentrations of the remaining five ions detected in LCW sites (Al, Zn, NO_3 , Fe, Cu) originated from other sources. All ion concentrations in the effluent with WQC were below chronic standards, except Cl and total Al species, which were below acute standards (Table 1.2). Al increased while Cl decreased in concentration downstream of the effluent on November 24th, 2000 (Table 1.1) and

concentrations were substantially lower in the effluent in 2001 (Table 1.2). All DO₂ measurements were above 6.0 mg/L during field tests and surveys, except at PR1 (3.78 mg/L) on July 6, 2001 and LPR1 (3.27 mg/L) on August 7, 2001.

1.3.2 Benthic Macroinvertebrates

The more sensitive indices (%E, %EPT, %EPT-H and EPT-H richness) to the mining outfall were emphasized and are provided in Table 1.3. The most dramatic decline in any insect taxon was observed for Ephemeroptera. A 99.6 and 99.8% reduction in mean mayfly abundance was found at sites downstream of the effluent in Fall 2000 and Spring 2001, respectively. Mayflies were absent from all PR3, PR4, PR5, LCS6 and LCS7 samples and %E was significantly reduced at impacted sites relative to most reference sites. The highest SC levels in which mayflies were collected were recorded at OR1 on October 14, 2000 (5,140 μ S/cm) and July 7, 2001 (4,180 μ S/cm), although %E was significantly reduced. Analysis of Spring 2001 categorized data indicated a significant reduction in %E at a mean SC of $3,978 \pm 2092$ μ S/cm (Table 1.4a). Although impairment occurred at mining influenced sites in %EPT and EPT richness relative to reference sites in 2001, relative abundances were significantly lower at PR3, PR4 and PR5 and substantial impairment occurred at LCS6, LCS7 and LCS9 using %EPT-H and EPT-H richness. This trend was supported by the 2000 data at PR3, PR4 and PR5 but was not significant in LCM. Abundance, taxa richness, the Shannon-Weiner diversity index and HBI did not indicate impairment to the coal-processing effluent in either survey, and data were therefore excluded. In general, there was no discernible decline in %CF below PR3 compared to sites upstream of PR3 (Fig. 1.3b).

1.3.3 In Situ Toxicity Testing

Corbicula survival during both tests was relatively high ($\geq 76\%$) and no significant differences were found among sites. Significant impairment below the effluent outfall, however, was observed for *Corbicula* growth (Fig. 1.3a), most clearly indicated by the 98-day test; *Corbicula* at the reference sites, LCRS1 (4.90 ± 0.37 mm), LCRS2 (5.05 ± 0.36 mm), LCRS3 (3.43 ± 0.83 mm), grew significantly more than clams at PR4 (0.31 ± 0.09 mm), PR5 (0.45 ± 0.08 mm) and LCS6 (1.46 ± 0.04 mm), while recovery was observed at LCS7 (2.74 ± 0.90 mm). *Corbicula* growth was stunted in Fall 2000 due to relatively lower temperatures, but significantly impaired at sites below the effluent. Analysis of categorized data indicated significant reduction in 36-d *Corbicula* growth at 7648 ± 233 $\mu\text{S}/\text{cm}$, compromised by a large gap between groupings (Table 1.4a).

1.3.4 Correlation analysis

Mean SC was strongly positively correlated with TDS ($n = 28$, $r = 0.9991$, $p < 0.0001$), negatively correlated with EPT-H richness ($r = -0.8756$, $p < 0.0001$) and *Corbicula* growth ($r = -0.8229$, $p = 0.0010$) but not well related with the Shannon-Weiner index ($r = -0.2004$, $p = 0.4922$) or HBI ($r = 0.3131$, $p = 0.2773$). The correlation between *Corbicula* growth and mean SC was strengthened ($r = -0.9755$, $p = 0.0009$) when lower SC sites (< 3000 $\mu\text{S}/\text{cm}$), where no significant relationship occurred ($r = 0.3989$, $p = 0.7875$), and the high nutrient site (PR2: $1.37\text{mg}/\text{L}$ NO_3) were excluded. Clam growth ($r = -0.3338$, $p = 0.4191$) and EPT richness ($r = -0.5302$, $p = 0.1765$) were not significantly correlated with aluminum concentrations in the water column.

1.3.5 Water Column Toxicity Testing

For *Ceriodaphnia* acute tests, mean survival was $\geq 95\%$ in reference site water but substantially lower for impacted sites, ranging from 6 to 78% (Fig. 1.2). The mean

48-h LC₅₀ values (n=3) for PR3, PR4 and PR5 were 6,713±99, 6,807±108 and 6,912±990 µS/cm, respectively. Total *Ceriodaphnia* mortality was observed in the 100% effluent exposure in the chronic test, while all survived in the control through 3,710 µS/cm exposures (Table 1.4b). The NOAEC and LOAEC endpoints for *Ceriodaphnia* survival were 4,730 and 6,040 µS/cm, respectively. *Ceriodaphnia* mean reproduction was a more sensitive endpoint in that the NOAEC and LOAEC were 2,910±40 and 3,710±46 µS/cm, respectively. The IC₁₀ and IC₂₅ values for reproduction were 2458 (926 – 3091) and 3154 (2203 – 3446) µS/cm, respectively. The NOAEC and LOAEC endpoints for the *P. promelas* 7-d test were 4,843±36 and 6,180±63 µS/cm for survival, and 3,800±24 and 4,843±36 µS/cm for mean growth, respectively (Table 1.4c). The IC₁₀ and IC₂₅ values for growth were 3769 (2803 – 3964) and 4068 (3789 - 4245)µS/cm, respectively.

1.4 Discussion

The composition of the Meigs County Mine #31 discharge provided a unique opportunity for this preliminary investigation and bioassessment of the influence of TDS toxicity, measured as SC ($r = 0.9991$, $p < 0.0001$), on aquatic life because it was essentially neutral and composed primarily of dissolved ions (*e.g.*, SO₄ and Na) without WQC (US EPA 1999) and Cl (below acute WQC), which effectively created semi-saline conditions with remarkably high mean water hardness (792 ± 43 mg/L as CaCO₃). Hard water promotes chemical complexation of metals and can diminish the associated toxicity (Black et al. 1980). This phenomenon was observed for an open pit mine effluent in Wisconsin, in which the toxicity of relatively low metal concentrations was ameliorated by water hardness ≥ 155 mg/L as CaCO₃ (Masnado *et al.* 1995).

Total Al and Cl concentrations, measured in the Mine #31 effluent, were above chronic WQC. Mount *et al.* (1997) found the mean 48-h LC₅₀ values for *C. dubia* in NaCl and Na₂SO₄ solutions to be 1,960 mg/L (~1,189 mg/L Cl) and 3,070 mg/L (~2,076 mg/L SO₄²⁻ and ~994 mg/L Na⁺), respectively. These Cl levels were higher than in the coal-processing effluent, while Na and SO₄ concentrations were considerably lower, suggesting Na and SO₄ contributed to the bulk of the acute toxicity observed in the LCW. Thus, due to the magnitude of the Na and SO₄ acute toxicity in PR, ions above chronic WQC in the coal-processing effluent were of lesser concern. Total Al concentrations in the LCW, however, increased from effluent levels at downstream sites (Latimer 1999, this study) and thereby potentially confounded linkage between the effluent and macroinvertebrate impairment. Recovery of *in situ Corbicula* growth (Fig. 1.3a) and EPT richness (Table 1.3) at sites with increasing Al concentrations and the lack of significant relationships between Al concentration and those indices, implied Al was not a major contributor to the observed toxicity. Latimer (1999), based on correlation analysis and laboratory formulated salt solutions, also concluded it was unlikely that Al or Cl contributed significantly to macroinvertebrate impairment or toxicity to *C. dubia* in the LCW. In addition, because ion concentrations presented in this study included all metal species, bioavailable (i.e., dissolved) metals were likely overestimated.

Our macroinvertebrate surveys provided strong indications of impairment below the coal-processing effluent and identified indices that were indicative of impairment due to TDS toxicity. The generally sensitive EPT indices, however, did not indicate statistically significant impairment, due to the relatively high tolerance of hydropsychids, which compartmentalize contaminants (Cain *et al.* 2001). Percent EPT-H and EPT-H

richness provided much improved statistical resolution and indicated significant impairment at effluent impacted sites. Relative Ephemeroptera abundance was likely a more sensitive indicator of TDS toxicity but the metric was compromised by lowered abundance of mayflies at some LCM reference sites due to the previously addressed stressors in the watershed. The sensitivity of mayflies relative to other macroinvertebrate taxa found in this study was consistent with the literature (Specht *et al.* 1984, Pontasch *et al.* 1989, Diamond *et al.* 1992). Abundance and richness were not good indicators of the effluent toxicity due to the diversity of tolerant Coleoptera and Diptera, and the Shannon-Weiner index ($p = 0.4922$) and HBI ($p = 0.2773$) were not significantly related to SC. The Shannon-Weiner index likely indicated changes in community structure unrelated to pollutants (Huges 1978), while application of biotic indices (e.g., HBI) was inappropriate for assessing TDS associated impacts because taxa tolerance assignments were based on sensitivities to organic pollutants (Garcia-Criado *et al.* 1999).

Although *in situ* *Corbicula* survival may be an important indicator of toxicity in AMD impacted systems (Soucek *et al.* 2001), it was not predictive of the toxicity associated with the coal-processing effluent. *Corbicula* growth, however, was significantly reduced below the mining effluent site (PR3) in the 98-day *in situ* test and corresponded closely with SC ($p = 0.0009$). Clams at impacted sites grew less during months 2 and 3 (Fig. 1.3a), likely a result of increased sensitivity due to increased temperature and exposure duration. While clam growth was strongly negatively correlated with SC, other factors such as nutrient levels, temperature, substrate and DO₂ (Mattice and Wright 1986, McMahon and Williams 1986) likely influenced growth more

substantially at sites with mean SC levels <3000 $\mu\text{S}/\text{cm}$. This trend was evident at PR2 where nutrients (NO_3 : 1.37 mg/L) and growth (7.00 ± 0.73 mm) were high.

The >90% dilution of PR by the effluent during low flow conditions (Cherry *et al.* 1999) posed the possibility that food resources limited *Corbicula* growth. Soucek *et al.* (2001) found *in situ* *Corbicula* growth to be significantly positively correlated with %CF in a nutrient limited system ($r = 0.745$, $p = 0.0035$); therefore, the instream distribution of other filter-feeding taxa was deemed prudent to determine if food availability was limiting to clam growth. Percent CF (*e.g.*, tolerant hydropsychids and simuliids) was high at PR and LCM sites impacted sites, relative to reference sites, most likely due to the effluent induced increase in flow. Based on these data, clams at impacted sites had adequate food resources, making the TDS toxicity of the effluent the more plausible cause of impaired clam growth.

Close associations between biotic impairment and SC were also found in acute and chronic water column toxicity testing. Although impairment was observed in both *Pimephales* survival and growth, our results suggested fish were less sensitive to dissolved solids toxicity than *Ceriodaphnia* reproduction, based on NOAECs and IC values. *Ceriodaphnia* LOAECs indicated harmful effects at $\geq 3,710$ $\mu\text{S}/\text{cm}$. Masnado *et al.* (1995) and Mount *et al.* (1997) found similar relative sensitivities between these organisms to salt water solutions formulated in the laboratory.

1.5 Conclusions

The data presented in this study indicate that the coal-processing effluent discharged from Meigs County Mine #31 impaired aquatic fauna in the lower reaches of PR and LCM. The extent of this impairment was best assessed with (1) field surveys

using *in situ* *Corbicula* growth, EPT-H indices and %E, and (2) laboratory acute and chronic water column toxicity testing with *Ceriodaphnia*. Analyses of categorized data from our field surveys suggested that mayflies may be more sensitive to SC than *Corbicula* growth (36-d exposure), *Ceriodaphnia* and *Pimephales* endpoints. More specifically, correlation analysis of longer term clam growth (98-d exposure), *C. dubia* reproduction and instream distribution of mayflies at study sites suggested SC levels that exceeded ~3700 $\mu\text{S}/\text{cm}$ impaired sensitive aquatic fauna in the LCW. The toxic effects of TDS in other systems, however, cannot be concretely quantified without knowledge of the ionic composition of the solution (Mount *et al.* 1997).

Based on correlation analysis, toxicity due to dissolved solids in the mining effluent was the most plausible cause of benthic macroinvertebrate impairment, but other possible contributors included (1) chronic aluminum toxicity, (2) chronic chlorides toxicity, and (3) degradation of resource quality. Research is underway to establish a causal link between the SC of the mining effluent and the observed macroinvertebrate impairment and to determine which ion(s) were primarily responsible for the impairment.

Acknowledgments. The authors of this paper thank Dr. David Soucek and Matthew Hull for their assistance and suggestions, Brian Denson, Patrick Barry and T. Chad Merricks for their assistance in the field, and Dr. Fred Benfield and Dr. Carl Zipper for contributing to the quality of this work with their experience and advice.

1.6 References

American Public Health Association (APHA), American Water Works Association & Water Environment Federation (1995) Standard methods for the examination of water and waste water, 19th ed. American Public Health Association, Wash, D.C.

- Armitage PD (1980) The effects of mine drainage and organic enrichment on benthos in the River Nent system, northern Pennines. *Hydrobiologia* 74:119-128
- Barber MT, Gerritsen J, Snyder BD, Stribling JB (1999) Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, 2nd ed. EPA 841-B-99-002. US EPA; Office of Water; Washington, D.C.
- Black JA, Roberts RF, Johnson DM, Minicucci DD, Mancy KH, Allen HE (1980) The Significance of Physiological Variables in Aquatic Bioassays of Heavy Metals. *Water Quality Criteria for Freshwater Fish*. Butterworth, London. 259-275
- Cain DJ, Wallace WG, Luoma SN (2001) Bioaccumulation and compartmentalization in aquatic insects to metal tolerance. Abstracts, 22nd Annual National Meeting of the Society of Environmental Toxicology and Chemistry. Baltimore, MD, USA, November 11-15, 2001, p 226
- Cherry DS, Larrick SR, Gunhrie, RK, Davis, EM, Sherberger FF (1979) Recovery of invertebrate and vertebrate populations in a coal ash stressed drainage system. *Can J Fish Aquat Sci* 36:1086-1096
- Cherry DS, Currie RJ, Latimer HA, Cairns J Jr, Diz HR, Johnson DM, Yeager MM (1999) The Leading Creek Improvement Plan: Southern Ohio Coal Company Meigs Mine No. 31. Final report to Southern Ohio Coal Company and American Electric Power, Columbus Ohio 43216
- Clean Air Act. 1990. Public Law 101-549. U.S.C. 7641 et seq
- Clements WH (2000) Integrating effects of contaminants across levels of biological organization: an overview. *J Aquat Ecosyst Stress Recovery* 7: 113-116

- Currie RJ (1999) Identification of ecosystem stressors in developing an enhancement plan for the Leading Creek Watershed, Meigs County, Ohio. Virginia Polytechnic Institute and State University, doctoral dissertation
- Diamond JM, Winchester EL, Mackler DG, Gruber D (1992) Use of the Mayfly *Stenonema modestrum* (Heptageniidae) in Subacute Toxicity Assessments. Environ Toxicol Chem 11:415-425
- Garcia-Criado F., Tome A, Vega FJ, C Antolin (1999) Performance of some diversity and biotic indices in rivers affected by coal mining in northwestern Spain. Hydrobiologia 394:209-217
- Gulley DD (1996) TOXSTAT[®]. Version 3.3. University of Wyoming Department of Zoology and Physiology, Laramie, WY
- Huges BD (1978) The influence of factors other than pollution on the value of Shannon's diversity index for benthic-macroinvertebrates in streams. Water Res 12:359-364
- Kelly M (1988) *Mining in the Freshwater Environment*. Elsevier Applied Science, London, UK
- Latimer HA (1999) An ecotoxicological evaluation of active coal mining, sedimentation and acid mine drainage in three tributaries of the Leading Creek Watershed, Meigs County, Ohio. Virginia Polytechnic Institute and State University, masters thesis
- Masnado RG, Geis SW, Sonzogni WC (1995) Comparative acute toxicity of a synthetic mine effluent to *Ceriodaphnia dubia*, larval Fathead Minnow and the freshwater mussel *Anodonta imbecilis*. Environ Toxicol Chem 14:1913-1920
- Mattice JS, Wright LL (1986) Aspects of Growth of *Corbicula Fluminea*.

- American Malacological Bulletin Special Edition No. 2 (1986): 167-178
- Merritt RW, Cummins KW (1996) An introduction to the aquatic insects of North America, 3rd addition. Kendall/Hunt Publishing Company, Dubuque, IA 52002
- McMahon RF, Williams CJ (1986) A reassessment of growth rate, life span, life cycles and population dynamics in a natural population of field caged individuals of *Corbicula fluminea* (Muller) (Bivalvia: Corbiculacea). Am Malacol Bull Special Edition No. 2 (1986): 151-166
- Mitchell DR (1950) Coal Preparation, 2nd ed. The American Institute of Mining and Metallurgical Engineers, New York
- Mount DR, Gulley DD, Hockett JR, Garrison TD, Evans JM (1997) Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas*. Environ Toxicol Chem 16: 2009-2019
- Ohio EPA (1994) Ecological recovery endpoints for streams affected by the Meigs #31 mine discharges during July-September 1993. EAS/1994-1-1
- Pennak RW (1989) Freshwater invertebrates of the United States, 3rd edition. John Wiley & Sons, Inc. New York
- Pontasch, KW, Niederlehner BR, Cairns J Jr (1989) Comparisons of single species, Microcosm and Field Responses to a Complex Effluent. Environ Toxicol Chem 8: 521-532
- Radford DS, Graveland DN (1978) The water quality of some coal mine effluents and their effect on stream benthos and fish. Alberta Recreation, Parks and Wildlife. Fisheries Pollution Report Number 4
- Roback SS, Richardson JW (1969) The effects of acid mine drainage on aquatic

- insects. Proc Acad Nat Sci Phila 121:81-107
- Sall J, Lehman A (1996) JMP start statistics. SAS Institute. Duxbury Press,
Belmont, CA
- Specht WL, Cherry DS, Lechleiter RA, Cairns JC (1984) Structural, functional,
and recovery responses of stream invertebrates to fly ash effluent. Can J Fish
Aquat Sci 41:884-896
- Soucek DJ, Schmidt TS, Cherry DS (2001) In situ studies with Asian clams
(*Corbicula fluminea*) detect acid mine drainage and nutrient inputs in low order
streams. Can J Fish Aquat Sci 58:602-608
- Train, Russell (1976) Development document for interim final effluent limitations
guidelines and new source performance standards for the coal mining point source
category. US Environmental Protection Agency, Washington D.C. 20460
- US EPA (1993) Methods for measuring the acute toxicity of effluents and receiving
waters to freshwater and marine organisms, 4th ed. Washington D.C. EPA/600/4-
90/027F, Cincinnati, OH
- US EPA (1994) Short-term methods for estimating the chronic toxicity of effluents and
receiving water to freshwater organisms, 3rd ed. Washington D.C. EPA-600-4-91-
002, Cincinnati, OH
- US EPA (1999) National Recommended water quality criteria-correction. EPA-822-Z-
99-001. Office of Water; Washington, D.C.

Table 1.1: Mean physicochemical parameters for study sites, with ranges and standard deviations indicated.

Site	Conductivity ($\mu\text{S/cm}$)	TDS (mg/L)	Hardness (mg/L)	TSS (mg/L) ^c	pH ^a	Al (mg/L) ^b	Cl (mg/L) ^b
LCRS1	485 (340-720)	267 (225-350)	218 \pm 69	125 \pm 35	7.79 \pm 0.22	0.110	12.97
OR1	3528 (470-5140)	1950 (425-3075)	397 \pm 204	188 \pm 124	7.84 \pm 0.24	ND	ND
LCS4	801 (330-1620)	275 (200-400)	128 \pm 14	275 \pm 35	7.60 \pm 0.08	ND	ND
LCRS2	577 (310-1010)	283 (225-325)	170 \pm 43	225 \pm 71	7.48 \pm 0.20	0.264	46.08
LCRS3	549 (260-960)	258 (225-275)	169 \pm 46	338 \pm 265	7.51 \pm 0.18	0.079	54.33
LPR1	399 (340-490)	283 (250-325)	169 \pm 49	125 ^d	7.88 \pm 0.06	ND	ND
PR1	613 (320-1080)	375 (225-575)	213 \pm 109	100 ^d	7.62 \pm 0.04	ND	ND
PR2	2977 (760-6220)	1017 (550-1475)	574 \pm 91	113 \pm 18	8.33 \pm 0.25	0.165	323.93
PR3	8109 (7750-8750)	5792 (5725-5875)	792 \pm 43	213 \pm 53	8.10 \pm 0.12	0.209	792.07
PR4	7932 (7430-8700)	5183 (4500-5575)	760 \pm 52	200 \pm 0	8.07 \pm 0.11	0.228	771.95
PR5	7912 (7150-8640)	5463 (5250-6000)	742 \pm 60	238 \pm 18	8.03 \pm 0.10	0.261	789.47
LCS6	6387 (2570-8080)	3008 (1775-5225)	627 \pm 196	363 \pm 159	7.86 \pm 0.17	0.373	720.46
LCS7	5376 (1260-7960)	2667 (1400-4925)	569 \pm 264	188 \pm 88	7.72 \pm 0.11	0.337	622.97
LCS9	4886 (1720-7120)	2858 (1075-4325)	484 \pm 221	175 \pm 71	7.79 \pm 0.09	0.360	475.84

^a pH reported as median values

^b n=1, highest recorded concentration

^c n=2

^d n=1

Table 1.2: Dissolved ion concentrations in the mining effluent in samples taken November 24, 2000 and October 12, 2001. US EPA acute and chronic water quality criteria (WQC) were included, where available.

DISSOLVED ION	CONCENTRATION (mg/L)		ACUTE WQC (mg/L)	CHRONIC WQC (mg/L)
	2000	2001		
Conductivity	8750 μ S/cm	8010 μ S/cm	n/a	n/a
SO ₄	3671.76	n/a	n/a	n/a
Na	1952	1468	n/a	n/a
Cl	792.07	n/a	860	230
Ca	237.7	191.7	n/a	n/a
Mg	53.7	42.62	n/a	n/a
K	25.08	15.06	n/a	n/a
Fe	0.4394	0.3252	n/a	1
Mn	0.4074	0.1183	n/a	n/a
Al	0.2085	0.1270	0.750	0.087
Cu	0.0033	<0.0028	0.013	0.009
Zn	0.0045	0.0049	0.120	0.120

Table 1.3: Benthic macroinvertebrate indices from Fall 2000 (a) and Spring 2001 (b). One standard deviation from each mean is indicated and sites with the same letter designation were not statistically significant from one another. The dashed lines indicate where the coal-processing effluent enters each system.

(a)					
	SITE	%EPT	%EPT-H	EPT-H Richness	%E
LCM	LCRS1	44.1±2.5 AB	36.3±5.3 A	8.0±0.8 A	17.3±18.4 A
	OR1	21.5±16.2 B	2.7±2.3 BC	1.3±1.0 D	0.7±1.4 B
	LCS4	60.8±11.0 A	15.5±7.2 AB	5.0±1.4 ABCD	4.4±8.6 AB
	LCRS2	24.6±15.3 B	12.1±6.1 ABC	5.3±1.7 ABC	2.7±2.7 AB
	LCRS3	23.8±5.9 B	13.4±5.1 ABC	6.5±2.7 AB	7.0±3.7 A
	LCS6	24.0±13.8 B	3.0±4.2 BC	2.0±1.8 CD	0.0±0.0 B
	LCS7	40.7±27.2 AB	11.2±8.9 ABC	3.0±2.6 BCD	0.0±0.0 B
	LCS9	12.7±2.8 B	2.0±1.9 C	1.5±1.3 CD	0.3±0.5 B
	Parker Run	PR2	15.4±9.4 a	4.3±2.1 a	2.0±1.2 a
PR3		2.5±3.1 a	0.0±0.0 b	0.0±0.0 b	0.0±0.0 a
PR4		19.6±5.2 a	0.1±0.2 b	0.1±0.2 b	0.0±0.0 a
PR5		17.5±17.5 a	1.0±2.0 b	1.0±2.0 b	0.0±0.0 a
(b)					
	SITE	%EPT	%EPT-H	EPT-H Richness	%E
LCM	LCRS1	26.5±11.0 A	11.4±7.8 A	4.8±0.5 A	9.6±8.1 A
	OR1	20.8±8.9 AB	9.8±8.2 A	2.8±1.0 ABC	2.5±1.8 AB
	LCS4	26.8±12.7 AB	7.5±4.2 ABC	3.0±0.8 AB	5.8±2.2 A
	LCRS2	13.1±5.6 B	6.6±2.3 AB	2.3±0.5 ABCD	4.7±1.9 A
	LCRS3	41.6±20.7 A	13.5±3.0 A	2.5±1.9 ABCD	3.3±3.9 AB
	LCS6	17.9±8.7 AB	1.7±2.9 BC	0.3±0.6 D	0.0±0.0 B
	LCS7	42.7±3.2 A	1.5±1.2 BC	1.5±1.3 BCD	0.0±0.0 B
	LCS9	17.6±10.3 AB	0.5±0.6 C	0.8±0.5 CD	0.1±0.2 B
	Parker Run	LPR1	24.2±8.8 a	24.2±8.8 a	4.5±1.3 a
PR1		12.0±5.3 ab	12.0±5.3 b	3.3±1.3 ab	11.9±5.2 b
PR2		19.9±7.8 ab	16.6±7.5 ab	2.3±0.5 b	16.6±7.5 ab
PR3		2.4±3.6 b	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c
PR4		11.3±13.7 ab	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c
PR5		25.5±12.2 a	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c

Table 1.4: Relative abundance of indigenous Ephemeroptera and 36-d *in situ* *Corbicula* growth during July 2001 for selected conductivity categories (a). Laboratory conducted chronic toxicity tests for *Ceriodaphnia* (b) and *Pimephales* (c) in field-collected Meigs County Mine #31 effluent. Asterisks denote a significant difference from the reference or control.

(a)

Conductivity Category ($\mu\text{S}/\text{cm}$)	Mean Conductivity ($\mu\text{S}/\text{cm}$)	Included Study Sites	<i>Corbicula</i>		Ephemeroptera	
			n	Growth (mm)	n	Mean Percent Abundance
0 – 1000	397 \pm 110	LCRS1, LCRS2, LCRS3, LCS4	20	1.21 \pm 0.59	16	5.8 \pm 4.9
2001 – 3000	2607 \pm 1918	OR1	5	0.99 \pm 0.37	4	2.5 \pm 1.8
3001 – 4000	3978 \pm 2092	LCS6, LCS7, LCS9	14	1.09 \pm 0.66	11	0.0 \pm 0.0*
7001 – 8000	7648 \pm 233	PR3, PR4, PR5	15	0.28 \pm 0.12*	12	0.0 \pm 0.0*

(b)

Mean Conductivity ($\mu\text{S}/\text{cm}$)	Percent Survival	Mean Reproduction
290 \pm 11 (control)	100 \pm 0	26.7 \pm 9.6
1780 \pm 40	100 \pm 0	24.4 \pm 13.0
2300 \pm 38	100 \pm 0	24.8 \pm 2.9
2910 \pm 40	100 \pm 0	22.4 \pm 7.8
3710 \pm 46	100 \pm 0	14.6 \pm 4.8*
4730 \pm 45	80 \pm 52	4.5 \pm 4.9*
6040 \pm 62	30 \pm 48*	0.3 \pm 0.7*
7750 \pm 44	0 \pm 0*	0.0 \pm 0.0*

(c)

Mean Conductivity ($\mu\text{S}/\text{cm}$)	Percent Survival	Mean Growth (mg)
313 \pm 10 (control)	100 \pm 0	0.356 \pm 0.134
2357 \pm 30	95 \pm 10	0.514 \pm 0.145
2992 \pm 28	88 \pm 10	0.496 \pm 0.136
3800 \pm 24	90 \pm 8	0.407 \pm 0.080
4843 \pm 36	95 \pm 10	0.131 \pm 0.043*
6180 \pm 63	38 \pm 15*	0.167 \pm 0.048*
7900 \pm 63	10 \pm 12*	0.106 \pm 0.126*

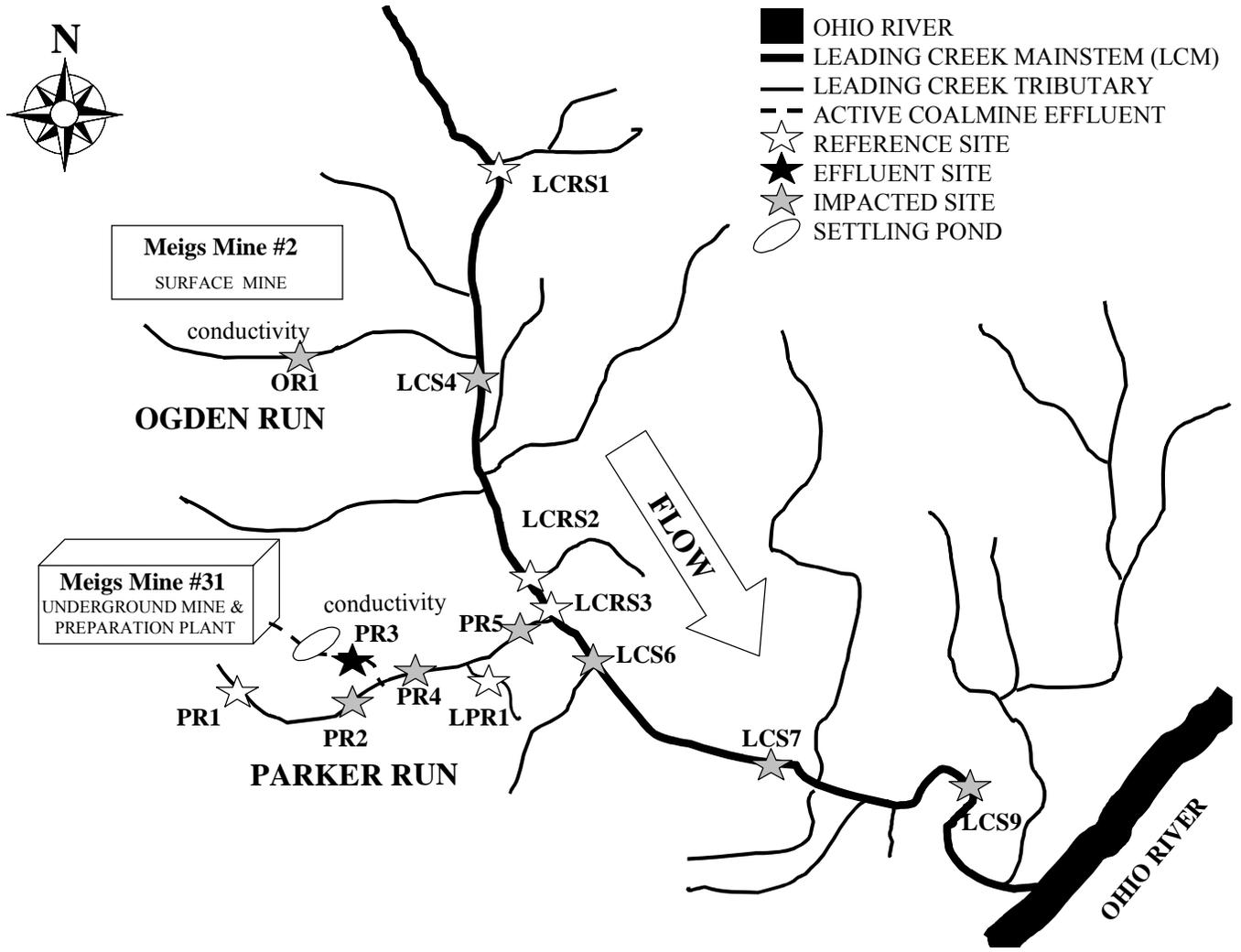


Fig. 1.1. Leading Creek Watershed study sites (not to scale).

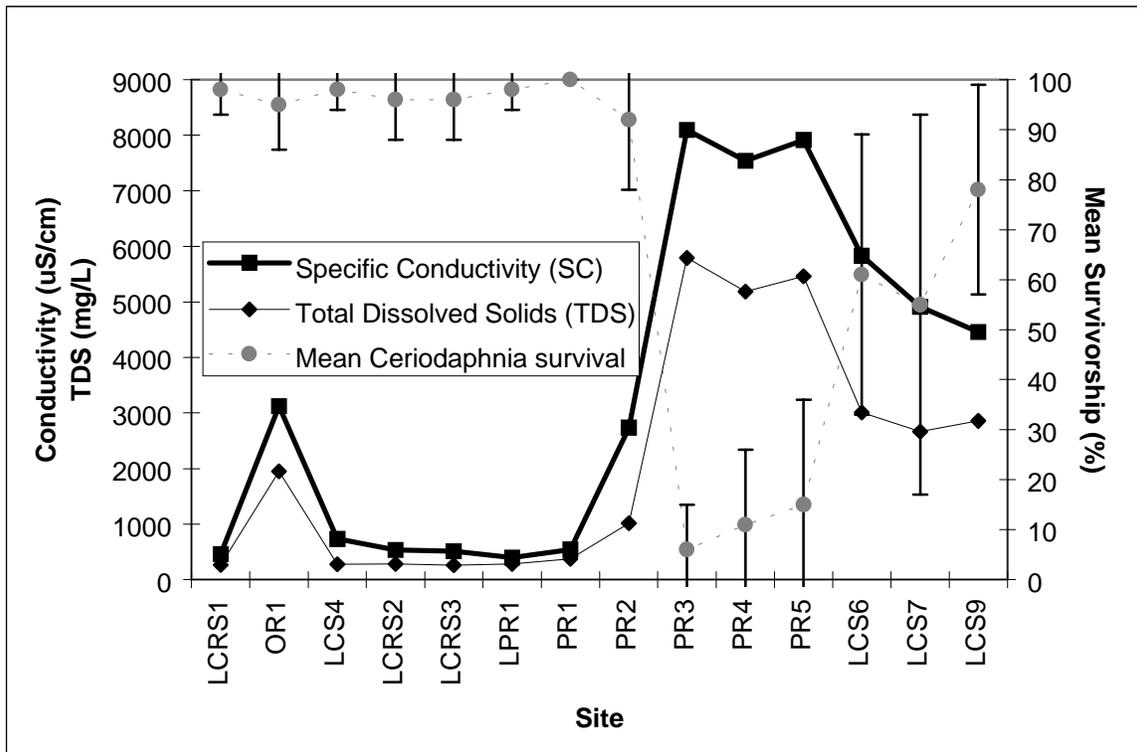
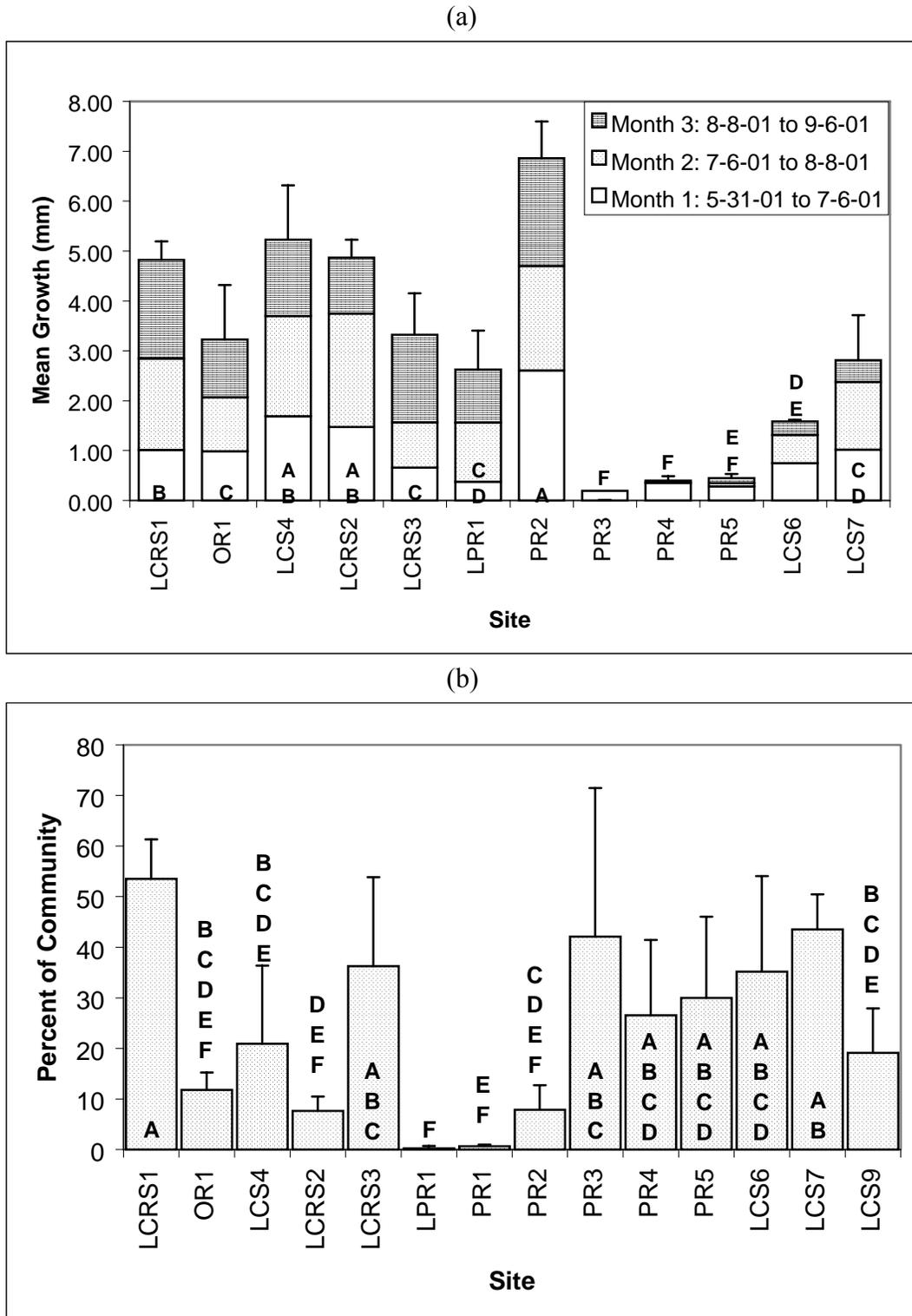


Fig. 1.2. Mean specific conductivity ($n \geq 4$), total dissolved solids ($n = 3$) and mean 48-h *Ceriodaphnia* survivorship ($n = 2 - 7$) for all Leading Creek Watershed sites. Spikes at OR1 and PR3 correspond to the mining activity. The ranges for SC and TDS data points, excluded for clarity, are provided in Table 1.1.

Fig. 1.3. *Corbicula fluminea* growth at study sites for the 98-day survey (a) and mean percentage of total collectors-filterers (b). Sites sharing the same letter designation were not significantly different from one another and bars represent one standard deviation from the mean.



**2 Evaluation of Ecologically Relevant Bioassays for a Lotic System impacted by a
Coal-mining effluent, using *Isonychia bicolor* [Walker]**

Abstract. Many studies investigating the ecotoxicological impacts of industrial effluents on freshwater biota utilize standardized test organisms such as *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas*. These organisms may not be the most predictive or ecologically relevant gauges of the responses of instream benthic macroinvertebrates to certain stressors. An indigenous species approach should be adopted, using a sensitive benthic collector-filterer following development of practical laboratory bioassays. In the Leading Creek Watershed (southeast Ohio), an aggregated ~99% reduction in mean mayfly abundance for all impacted sites was observed below a coal-mining effluent with mean specific conductivity (SC) of 8,109 (7,750-8,750) $\mu\text{S}/\text{cm}$. The mayfly, *Isonychia bicolor*, was exposed for 7-days to a simulation of this effluent, in lotic microcosms. Based on lowest observable adverse effect concentrations, *I. bicolor* survival was a more sensitive endpoint to SC (1,562 $\mu\text{S}/\text{cm}$) than were 7-day *C. dubia* survival and fecundity (3,730 $\mu\text{S}/\text{cm}$). *Isonychia* molting, a potentially more sensitive endpoint, was also examined. Using traditional test organisms to assess discharges to surface water alone may not adequately protect benthic macroinvertebrate assemblages in systems impaired by discharges high in SC.

2.1 Introduction

Field studies assessing the severity of unknown chemical stressors on aquatic biota often are complicated by numerous biotic and abiotic factors that may confound bioassessment. To mitigate this problem, researchers utilize a whole effluent toxicity testing (WETT) approach to observe the individual responses of organisms in controlled laboratory experiments. Compounding the inherent loss of ecological relevance in such experiments (Clements, 2000), standardized test organisms such as lentic cladocerans

(e.g., *Ceriodaphnia dubia*, *Daphnia magna*) and relatively tolerant fish species (e.g., *Cyprinus carpio*, *Lepomis macrochirus*, *Pimephales promelas*) may further limit the applicability of extrapolating laboratory responses to instream benthic macroinvertebrate assemblages (Rosenberg and Resh, 1996). Moreover, the most commonly used test organisms may not adequately protect sensitive aquatic benthos from certain pollutants. For instance, *C. dubia* and *P. promelas* ranked only 6th and 14th most sensitive to copper of the 17 organisms used in acute toxicity testing for the development of site-specific criteria in the Clinch River, Virginia (Cherry *et al.*, 2002).

The elevated specific conductivity (SC) levels associated with a continuously discharged coal-mining effluent in Meigs County, Ohio have been reported in Cherry *et al.* (1999). The mean SC of this effluent, 8,109 (7,750-8,750) $\mu\text{S}/\text{cm}$, was only slightly diluted by the receiving system (Kennedy *et al.*, in press). Although SC levels were strongly negatively correlated with *in situ* growth of *Corbicula fluminea* ($r = -0.8229$, $p = 0.0010$) and EPT (Ephemeroptera, Plecoptera, Trichoptera) minus Hydropsychidae (EPT-H) richness ($r = -0.8756$, $p < 0.0001$), Ephemeroptera, reduced in abundance by >99% below the effluent, was the most severely influenced indigenous taxon (Kennedy *et al.*, in press).

The sensitivity of insects in the order Ephemeroptera to both natural and anthropogenic perturbations is well documented (e.g., Pontasch and Cairns, 1988; Short *et al.*, 1991; Williams and Williams, 1998). Although toxicity tests with mayflies have been conducted in the laboratory (e.g., Peters *et al.*, 1985; Sherberger *et al.*, 1979; Diamond *et al.*, 1992; Dobbs *et al.*, 1994), few have addressed sensitivities to SC, total

dissolved solids (TDS) or salinity (Goetsch and Palmer, 1997; Williams and Williams, 1998; Chadwick and Feminella, 2001).

The objective of this study was to formulate a solution simulating the coal-mining discharge, validated by *Ceriodaphnia* tests, to serve as the medium for *in vitro* chronic toxicity tests using an ecologically relevant test organism. The mayfly, *Isonychia bicolor* (Walker), was selected for this study because of its high sensitivity (4th overall) to copper (e.g., Cherry *et al.*, 2002), availability, and use in previous studies (Sherberger *et al.*, 1977; Peters *et al.*, 1985; Sibley, 1991; Diamond *et al.*, 1990; Dobbs *et al.*, 1993; Kobuszewski and Perry, 1994). We compared *I. bicolor* endpoints in terms of SC, used to represent TDS concentrations, to (1) *C. dubia* responses, to determine if this standardized test organism was protective of sensitive, instream, benthic macroinvertebrates and (2) indigenous mayfly population responses to dissolved solids in the field, to determine the validity of laboratory and field comparisons. It was not the intention of this study to suggest *C. dubia* should be replaced by *I. bicolor* as a WETT organism, but rather supplement its use in a system with a high dissolved solids perturbation.

2.2 Methods

2.2.1 Study sites

Fourteen study sites, described in Kennedy *et al.* (in press), were selected in the Leading Creek Watershed (LCW) to assess the downstream ramifications of the coal-mining effluent. The tributaries, Ogden Run (OR) and Parker Run (PR), were the receiving systems for mining effluents and the primary sources of highly conductive waters entering Leading Creek Mainstem (LCM). Of the 14 sites selected, five were considered reference stations (LCRS1, LCRS2, LCRS3, PR1, LPR1) due to their location

above the discharge, and eight (OR1, LCS4, PR2, PR4, PR5, LCS6, LCS7, LCS9) served to assess impact caused by the effluent outfall site (PR3), immediately received by PR.

2.2.2 Water chemistry analyses

All water chemistry analyses were performed in the field or in the laboratory within 24 h of collection. Conductivity and dissolved oxygen (DO₂) were measured in the field using an Orion[®] model 122 meter and a Yellow Springs (RDP, Dayton, OH, USA) model 54A meter, respectively. The pH was determined with an Accumet[®] (Fisher Scientific, Pittsburgh, PA, USA) meter equipped with an Accumet[®] gel-filled combination electrode (accuracy < ± 0.05 pH @ 25°C). Alkalinity and hardness (mg/L as CaCO₃) were determined by simple titration of 50 mL samples (APHA, 1995).

2.2.3 Dissolved ion analysis

Samples were filtered and submitted to the Virginia Tech Department of Environmental Engineering to determine dissolved anion concentrations via ion chromatography. Cations were analyzed by inductively coupled plasma (ICP) spectrometry at the Virginia Tech Soil Testing Laboratory (APHA 1995). Ions analyzed were Al, Ca, Cl, Cr, Cu, Fe, K, Mg, Mn, Na, NO₃, P, SO₄ and Zn. Total dissolved solids (TDS) were determined as described in APHA (1995), with slight modifications.

Samples were heated in a Fisher Isotemp oven[®] (100 series model 1266) at 80°C, to prevent boiling over, prior to a 1 h increase to 180°C.

Due to the strong linear correlation between TDS and SC ($r = 0.9989$, $p < 0.0001$) in our *Ceriodaphnia* tests, this study used SC levels, and conversions were performed with the following equation derived from this correlation:

$$SC = 1.3072(TDS) + 169.15$$

2.2.4 Simulated coal-mining effluent

The simulated effluent represented the total ion concentrations in the Mine #31 effluent sample taken November 24th, 2000 (Table 2.1), and was used to (1) isolate known inorganic ions from any unknown constituents and (2) provide an easily replicable medium for testing. It was formulated using reagent grade $\text{Al}_2(\text{SO}_4)_3$, CaCl_2 , $\text{Fe}_2(\text{SO}_4)_3$, MgSO_4 , MnSO_4 , KBr , KCl , NaCl and Na_2SO_4 dissolved in filtered water for *C. dubia* tests and unfiltered water for *I. bicolor* tests, based on conditions to which organisms were acclimated. Water was collected from an unpolluted reference stream, Sinking Creek, accessed at Newport, VA. Copper and Zn were not added because concentrations were higher in Sinking Creek water (SCW) than in the mining discharge, although still well below chronic WQC. Ion concentrations in SCW were accounted for in all calculations.

2.2.5 Test organisms

Ceriodaphnia dubia, test organism recommended by the US Environmental Protection Agency (EPA), has been commonly used in aquatic ecotoxicological studies and is relatively more sensitive to laboratory formulated salt solutions than *D. magna* and *P. promelas* (Masnado *et al.*, 1995; Dickerson *et al.*, 1996; Mount *et al.*, 1997).

Isonychia bicolor is a mayfly in the family Isonychiidae (Merritt and Cummins, 1996), but was previously classified in the families Heptageniidae, Baetidae, Siphonuridae and Oligoneuriidae (Kondratieff and Voshell, 1984). *Isonychia* is bivoltine, consisting of a larger, over-wintering cohort with spring emergence and a smaller summer/fall cohort that matures and emerges rapidly (Kondratieff and Voshell, 1984). Although strong swimmers, these mayflies seek cover, clinging to leaf packs, cobble and other available substrata (Kondratieff and Voshell, 1984). *Isonychia*, especially the over-wintering

cohort, was desirable for testing because it was easy to collect, larger in size and directly exposed to the water column with a relatively large gill surface area, potentially sensitizing it to osmoregulatory stress (Peters *et al.*, 1985). Organisms from the spring/fall cohort were too small for experimental apparatuses and were observed to emerge at a higher frequency during our preliminary studies.

2.2.6 Ceriodaphnia toxicity tests

Ceriodaphnia were obtained from SCW reserve cultures and cultured individually, according to US EPA (1994), for at least 2 weeks prior to testing. For direct comparisons of the relative sensitivities between test organisms, static, 7-d *C. dubia* tests, conducted according to US EPA (1994), utilized the simulated effluent at similar exposures to the 7 d *Ceriodaphnia* tests in field-collected, coal-mining effluent and the 7 d *I. bicolor* tests. Filtered SCW was used as the diluent (0.25 serial dilution) and control. All tests were 7 d so that *Ceriodaphnia* was exposed to the simulated effluent for the same duration as *Isonychia*. Tests were conducted at $25.0 \pm 1.0^\circ\text{C}$ to adhere to the protocol of most National Pollution Discharge Elimination System (NPDES) permits and to avoid delayed brood patterns at cooler temperatures.

2.2.7 Isonychia toxicity tests

Isonychia were collected from a riffle area in Sinking Creek, as in Peters *et al.*, 1985, using D-frame dip-nets (Wildco, 425-A40) with flat, 800 x 900 μm mesh backs to minimize injury, and immediately transported to the laboratory (~15 min) in a cooler containing site-collected water. Mayflies were acclimated to laboratory conditions for 6-9 d in 5-L aquariums containing SCW, agitated/aerated by two stir bars and airlines, and the temperature was increased by $\leq 2.0^\circ\text{C}/\text{day}$ to the desired test temperature. Organisms were fed daily with ~6 mL of yeast, cerophyll, trout chow and ground Tetra-min[®]

(YCTT) passed through cheesecloth to remove larger particles, because *I. bicolor* consumes primarily 0.1 – 0.7 μm detrital particles (Wallace and O'Hop, 1979), and water was renewed (~60%) every 48-h.

The experimental apparatuses (Figure 2.1), or simulated lotic microcosms (SLMs), were modifications from previous studies (Lechleitner *et al.*, 1985; Farris *et al.*, 1991), adopted based on similar LC_{50} values obtained for *I. bicolor* in the flow-through and bioassay methodologies used by Peters *et al.* (1985). To satisfy the flow requirement of *I. bicolor* for filter-feeding in longer term bioassays (Pontasch and Cairns, 1988), a stir bar in a screen (~1 mm^2 openings) covered, glass petri dish, all within a 1-L beaker, provided flow and aeration, although SLMs were also aerated to assure adequate DO_2 levels (6.5 – 9.0 mg/L). Quick Count[®] plastic canvas (Uniek, Inc., Waunakee, WI, USA) sheets were manipulated into cylinders, fastened with plastic cable ties and placed into each SLM to provide flow heterogeneity and suitable substrate for the clinging habit of *I. bicolor*. Each replicate SLM contained 10 test organisms and was placed on a stir plate in an incubator (12:12 h light:dark photoperiod).

Tests, conducted in the simulated effluent for 7 d, were static with induced current, renewed daily (60%), and used unfiltered SCW as the diluent (0.5 serial dilution) and control. To utilize the more desirable over-wintering cohort, four tests were performed from Fall 2001 and Winter 2002 (Table 2.2). Test I (t=6; n=3) was conducted at the temperature of SCW at mayfly collection ($12.0 \pm 2.0^\circ\text{C}$). Tests II (t=6, n=3), III (t=4, n=4) and IV (t=5, n=4) were run at $20.0 \pm 2.0^\circ\text{C}$ to more closely represent summer conditions at Parker Run sites, thus increasing sensitivities to stressors and stimulating growth (i.e., molting). Test organisms in each SLM were fed ~2 mL of YCTT daily.

Endpoints used were percent survival and mean exuvia/replicate (ex/rep), as in Diamond *et al.* (1992). An additional endpoint, mean exuvia/organism/day (ex/org/d) was examined because it was computationally independent of survivorship. Additionally, ex/rep and ex/org/d were also examined excluding day 1 data, because between 2 and 8 times more exuvia were observed for day 1 than any other test day during the 20°C bioassays. Dead organisms and exuvia were removed daily.

2.2.8 Instream Ephemeroptera Distribution

Four replicate qualitative samples were collected with 800 µm D-frame nets (Wildco 425-D10) during two separate field excursions from 12-LCW sites on October 14, 2000 (10.1 – 17.2°C) and from 14-LCW sites on July 6, 2001 (19.9 – 25.7°C). Collection of each replicate involved thorough five-minute sampling of all available habitat types. Samples were preserved in ~70% ethanol and returned to the laboratory for processing and identification to the lowest practical taxonomic level using standard keys (Merritt and Cummins, 1996; Pennak, 1989). The Fall 2000 and Spring 2001 data were combined, ranked and categorized into 500-1000 µS/cm intervals to gauge the influence of SC on relative Ephemeroptera abundance (%E).

2.2.9 Statistical analysis

Toxicity tests and categorized field data were analyzed using TOXSTAT[®] (Gulley, 1996), with application of appropriate parametric (Dunnett's Test, T-test with Bonferroni Adjustment) or nonparametric (Steel's-Many One Rank Test, Wilcoxon Rank Sum with Bonferroni Adjustment) analyses ($\alpha=0.05$), to determine no observable adverse effect concentration (NOAEC) and lowest observable adverse effect concentration (LOAEC) endpoints. Test selection was performed according to the flow charts provided in EPA (1994). Fishers Exact Test was used to determine significant differences

Ceriodaphnia survivorship. Emergence was not a concern in *I. bicolor* tests I or II, but was accounted for in statistical analysis of data from tests III and IV, in which some early emergence was observed (at most 1 to 2 emergences/replicate), likely due to the increase in degree-days during the acclimation to 20°C (Sweeney, 1978; Chadwick *et al.*, 2001). Correlation analyses were conducted using JMP IN[®] (Sall and Lehman, 1996).

2.2.10 Quality assurance – quality control (QA-QC)

The accuracy of the calculations for the simulated effluent was checked by balanced meq/L and the measured ionic composition of simulated effluent was compared to the field-collected effluent. During all chronic toxicity testing, treatments were monitored daily for DO₂, pH and SC. A 7-d cumulative composite sample of treatments was taken at the end of test III and cumulative samples were taken from each exposure at the end of test IV. Samples were analyzed at the Virginia Tech Water Quality Laboratory for total ammonia ([NH₃] + [NH₄⁺]) concentrations and water quality criteria were determined as described in US EPA (1999). Unionized ammonia concentrations were calculated according to EPA (1999).

2.3 Results

2.3.1 Ceriodaphnia toxicity tests

Survival for *Ceriodaphnia* in the controls was ≥ 90% for all tests (Table 2.3). Survivorship NOAEC and LOAEC endpoints in the coal-mining and simulated effluents were slightly inconsistent in both testing media, although no statistical differences from the control were found at ≤ 3700 μS/cm. The NOAEC and LOAEC endpoints for reproduction were 3,014 ± 27 (~2,176 mg/L) and 3,730 ± 26 μS/cm (~2,727 mg/L) in the simulated effluent, which compared well with the endpoints of 2,910 ± 40 (~2,097 mg/L)

and $3,254 \pm 26 \mu\text{S}/\text{cm}$ ($\sim 2,360 \text{ mg}/\text{L}$) derived for the mining effluent. For all tests, the DO_2 ranged from 7.0 - 8.1 mg/L and pH ranged from 8.17 - 8.49.

2.3.2 Isonychia toxicity tests

Feeding behavior, excretory activity, molting and mortality, including dead organisms still partially within exuvia, were observed throughout testing. All four 7-d tests indicated a dose-dependent response and strong negative correlations between the SC of the simulated effluent and mean survival (Figure 2.2). The NOAEC and LOAEC endpoints at $12.0 \pm 2.0^\circ\text{C}$ were $2,734 \pm 30$ ($\sim 1,962 \text{ mg}/\text{L}$) and $4,973 \pm 42 \mu\text{S}/\text{cm}$ ($\sim 3,675 \text{ mg}/\text{L}$), respectively, with $95.0 \pm 7.1\%$ survival in the control.

There were no significant differences in survivorship between the controls of the 3 tests conducted at $20.0 \pm 2.0^\circ\text{C}$ ($\geq 95\%$ survival), making comparisons between tests possible. The NOAECs at $20 \pm 2.0^\circ\text{C}$ were 619 ± 4 ($\sim 344 \text{ mg}/\text{L}$) and $550 \pm 6 \mu\text{S}/\text{cm}$ ($\sim 291 \text{ mg}/\text{L}$) for tests III and IV, respectively, while no NOAEC was determined for test II (Table 2.4). The LOAECs at $20 \pm 2.0^\circ\text{C}$ were $1,562 \pm 42$ ($\sim 1066 \text{ mg}/\text{L}$; lowest SC exposure in test II), 966 ± 8 ($\sim 610 \text{ mg}/\text{L}$) and $987 \pm 11 \mu\text{S}/\text{cm}$ ($\sim 626 \text{ mg}/\text{L}$) for tests II, III and IV, respectively. In the $18,040 \mu\text{S}/\text{cm}$ ($\sim 13,671 \text{ mg}/\text{L}$) exposure, 100% mortality was observed after 4 d, and a statistically significant reduction was observed by day 1. Controls were continued beyond termination of all tests, and survival remained $\geq 80\%$ after 12-15 d. Exuvia/rep and ex/org/d also indicated a dose-dependant relationship with SC, although somewhat inconsistent, showing both less sensitivity (Table 2.4a) and more sensitivity (Table 2.4c) than survivorship. The exclusion of day 1 data made slight differences in NOAEC and LOAEC endpoints in tests II and III, but did not influence

endpoints derived for test IV. Other water chemistry parameters were similar between all treatments and replicates, and ranges are provided in Tables 2.1 and 2.2.

2.3.3 Instream Ephemeroptera distribution

Combined Fall 2000 and Spring 2001 data showed that elevated SC levels had a distinct adverse influence on %E (Figure 2.3). The category of 2001-3000 $\mu\text{S}/\text{cm}$ was substantially reduced compared to the reference category (0-500 $\mu\text{S}/\text{cm}$), although a statistical significance was not obtained until the next category (Table 2.5). Overall, categorized SC and %E were significantly inversely correlated ($r = -0.8443$, $p = 0.0168$).

2.3.4 Quality assurance – quality control (QA-QC)

Ion concentrations in the simulated effluent were within 20% of the coal-mining effluent, except for Ca, Al and Fe (Table 2.1). Starting with the control, the 7-d aggregate unionized ammonia (NH_3) concentrations were 0.068, 0.071, 0.097, 0.087, 0.076 and 0.088 mg/L for test IV and were not significantly correlated with percent survival ($r = -0.1484$, $p = 0.8118$).

2.4 Discussion

The purpose of our laboratory conducted *Isonychia* tests was to (1) use a representative benthic organism to isolate TDS, measured as SC ($r = 0.9989$, $p < 0.0001$), as a stressor from potential field-encountered confounding factors (e.g., habitat quality, sediment toxicity) and (2) account for the severe reduction in mayflies below the coal-mining effluent discharged from Meigs County Mine #31 at sites with SC levels at the *Ceriodaphnia* NOAEC endpoint of $2,910 \pm 40 \mu\text{S}/\text{cm}$ (Kennedy *et al.*, in press). We found that both *Ceriodaphnia* and *Isonychia* were sensitive to elevated levels of dissolved solids, in the absence of WQC metals. Survival of *Isonychia* was significantly reduced at substantially lower levels than observed for *C. dubia* endpoints. Cherry *et al.* (2002),

also found *Isonychia* to be more sensitive than *Ceriodaphnia* to acute copper exposure. It was not our intention to replace *C. dubia*, but rather supplement standardized testing of mining effluents.

Water chemistry parameters during testing were adequate for the survival of aquatic life. Although the hydrated salts used resulted in lower than expected Ca, Al and Fe concentrations, 7 d *Ceriodaphnia* testing indicated that the simulated effluent was representative of the toxicity of the coal-mining discharge (Table 2.3) and that the associated toxicity was predominately due to known ions rather than unknown constituents. These results also suggested that Al toxicity was not as important a contributor to the coal-mining effluent toxicity as the three most abundant ions in the effluent (SO₄, Na, Cl) because it was below detection limits (<0.0026 mg/L) in the simulated effluent. Other inorganic constituents were below or did not have chronic WQC.

Isonychia endpoints were considerably more sensitive to dissolved solids than *Ceriodaphnia* endpoints and mayflies were more sensitive at 20.0 ± 2.0°C than at 12.0 ± 2.0°C. The NOAEC endpoints at 20.0 ± 2.0°C for *Isonychia* survival (619 ± 4 µS/cm) and ex/rep (619 ± 4 µS/cm) in the simulated effluent were substantially lower (i.e., more sensitive) than the most sensitive *Ceriodaphnia* endpoint, mean reproduction (Kennedy *et al.*, in press), in both the coal-mining effluent (2910 ± 40 µS/cm) and simulated effluent (3014 ± 7 µS/cm). Goetsch and Palmer (1997), using a South African *Tricorythus* species in a Na₂SO₄ solution, found a similar 96-h LOAEC endpoint of 100 mS/m (1,000 µS/cm) to our 7-d LOAEC endpoints of 966 ± 8 and 987 ± 11 µS/cm. Their similar endpoints obtained over a shorter duration can probably be explained by the use of a

different species and solution of lower water hardness, and thus greater toxicity (Black, 1980; Masnado *et al.*, 1995; Mount *et al.*, 1997).

According to previous researchers (Sherberger *et al.*, 1977; Diamond *et al.*, 1990), the frequency of molting in *Isonychia* may be a more sensitive endpoint than their survival. Our results supported this claim using mean ex/rep (Table 2.4), but the relationship was slightly degraded using mean ex/org/d, suggesting ex/rep was strongly survivorship dependent (i.e., fewer molts at higher exposures due to fewer live organisms). Although considerably more *Isonychia* exuvia were observed after day 1 in this study and a previous one (Diamond *et al.*, 1990) suggesting a handling response to loading organisms into test chambers, exclusion of these data made little difference in NOAEC and LOAEC endpoints (Table 4c). Derivatives of *Isonychia* molting do appear to indicate a dose-dependent response to SC levels, but further modifications in test duration and replication are required to establish an endpoint as statistically resolute as *Isonychia* survivorship. Therefore, our suggested safe SC levels were based primarily on survivorship.

Specific conductivity ($0.9009 \leq r \leq 0.9883$) accounted for *I. bicolor* mortality in the four 7 d tests better than potential confounders. Toxicity due to NH₃ in the *I. bicolor* bioassays was unlikely ($r = -0.1484$, $p = 0.8118$) because macroinvertebrates are less sensitive to NH₃ toxicity than the fish species on which standards are based (Grammeter and Frutiger, 1990) and NH₃ toxicity tends to decrease with increasing ionic strength (Williams *et al.*, 1986). Thermal shock during the acclimation process was not a concern in tests I and II, but the collection temperatures for tests III and IV required more

acclimation to 20°C. However, *I. bicolor* is tolerant to thermal shock (Sherberger *et al.*, 1979) and there were no differences in control survival between all tests.

Instream mayfly distributions in the LCW suggested that *Isonychia* endpoints may be only slightly overprotective. Our analysis of the field distribution of other mayfly taxa in the LCW (e.g., baetids, caenids, heptageniids, leptophlebiids) addressed the concern of field-collected organisms tested in the laboratory. Although there was considerable variability in our %E data, mayflies generally were not collected at sites with SC levels >2607 µS/cm (Figure 2.3). All mayflies found at sites with SC levels > 3433 µS/cm were collected during October 2000 when temperatures were lower (10.1 – 17.2°C), making organisms more metabolically tolerant to TDS toxicity (this study). Categorized instream mayfly distribution in the LCW, suggested the range of 2001-3000 µS/cm substantially reduced relative mayfly abundance, although confounding factors inherent to analyses of field data compromised the detection of statistical significance. Chapman *et al.* (2000) suggested that most aquatic organisms should tolerate TDS concentrations up to 1,000 mg/L (~1,476 µS/cm) at $23.0 \pm 1.0^\circ\text{C}$, although the *Chironomus tentans* used to obtain this endpoint are considered more tolerant than mayflies. In summation, the LOAEC endpoint from test II (1562 ± 42 µS/cm; 1066 mg/L) may be more appropriate for cooler conditions in the LCW.

Our *in vitro* toxicity tests with *Isonychia* resulted in endpoints that were more ecologically relevant and protective of the most sensitive benthic assemblages in regard to TDS perturbations (Pontasch *et al.*, 1989). During the teneral stage, organisms may be more susceptible to osmoregulatory stress because they lack a sclerotized exoskeleton that serves as a barrier to diffusive gradients. Morris *et al.* (1987), however, suggested

that alterations in gill phospholipid composition within 1-3 days after molting may counteract increased cuticle permeability in the amphipod, *Gammarus duebeni*.

Mayflies, however, are especially sensitive to osmoregulatory stress because they are more permeable, a direct function of the number of exposed cells not under the epithelial layer, than predicted based on their body weight (Buchwalter, Jenkins and Curtis, 2002).

Overall, the mechanism of mortality due to high TDS was probably dehydration of gill and internal tissue, salt accumulation (Ingersoll *et al.*, 1992) and compromised osmoregulatory function of coniform chloride cells through cytoplasmic degradation and vacuole formation in hypertonic solution (Wichard *et al.*, 1973; Peters *et al.*, 1985).

Alterations in the number of chloride cells in the tracheal gills of a *Callibaetis sp.* in water with gradually altered TDS levels indicate mayflies have some adaptive ability to regulate salt balance (Wichard *et al.*, 1973). Given salinity acclimated organisms are generally more tolerant (Hart *et al.*, 1991; Koel and Peterka, 1995), degradation of chloride cells in water with rapidly altered TDS concentrations suggests rate of change in TDS may be more toxic than TDS alone (Wichard *et al.*, 1973). This was not a concern for our laboratory to field comparisons because of the consistency of SC levels at LCW study sites would force colonizing mayfly larvae to tolerate an extreme change in TDS. If TDS shock related mortality did occur in our *I. bicolor* bioassays, it was not evident at treatments $\leq 9049 \mu\text{S}/\text{cm}$ during the first 3-4 days of testing (Figure 2.2).

Testing with *I. bicolor* elicits high control survivorship ($\geq 95\%$), significant results, enhanced ecological relevance and more protective endpoints in terms of SC than the laboratory tests with *C. dubia*, *P. promelas* and *in situ* tests with *C. fluminea* used in Kennedy *et al.* (in press). However, *C. dubia* tests were remarkably consistent, replicable

and efficient. Specific conductivity levels up to $\sim 900 \mu\text{S}/\text{cm}$ ($559 \text{ mg}/\text{L}$) appear to be safe for sensitive benthic assemblages. However, conspicuous reductions will likely occur between $\sim 1,500$ to $2,500 \mu\text{S}/\text{cm}$ ($\sim 1018 - 1783 \text{ mg}/\text{L}$), based on *Isonychia* survivorship, instream mayfly distributions in the LCW and endpoints obtained from previous research. Deviations in this range will occur with site-specific ionic composition, water hardness (Mount *et al.*, 1997; Goodfellow *et al.*, 2000) and temperature (this study). Overall, our results suggest NPDES permits for surface water discharges relying solely on standardized test organisms may not protect sensitive biota, such as mayflies, in the receiving system. Future research should focus on sodium, sulfate and chlorides dominated solutions to establish protective limits on TDS levels for coal-mining discharges, integrating the influence of hardness. We are currently pursuing such research by testing the relative toxicity of a series of laboratory formulated salt solutions.

Acknowledgements

The authors thank Brian Denson, Matthew Hull, Patrick Barry and T. Chad Merricks for suggestions on the experimental design, assistance in the laboratory and field. We also thank Dr. David Soucek, Dr. E. Fred Benfield, Dr. Jerry Diamond and Dr. Reese Voshell for their expert advice.

2.5 References

American Public Health Association (APHA): American Water Works Association and Water Environment Federation, 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Wash, D.C.

- Black, J.A., Roberts, R.F., Johnson, D.M., Minicucci, D.D. and Mancy, K.H., Allen, H.E.: 1980. The Significance of Physiological Variables in Aquatic Bioassays of Heavy Metals. *Water Quality Criteria for Freshwater Fish*. Butterworth, London. 259-275.
- Buchwalter, D.B., Jenkins, J.J. and Curtis, L.R.: 2002. Respiratory strategy and temperature influences on contaminant and water uptake rates in aquatic insects: Implications for stressor-specific diagnostics. Annual Meeting of the North American Benthological Society, Pittsburgh, PA (USA), May 28-Jun 1, 2002 (World Meeting Number 000 5892).
- Chadwick, M.A. and Feminella, J.W.: 2001. Influence of salinity and temperature on the growth and production of a freshwater mayfly in the Lower Mobile River. *Limnol Oceanog.* **46**, 532-542.
- Chapman, P.M., Bailey, H. and Canaria, E.: 2000. Toxicity of total dissolved solids associated with two mine effluents to *Chironomid* larvae and early life stages of rainbow trout. *Environ. Toxicol. Chem.* **19**, 210-214.
- Cherry, D.S., Currie, R.J., Latimer, H.A., Cairns, J., Diz, R.B, Johnson, D.M. and Yeager, M.M.: 1999. Leading Creek Improvement Plan. Final report to Southern Ohio Coal Company and American Electric Power, Columbus Ohio.
- Cherry, D.S., Van Hassel, J.H., Farris, J.L., Soucek, D.J. and Neves, R.J.: 2002. Site-specific derivation of the acute copper criteria for the Clinch River, Virginia. *Hum. Ecol. Risk Assess.* **8**, 591-601.
- Clements, W.H.: 2000. Integrating effects of contaminants across levels of biological organization: an overview. *J. Aquat. Ecosyst. Stress Recovery* **7**, 113-116.

- Diamond, J.M., Mackler, D.G., Collins, M. and Gruber, D.: 1990. Derivation of a freshwater silver criteria for the New River, Virginia, using representative species. *Environ. Toxicol. Chem.* **9**, 1425-1434.
- Diamond, J.M., Winchester, E.L., Mackler, D.G. and Gruber, D.: 1992. Use of the Mayfly *Stenonema modestrum* (Heptageniidae) in Subacute Toxicity Assessments. *Environ. Toxicol. Chem.* **11**, 415-425.
- Dickerson, K.K., Hubert, W.A. and Berman, H.L.: 1996. Toxicity assessment of water from lakes and wetlands receiving irrigation drain water. *Environ. Toxicol. Chem.* **15**, 1097-1101.
- Dobbs, M.G., Farris, J.L., Reash, R.J., Cherry, D.S. and Cairns, J.: 1994. Evaluation of the resident-species procedure for developing site-specific water quality criteria for copper in Blaine Creek, Kentucky. *Environ. Toxicol. Chem.* **13**, 963-971.
- Farris, J.L., Cherry, D.S. and Neves, R.J.: 1991. Validation of copper concentrations in laboratory testing for site-specific copper criteria in the Clinch River. Final report to American Electric Power.
- Goetsch, P.A. and Palmer, C.G.: 1996. Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. *Arch. Environ. Contam. Toxicol.* **32**, 32-41.
- Goodfellow, W.L., Ausley, L.L., Burton, D.T., Denton, D.L., Dorn, P.B., Grothe, D.R., Heber, M.A., Norberg-King and T.J., Rodgers, J.H. Jr.: 2000. Major ion toxicity in effluents: a review with permitting recommendations. *Environ. Toxicol. Chem.* **19**, 175-182.
- Grammeter, S. and Frutiger, A.: 1990. Short-term toxicity of NH₃ and low oxygen to

- benthic macroinvertebrates of running waters and conclusions for wet weather water pollution control measures. *Water Sci. Technol.* **10/11**, 291-296.
- Gulley, D.D.: 1996. TOXSTAT®. Version 3.3. University of Wyoming Department of Zoology and Physiology, Laramie, WY.
- Hart, B.T., Bailey, P., Edwards, R., Hortle, K., James, K., McMahon, A., Meredith, C. and Swadling, K.: 1991. A review of the salt sensitivity of the Australian freshwater biota. *Hydrobiologia* **210**, 105-144.
- Ingersoll, C.G., Dwyer, F.J., Burch, S.A., Nelson, M.K., Buckler, D.R. and Hunn, J.B.: 1992. The use of freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drain water. *Environ. Toxicol. Chem.* **11**, 503-511.
- Kennedy, A.J., Cherry, D.S. and Currie, R.J.: in press. Field and Laboratory Assessment of a Coal Processing Effluent in the Leading Creek Watershed, Meigs Co., Ohio. *Arch. Environ. Contam. Toxicol.* in press
- Kobuszewski, D.M. and Perry, S.A.: 1994. Secondary production of *Rhyacophila*, *Ameletus* sp., and *Isonychia bicolor* from streams of low and circumneutral pH in the Appalachian Mountains and West Virginia. *Hydrobiologia* **273**, 163-169.
- Koel, T.M. and Peterka, J.J.: 1995. Survival to hatching of fishes in sulfate-saline waters, Devils Lake, North Dakota. *Can. J. Fish Aquat. Sci.* **52**, 464-469.
- Kondratieff, B.C. and Voshell, J.R.: 1984. The north and central species of *Isonychia* (Ephemeroptera: Oligoneuriidae). *Transactions of American Entomology* **110**, 129-244.
- Lechleitner, R.A., Cherry, D.S., Cairns, J.C. Jr. and D.A. Stetler: 1985. Ionoregulatory

- and toxicological responses of stonefly nymphs (Plecoptera) to acidic and alkaline pH. *Arch. Environ. Contam. Toxicol.* **14**, 179-185.
- Masnado, R.G., Geis, S.W. and Sonzogoni, W.C.: 1995. Comparative acute toxicity of a synthetic mine effluent to *Ceriodaphnia dubia*, larval Fathead Minnow and the Freshwater mussel *Anodonta imbecilis*. *Environ. Toxicol. Chem.* **14**, 1913-1920.
- Merritt, R.W. and Cummins, K.W.: 1996. An introduction to the aquatic insects of North America, 3rd addition. Kendall/Hunt Publishing Company, Dubuque, IA 52002.
- Morris, R.J., Lockwood, A.P.M., Dyball, D. and Bolt, S.R.L.: 1987. Changes in the fatty acid composition of the gill phospholipids in *Gammarus duebeni* during moult: Evidence for reduced permeability of the gill membrane. *Com. Biochem. Physiol.* **88B**, 257-260
- Mount, D.R., Gulley, D.D., Hockett, J.R., Garrison, T.D. and Evans, J.M.: 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (Fathead Minnows). *Environ. Toxicol. Chem.* **16**, 2009-2019.
- Pennak, R.W.: 1989. Freshwater invertebrates of the United States, 3rd edition. John Wiley & Sons, Inc. New York.
- Peters, G.T., Cherry, D.S. and Cairns, J.C. Jr.: 1985. Responses of *Isonychia bicolor* to alkaline pH: an evaluation of survival, oxygen consumption, and chloride cell ultrastructure. *Can. J. Fish. Aquat. Sci.* **42**, 1088-1095.
- Pontasch, K.W. and Cairns, J. Jr.: 1988. Establishing and maintaining laboratory-based microcosms of riffle insect communities: their potential for multispecies toxicity

- tests. *Hydrobiologia* **175**, 49-60.
- Rosenberg, D.M. and Resh, V.H.: 1996. Use of aquatic insects in biomonitoring. *In: An introduction to the aquatic insects of North America*, 3rd addition. Kendall/Hunt Publishing Company, Dubuque, IA 52002. pp. 87-97.
- Sall, J. and Lehman, A.: 1996. JMP start statistics. SAS Institute. Duxbury Press, Belmont, CA.
- Sherberger, F.F., Benfield, E.F., Dickson, K.L. and Cairns, J. Jr.: 1977. Effects of thermal shocks on drifting aquatic insects: a laboratory simulation. *J. Fish. Res. B. Can.* **34**, 529-536.
- Short, T.M., Black, J.A. and Birge, W.J.: 1991. Ecology of a saline stream: community responses to spatial gradients of environmental conditions. *Hydrobiologia* **226**, 167-178.
- Sibley, P.K. and Kaushik, N.K.: 1991. Toxicity of microencapsulated permethrin to selected nontarget aquatic invertebrates. *Arch. Environ. Contam. Toxicol.* **20**, 168-176.
- Sweeney, B.W.: 1978. Bioenergetic and development response of a mayfly to thermal variation. *Limnol. Oceanogr.* **23**, 461-477.
- US EPA: 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms, 3rd ed. Washington D.C. EPA-600-4-91-002.
- US EPA: 1999. Update of ambient water quality for Ammonia. Office of Water, Office of Science and Technology, Washington, D.C. EPA-822-R-99-014.
- Wallace, J.B. and O'Hop, J.: 1979. Fine particle suspension-feeding capabilities of

- Isonychia* spp. (Ephemeroptera: Siphonuridae). *Ann. Entomol. Soc. Am.* **72**, 353-357.
- Wichard, W., Tsui, P.T.P. and Komnick, H.: 1973. Effect of different salinities on the coniform chloride cells of mayfly larvae. *J. Insect Physiol.* **19**, 1825-1835.
- Williams, D.D. and Williams, N.E.: 1998. Aquatic insects in an estuarine environment: densities, distribution and salinity tolerance. *Freshwater Biol.* **39**, 411-421.
- Williams, K.A., Green, D.W.J. and Pascoe, D.: 1986. Studies on the Acute Toxicity of Pollutants to Freshwater Macroinvertebrates: 3. Ammonia. *Arch. Hydrobiologie* **106**, 61-70.

Table 2.1. Comparison of mean water physicochemical parameters and total ions in the coal-mining effluent (sampled November 24, 2000), the simulated effluent and the diluent.

Parameter	Coal mining effluent	Simulated effluent	Percent Difference	Filtered Diluent
Conductivity	8750	8890	1.6	240
Hardness	770	659	14.4	143
Alkalinity	162	142	12.3	141
pH	7.91	8.38	5.9	8.12
SO ₄ ²⁻	3671.76	3714	1.2	2.84
Na ⁺	1952	2118	8.5	2.862
Cl ⁻	792.07	685.1	13.5	3.52
Ca	237.7	187.5	21.1	35.6
Mg	53.7	51.4	4.3	11.00
K	25.08	28.48	13.6	2.269
Br	1.63	n/a	n/a	0.53
Fe	0.4390	0.1927	56.1	0.0111
Mn	0.4070	0.3551	12.8	0.0011
Al	0.209	ND	n/a	ND
Zn	0.005	0.0076 [†]	52	ND
Cu	0.003	0.011 [†]	266.7	ND

[†] Ambient concentration in unfiltered SCW

Table 2.2. Collection, acclimation and laboratory testing conditions (given as ranges) for *Isonychia bicolor* tests.

Test Number	Collection Parameters			Test Parameters				
	Date	Temperature (°C)	Conductivity (µS/cm)	Initiation Date	Test Temperature (°C)	Mean Simulated Effluent Conductivity (µS/cm)	pH	DO ₂
I	10-19-01	12.5	260	10-25-01	12.0 ± 2.0	9020 ± 44	7.93-8.24	6.2-9.0
II	12-5-01	12.0	260	12-11-01	20.0 ± 2.0	9049 ± 71	7.91-8.35	6.1-9.0
III	1-3-02	1.9	260	1-12-02	20.0 ± 2.0	1637 ± 14	7.89-8.23	7.1-8.2
IV	1-29-02	7.0	240	2-6-02	20.0 ± 2.0	3039 ± 11	7.88-8.38	7.2-8.5

Table 2.3. Comparison of NOAEC and LOAEC endpoints for 7-d mean *Ceriodaphnia dubia* survival and reproduction in the coal-mining effluent and the simulated effluent. Tests were analyzed individually and asterisks denote a significant difference from the control.

<i>C. dubia</i> in coal mining effluent				<i>C. dubia</i> in simulated effluent			
Test	Mean Conductivity ($\mu\text{S}/\text{cm}$)	Mean Survivorship (%)	Mean Reproduction	Test	Mean Conductivity ($\mu\text{S}/\text{cm}$)	Mean Survivorship (%)	Mean Reproduction
I	290 \pm 11	100 \pm 0	34.1 \pm 9.6	II	227 \pm 5	100 \pm 0	37.2 \pm 4.5
II	294 \pm 8	90 \pm 32	40.4 \pm 9.3	I	247 \pm 8	100 \pm 0	39.7 \pm 3.9
I	1780 \pm 40	100 \pm 0	40.5 \pm 13.0	I	1550 \pm 76	100 \pm 0	39.3 \pm 4.7
I	2300 \pm 38	100 \pm 0	35.3 \pm 2.9	II	1551 \pm 9	100 \pm 0	36.1 \pm 3.6
II	2553 \pm 21	100 \pm 0	33.6 \pm 11.0				
I	2910 \pm 40	100 \pm 0	31.0 \pm 7.8	I	3014 \pm 27	90 \pm 32	33.1 \pm 10.7
II	3254 \pm 26	100 \pm 0	29.6 \pm 7.5*	II	3027 \pm 23	100 \pm 0	35.1 \pm 3.4
I	3710 \pm 46	90 \pm 32	22.3 \pm 4.8*	II	3730 \pm 26	100 \pm 0	30.5 \pm 2.8*
II	4146 \pm 35	100 \pm 0	24.2 \pm 5.4*	I	4530 \pm 41	30 \pm 48*	9.8 \pm 9.6*
I	4730 \pm 45	60 \pm 52*	5.5 \pm 4.9*	II	4530 \pm 29	100 \pm 0	23.0 \pm 4.6*
II	5304 \pm 44	100 \pm 0	9.2 \pm 4.2*	I	6006 \pm 46	60 \pm 52*	1.1 \pm 2.2*
I	6040 \pm 62	30 \pm 48*	0.3 \pm .7*	II	6017 \pm 20	80 \pm 42	1.1 \pm 1.8*
II	6767 \pm 32	50 \pm 53	0.0 \pm 0.0*				
I	7750 \pm 44	0 \pm 0*	0.0 \pm 0.0*	I	8863 \pm 46	0 \pm 0*	0.0 \pm 0.0*

Table 2.4. Mean *Isonychia bicolor* survival and four molting endpoint variations for test II (a), test III (b) and test IV (c), conducted at $20.0 \pm 2.0^\circ\text{C}$.

(a)

Mean Specific Conductivity ($\mu\text{S/cm}$)	Mean Survival (%)	Exuvia/replicate		Exuvia/organism/day	
		days 1-7	days 2-7	days 1-7	days 2-7
263 \pm 5	96.7 \pm 5.8	10.7 \pm 0.6	4.0 \pm 1.0	0.15 \pm 0.01	0.07 \pm 0.02
1562 \pm 42	73.3 \pm 5.8*	8.0 \pm 1.0	5.7 \pm 0.6	0.13 \pm 0.02	0.11 \pm 0.02
2757 \pm 70	50.0 \pm 20.0*	3.0 \pm 0.0*	1.7 \pm 1.5	0.05 \pm 0.00	0.03 \pm 0.03
4917 \pm 49	43.3 \pm 15.3*	9.3 \pm 2.1	4.3 \pm 1.0	0.14 \pm 0.10	0.07 \pm 0.02
9049 \pm 71	13.3 \pm 5.8*	6.3 \pm 3.2*	4.3 \pm 2.5	0.10 \pm 0.05	0.08 \pm 0.05
18040 \pm 84	0.0 \pm 0.0*	0.7 \pm 1.2*	0.0 \pm 0.0*	0.08 \pm 0.14	0.00 \pm 0.00*

(b)

Mean Specific Conductivity ($\mu\text{S/cm}$)	Mean Survival (%)	Exuvia/replicate		Exuvia/organism/day	
		days 1-7	days 2-7	days 1-7	days 2-7
260 \pm 0	95.0 \pm 10.0	10.5 \pm 1.3	7.3 \pm 1.5	0.15 \pm 0.02	0.12 \pm 0.02
619 \pm 4	91.9 \pm 10.6	8.5 \pm 2.6	4.8 \pm 1.7*	0.13 \pm 0.01	0.09 \pm 0.03
966 \pm 8	69.2 \pm 16.4*	6.0 \pm 2.6*	4.3 \pm 1.7*	0.10 \pm 0.04*	0.09 \pm 0.02
1637 \pm 14	52.8 \pm 21.6*	4.5 \pm 1.9*	1.5 \pm 1.3*	0.07 \pm 0.03*	0.03 \pm 0.03*

(c)

Mean Specific Conductivity ($\mu\text{S/cm}$)	Mean Survival (%)	Exuvia/replicate		Exuvia/organism/day	
		days 1-7	days 2-7	days 1-7	days 2-7
244 \pm 5	95.0 \pm 5.8	11.0 \pm 2.2	8.0 \pm 1.8	0.16 \pm 0.03	0.14 \pm 0.03
550 \pm 6	84.6 \pm 14.2	7.8 \pm 1.3*	5.0 \pm 1.8*	0.11 \pm 0.02*	0.08 \pm 0.03*
987 \pm 11	62.2 \pm 18.4*	6.3 \pm 1.0*	4.8 \pm 0.5*	0.12 \pm 0.03	0.11 \pm 0.02
1693 \pm 10	45.9 \pm 16.9*	4.5 \pm 1.0*	3.8 \pm 0.5*	0.08 \pm 0.02*	0.08 \pm 0.02*
3039 \pm 11	15.0 \pm 11.5*	4.3 \pm 0.5*	1.8 \pm 1.0*	0.08 \pm 0.04*	0.05 \pm 0.03*

Table 2.5. Mean Relative Ephemeroptera abundance (%E) for categorized Leading Creek Watershed sites during Fall 2000 and Spring 2001. Asterisks denote a significant difference from the reference group.

Specific Conductivity Category ($\mu\text{S}/\text{cm}$)	Number of Sites	Mean Specific Conductivity ($\mu\text{S}/\text{cm}$)	Mean %E
0-500 (reference)	6	402 \pm 60	9.8 \pm 8.7
501-1000	3	710 \pm 136	9.0 \pm 11.8
1001-2000	2	1310 \pm 156	10.5 \pm 9.9
2001-3000	1	2607 \pm 0	2.5 \pm 1.8
3001-4000	3	3707 \pm 267	0.1 \pm 0.2*
4001-5000	3	4517 \pm 252	0.3 \pm 0.9*
5001-9000	8	7606 \pm 998	0.0 \pm 0.0*

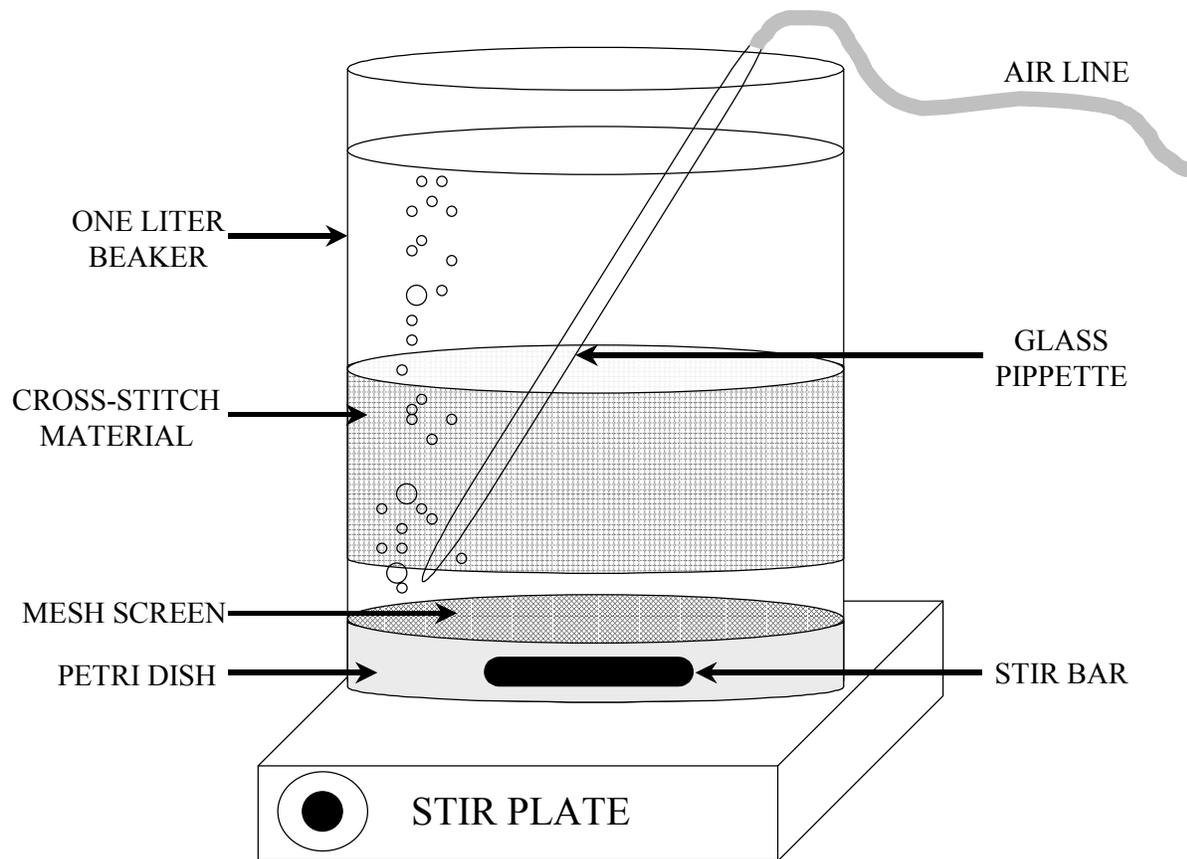
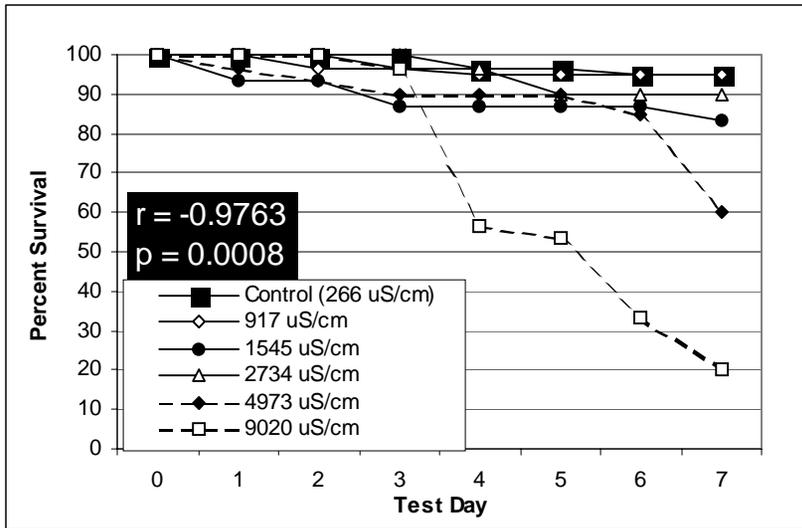
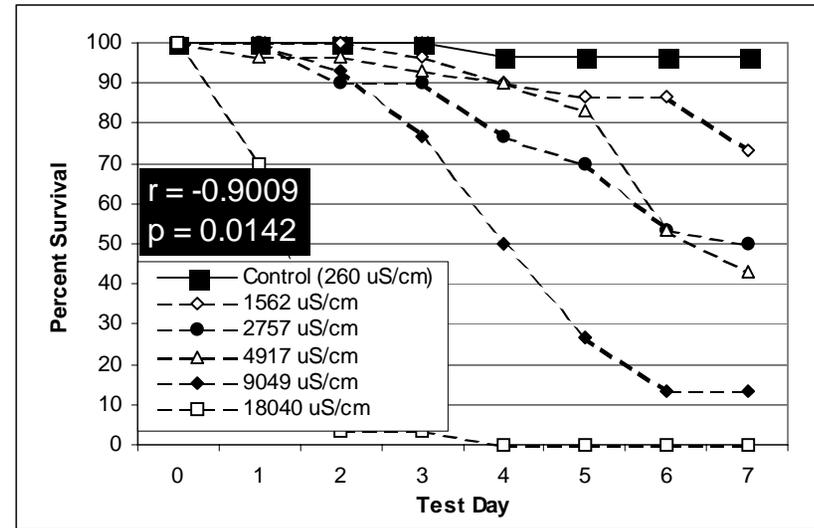


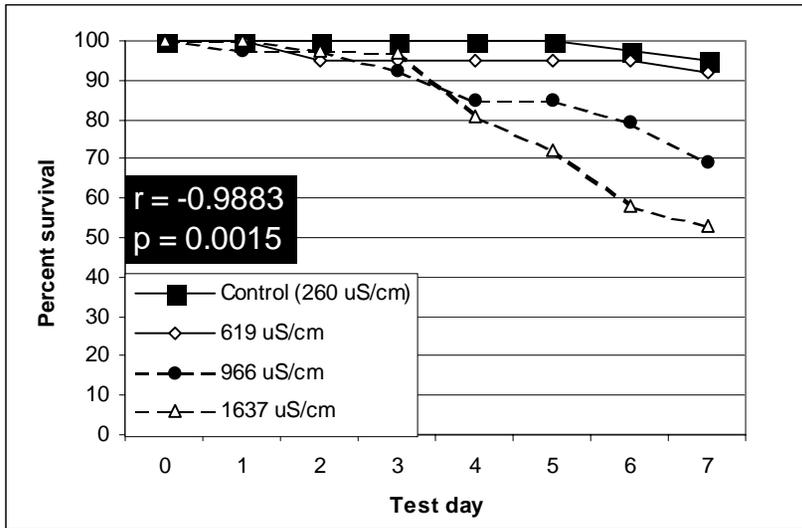
Fig. 2.1. Simulated Lotic Microcosm (SLM) used for toxicity testing with *Isonychia bicolor*.



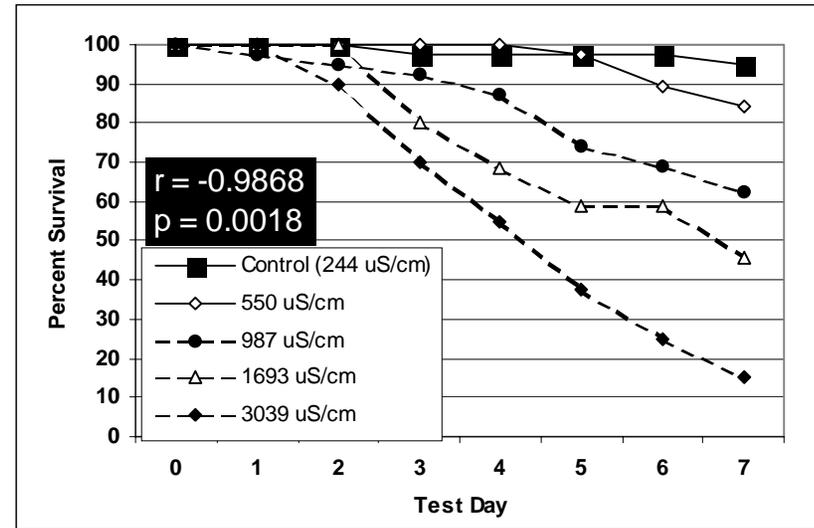
(a)



(b)



(c)



(d)

Fig. 2.2. Seven-day survivorship for *Isonychia bicolor* at 12°C for test I (a), and at 20°C for test II (b), test III (c) and test IV (d). Dashed lines indicate a significant difference from the control and inverse correlations between survival and SC are indicated in black.

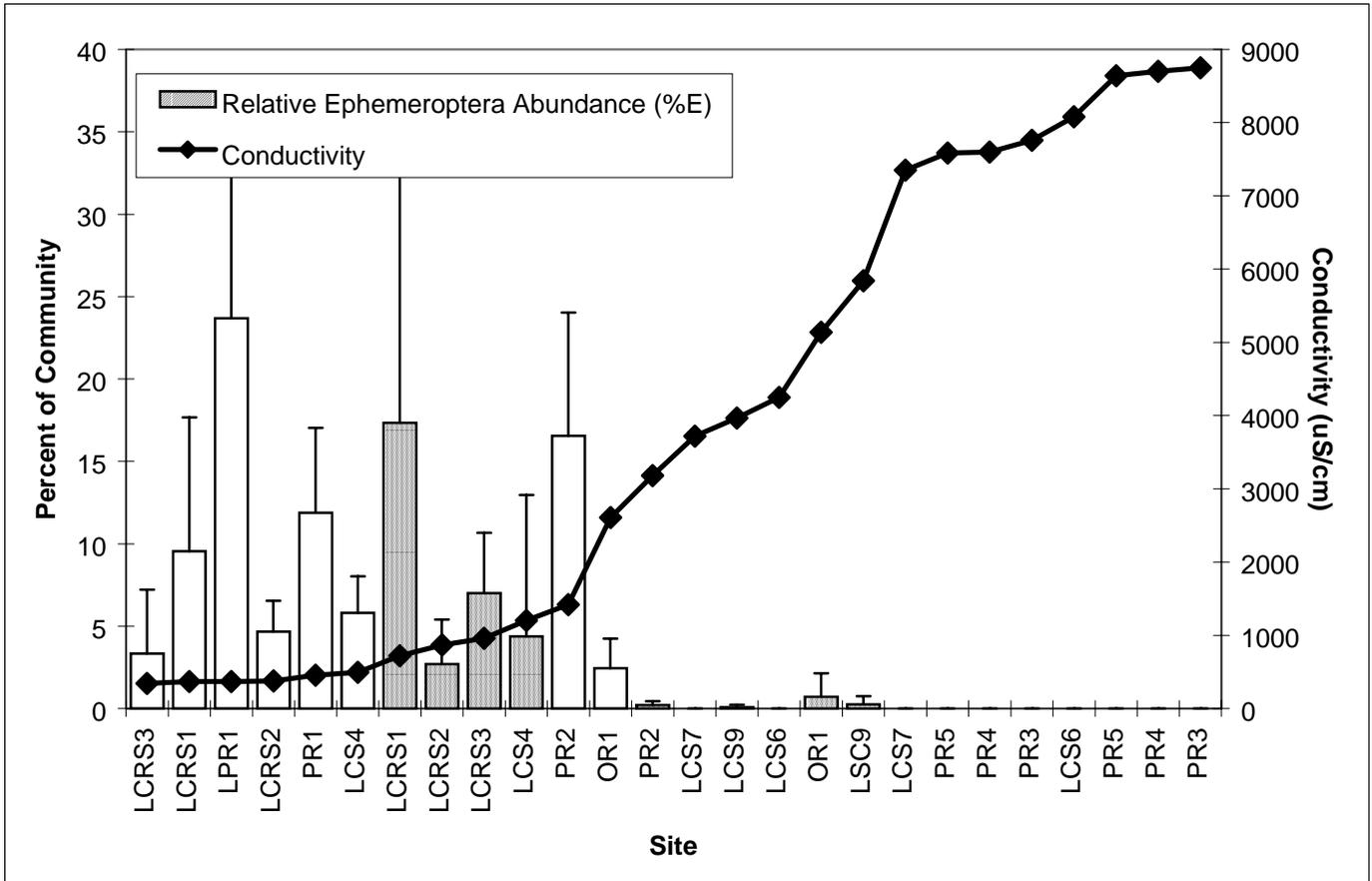


Fig. 2.3. Mean relative Ephemeroptera abundance (%E) at Leading Creek Watershed study sites for Fall 2000 (shaded bars) and Spring 2001 (white bars) data, ranked by specific conductivity.

**3 Stressor Identification and Evaluation of the Relative Ionic Contribution of a
Coal-mine effluent to Biotic Impairment**

Abstract – The U.S. Environmental Protection Agency has defined national instream water quality criteria (WQC) for 157 pollutants (US EPA, 1999). However, WQC for total dissolved solids (TDS) concentrations do not currently exist. Some water treatment processes, including pH modifications at active coal-mining and preparation facilities, discharge wastewaters of potentially adverse TDS levels. The strong correlations between specific conductivity (SC), a surrogate for TDS, and metrics of biotic impairment ($r = -0.6867$ to -0.8321), in addition to the application of causal considerations for strength of evidence analysis, indicated that the coal-mine effluent was the primary stressor in the mid-reaches of Leading Creek Watershed, Meigs County, Ohio. Acute and chronic testing with *Ceriodaphnia dubia* in laboratory manipulated media indicated that the majority of the effluent toxicity could be attributed to the most abundant ions in the discharge, sodium (1952 mg/L) and/or sulfate (3671.76 mg/L), although the high water hardness (792 ± 43 mg/L as CaCO_3) of the effluent had an ameliorative effect. Based on this laboratory testing at these concentrations, $\text{Na}^+/\text{SO}_4^{2-}$ dominated dissolved solids were acutely toxic at ~ 7000 $\mu\text{S}/\text{cm}$ (5167 mg/L), with chronic toxicity occurring at ~ 3200 $\mu\text{S}/\text{cm}$ (2342 mg/L). At a lower hardness of 88 mg/L as CaCO_3 , acute and chronic toxicity endpoints were substantially reduced, occurred at 5100 $\mu\text{S}/\text{cm}$ (3754 mg/L) and ~ 2100 $\mu\text{S}/\text{cm}$ (1523 mg/L), respectively. Point source discharges causing instream TDS concentrations to exceed these levels risk impairment to aquatic life.

3.1 INTRODUCTION

Water quality criteria (WQC) for the protection of aquatic life have not been defined for sodium (Na^+), sulfate (SO_4^{2-}) or total dissolved solids (TDS) concentrations

(US EPA 1999). WQC are safe acute and chronic standards developed by the U.S. Environmental Protection Agency (EPA), based on carefully reviewed studies. Dissolved solids, however, can reach concentrations in discharges from coal preparation and treatment facilities that are toxic to sensitive aquatic fauna, especially in regions where large amounts of pyritic minerals are associated with the coal seams (Kennedy *et al.* in press). Traditional bioassessment of a watershed alone, however, does not provide a sound causal relationship between any one potential stressor and biotic impairment, especially when the stressor is poorly studied and consequently lacks WQC.

Anthropogenic environmental impacts in the Leading Creek Watershed (LCW), Meigs County, Ohio, have been studied by a number of researchers (Cherry *et al.*, 1999, Currie, 1999; Latimer, 1999; Kennedy *et al.*, in press). During the 2000 and 2001 field seasons, the discharge from Meigs Mine #31, an underground long-wall coal mine and preparation facility, had a mean specific conductivity (SC) of 8109 (7750-8750) $\mu\text{S}/\text{cm}$. This effluent was characterized by high SO_4^{2-} (3672 mg/L), Na^+ (1952 mg/L), chlorides (792 mg/L), calcium (238 mg/L) and water hardness (792 ± 43 mg/L as CaCO_3) concentrations, circum-neutral pH (8.10 ± 0.12) and low metals concentrations (Kennedy *et al.*, in press). The elevated SC, traceable throughout the lower reaches of the LCW, was the result of wastewater treatment with basic reagents (e.g., NaOH, CaO) intended to neutralize acidity created by the oxidation of the reduced sulfur (S^{2-}) present in the coal and adjacent rock strata. In addition to neutralizing the pH of acidic discharges, coal mining and processing facilities are also required to reduce concentrations of Fe and Mn to 3.5 and 2.0 mg/L, respectively (Zipper, 2000). The Mn-regulatory requirement was instituted due to the difficulty associated with removing Mn from solution, using the

logic that these efforts would ensure removal of other more toxic acid soluble metals, such as Al, Cu, Zn and Fe (Kleinmann, 1988). The resulting effluent is commonly high in TDS, dominated by the cations added as alkaline reagents (Na^+ and/or Ca^{2+}) and the anions, originally sourced in the coal and mineral strata, produced by the reactions resulting from the oxidation of reduced S^{2-} and subsequent treatment (SO_4^{2-} , Cl^-). Kennedy *et al.* (in press) found significant biotic impairment to the *in situ* growth of *Corbicula fluminea*, EPT-Hydropsychidae richness, and relative Ephemeroptera abundance below the effluent raceway discharging into Parker Run, a tributary of Leading Creek. Additionally, they found TDS related acute toxicity ($\text{LC}_{50} = 6,713 \pm 99 \mu\text{S/cm}$) and chronic reproductive impairment ($\text{LOAEC} = 3710 \pm 46 \mu\text{S/cm}$) in tests using *Ceriodaphnia dubia*.

Inferential uncertainty caused by other stressors in the LCW, such as untreated acid mine drainage (AMD) from abandoned mined land (AML), poor agricultural practices and potential sediment toxicity from two emergency dewatering events of Mine #31 in 1993 and 1997 (Cherry *et al.*, 1999), confounded direct linkage between field-observed biotic impairment and the treated mine effluent. Therefore, a weight of evidence approach to better establish stressor/impairment relationships (US EPA 2000) and testing in laboratory manipulated media, were applied to investigate the potential relationships between TDS and biotic impairment. The objectives of this study were to determine (1) cause for the relationship between the coal-mine effluent and biotic impairment observed in previous study, (2) the contributions of the dominant dissolved constituents to the toxicity of the mine effluent, and (3) the influence of hardness on TDS

toxicity, in response to the discretion of wastewater-treatment managers to use Na^+ or Ca^{2+} basic reagents (Skousen *et al.*, 2000).

3.2 MATERIALS AND METHODS

3.2.1 Study sites

Fourteen study sites (Fig. 3.1), were selected in the Leading Creek Watershed (LCW) to assess the impact of the Meigs Mine #31 effluent. The tributaries, Ogden Run (OR) and Parker Run (PR), were the receiving systems for mine effluents and the primary sources of highly conductive waters entering the Leading Creek Mainstem (LCM). Five sites were considered reference stations (LCRS1, LCRS2, LCRS3, PR1, LPR1) and eight served to assess impact below the surface mine effluent received by OR (OR1, LCS4), and the underground mine effluent raceway (PR3) immediately received by PR (PR2, PR4, PR5, LCS6, LCS7, LCS9).

3.2.2 Physicochemical parameters

All water chemistry analyses were performed in the field or the laboratory within 24 hours of collection. Conductivity and dissolved oxygen (DO_2) were measured in the field using an Orion[®] model 122 meter and a Yellow Springs (RDP, Dayton, OH, USA) model 54A meter, respectively. The pH was determined with an Accumet[®] (Fisher Scientific, Pittsburgh, PA, USA) meter equipped with an Accumet[®] gel-filled combination electrode (accuracy $< \pm 0.05$ pH @ 25°C). Alkalinity and hardness (mg/L as CaCO_3) were determined titrimetrically (APHA, 1995). Flow measurements (m/s) were taken with a Flo-mate[™] model 2000 portable flowmeter (Marsh-McBirney Inc., Frederick, MD) and discharge was calculated in millions of gallons per day (MGD). Mean quantitative sediment depth was determined by dropping a five pound weight three times from a set distance onto a ~1 cm diameter bar, with three replicates per site. The

SC, DO₂, and pH of out-water, with the addition of alkalinity and hardness for in-water, were measured at test initiation and termination for acute toxicity tests and daily for chronic tests.

3.2.3 Dissolved ion analysis

Samples were filtered and submitted to the Virginia Tech Department of Environmental Engineering to determine dissolved anion concentrations via ion chromatography. Cations were analyzed by inductively coupled plasma (ICP) spectrometry at the Virginia Tech Soil Testing Laboratory (APHA 1995). Ions analyzed were Al, Ca, Cl, Cr, Cu, Fe, K, Mg, Mn, Na, NO₃, P, SO₄ and Zn. Total dissolved solids (TDS) were determined as described in APHA (1995), with slight modifications. Samples were heated in a Fisher Isotemp oven[®] (100 series model 1266) at 80°C, to prevent boiling over, prior to a 1 h increase to 180°C.

3.2.4 Habitat assessment

Habitat assessments were conducted on July 5th, 2001, using U.S. EPA Rapid Bioassessment Protocols (RBP) for use in Streams and Wadeable Rivers (EPA 841-B-99-002) and on July 1st, 2002, using the Qualitative Habitat Evaluation Index (QHEI) recommended by the Ohio EPA (OH EPA 1989). These two protocols were used to minimize subjectivity and to apply approaches with both a national and local scope to determine if habitat quality contributed significantly to biotic impairment.

3.2.5 Sediment toxicity tests

Sediment was collected from LCW sites from approximately the top 5 cm of bottom substrate, passed through a sieve (US #10, 2mm) to remove large debris, and stored in plastic zipper bags at 4°C. Sediment toxicity testing used *Daphnia magna*, cultured according ASTM (E 1706-95b), in bioassay chambers (50 mL beakers)

containing sediment (20% of total volume) and water collected from Sinking Creek, an unpolluted reference stream near Newport, VA. After sediment was loaded into bioassay chambers, it was allowed to settle for 24 h so suspended particulates would not clog the filtering apparatus of test organisms. Testing involved eight replicates per site, each containing one test organism, with 10 days of exposure to the sediments at $25.0 \pm 1.0^\circ\text{C}$, using the endpoints survivorship and mean reproduction (ASTM, E 1706-95b). Daily water renewal (~80%) was performed carefully to minimize stirring the sediment. Feeding with 0.36 mL/30mL of a 1:1 mixture of *Selenastrum capricornutum* and YCT (yeast, cerophyll and trout chow) was performed daily following renewal.

3.2.6 Establishing causal relationships

Stressors that potentially confounded establishing a causal relationship between the coal-mine effluent and biotic impairment were defined (Fig. 3.2) and investigated. In addition, guidelines for establishing causality through strength of evidence analysis were followed according to the U.S. EPA Stressor Identification Guidance Document (2000), which is summarized by Suter *et al.* (2002), with direct application to the LCW (Table 3.1).

3.2.7 Laboratory formulated effluents

A simulated effluent (SE) was formulated representing the total ion concentrations in the Meigs Mine #31 effluent (ME#31) sample taken on November 24th, 2000. This testing solution served to (1) isolate known inorganic components from unknown constituents/confounders and (2) provide a consistent and easily replicable medium for testing. To assure that our analysis of the ME#31 was inclusive of the majority of ions in solution, the meq/L of cations and anions were compared, as in Tietge *et al* (1997). The SE was formulated using reagent grade $\text{Al}_2(\text{SO}_4)_3$, CaCl_2 , $\text{Fe}_2(\text{SO}_4)_3$,

MgSO₄, MnSO₄, KBr, KCl, NaCl and Na₂SO₄. Mock solutions of the SE served to isolate the components that were most important to determining the toxicity of the ME#31 by comparing toxic acute and chronic endpoints. Based on the relevant ionic concentrations of the ME#31, Mock Solution I (MS-I) was formulated with three salts (Na₂SO₄, CaSO₄, MgSO₄) and Mock Solution II (MS-II) consisted only of Na₂SO₄ to determine which ions were most predictive of the ME#31 toxicity and to eliminate those that were less crucial. For all testing media, salts were dissolved in moderately hard, reconstituted (EPA¹⁰⁰) water (EPA, 1994), with consideration to ion concentrations already in the solvent and the adjustments for hydrated salts. The nominal concentrations (meq/L) of the SE and mock solutions were calculated and determined to be equal for cations and anions.

3.2.8 Water hardness study

To determine the role of water hardness on Na₂SO₄ toxicity, a series of 10 solutions of increasing hardness, ranging from 83 to 834 mg/L as CaCO₃, were formulated (Table 3.2a). The proportions of CaSO₄ and MgSO₄ used to adjust the hardness of each solution were based on the set ratio of Ca²⁺ and Mg²⁺ ($[Ca^{2+}] / [Mg^{2+}] = 4.4$) derived from their relative concentrations in the coal-mine effluent. The hardness series was used in two different matrices of 48-h toxicity tests, to focus on the acutely toxic nature of the effluent. Matrix I consisted of the 10 solutions of the hardness series, each adjusted to a SC level of ~8900 μS/cm with Na₂SO₄. For Matrix II, however, a set amount of Na₂SO₄ (6 g/L) was dissolved in each of the 10 solutions of the hardness series, resulting in slightly variable SC levels (8120 – 8770 μS/cm).

Utilizing the LC₅₀ values acquired from 48-h tests with *C. dubia*, the 20 data points acquired using these two matrices were applied to illustrate that the relationship

between hardness and TDS toxicity was not a function of methodology and to develop a model predicting Na₂SO₄ dominated toxicity. This model was applied to all acute toxicity testing conducted in this study and was considered adequately predictive if the model LC₅₀ was within the 95% confidence intervals of the observed LC₅₀, as in Tietge *et al.* (1997). In addition, conservative, safe, acute, standards were derived by plotting the treatment levels in which ≥85% survivorship was observed in all tests from both matrices against hardness.

3.2.9 Water column toxicity testing

All tests used <24-h old *Ceriodaphnia dubia*, cultured at Virginia Tech according to EPA (1994), and were conducted in 50 mL beakers at 25.0 ± 1.0°C utilizing EPA¹⁰⁰ water for controls and 0.25 serial dilutions. The acute toxicity of the ME#31, SE, MS-I and MS-II were determined by 48-h tests, conducted as described in US EPA (1993). Chronic toxicity tests were static, renewed daily, and conducted for 7-days according to US EPA (1994), and test organisms were fed daily with 0.36 mL/30 mL of a 1:1 mixture of *S. capricornutum* and YCT. *Ceriodaphnia* was selected for testing because it was more sensitive, and thus more protective of aquatic life, than other U.S. EPA recommended test organisms (i.e., *Daphnia magna*, *Pimephales promelas*) to salt solutions in other studies (Cowgill and Milazzo, 1991; Masnado *et al.*, 1995; Dickerson *et al.*, 1996; Mount *et al.*, 1997; Kennedy *et al.*, in press).

3.2.10 Statistical analysis

Toxicity tests were analyzed using TOXSTAT[®] (Gulley 1996), with application of the appropriate parametric (Dunnett's Test, T-test with Bonferroni Adjustment) or nonparametric (Steel's-Many One Rank Test, Wilcoxon Rank Sum with Bonferroni Adjustment) analyses ($\alpha=0.05$) to determine the no observable adverse effect

concentration (NOAEC), lowest observable adverse effect concentration (LOAEC), lethal concentration at which 50% mortality was observed (LC₅₀) and the inhibition concentration at which reproduction was inhibited by 25% (IC₂₅). Test selection was performed according to the flow charts provided in EPA (1994). Pearson and Spearman Rank correlation analyses were conducted using JMP IN[®] (Sall and Lehman, 1996) and analyses using chronic *Ceriodaphnia* reproduction data excluded controls and treatment levels above 7000 µS/cm, where complete mortality of test organisms skewed the linear relationship.

3.3 RESULTS

3.3.1 Physicochemical parameters

A detailed water chemistry analysis for LCW sites during the entire study period was provided in Kennedy *et al.* (in press). Conductivity was the only parameter that substantially varied among LCW study sites, while DO₂, pH and temperature remained relatively consistent (Table 3.3). The mine effluent contributed a substantial portion of total discharge (MGD) in both PR and LCM (Table 3.3). The effluent contributed 63-70% of the water at PR4 and 10-52% of the water at LCS6, although this dilution underestimated SC levels. Quantitative sediment depth measurements indicated that LPR1, PR5 and LCS9 were subject to substantial deposition. The pH (7.74 - 8.17), and DO₂ (7.0 – 8.1 mg/L) for laboratory toxicity tests with *Ceriodaphnia* did not vary significantly between treatment levels and were adequate for the persistence of aquatic life.

SC values were used in this study as a surrogate for TDS due to their close linear correlations at LCW sites (n = 28, r = 0.9991, p < 0.0001), the second chronic water column toxicity test in the coal-mine effluent (n = 6, r = 0.9993, p < 0.0001) and the first

chronic water column toxicity test in the SE ($n = 7$, $r = 0.9990$, $p < 0.0001$). Reported TDS levels were extrapolated from SC values using the linear equation derived from the aggregation of data from these correlation analyses ($n = 41$, $r = 0.9987$, $p < 0.0001$):

$$\text{TDS (mg/L)} = 0.7435 * (\text{SC} - 50.2855) \quad (1)$$

3.3.2 Biotic response

Data for metrics of biotic impairment from Kennedy *et al.* (in press) are summarized in Fig. 3.3. Percent EPT did not respond to the mine effluent induced increase in SC, while %EPT-Hydropsychidae (%EPT-H) and %Ephemeroptera (%E) were significantly impaired at impacted sites; EPT-H taxa were completely eliminated from PR impacted sites, with slight recovery at LC impacted sites, while Ephemeroptera were eliminated from all impacted LCW sites. Three-month *Corbicula* growth showed similar impairment in PR impacted sites, but recovered more rapidly in the LCM. The potential stressors in Fig. 3.2 (i.e., SC, sediment toxicity, habitat quality, DO₂, temperature, current velocity) were correlated (r) with metrics of biotic impairment (Table 3.3). Conductivity was the only appropriate parameter to consistently show significant relationships with the metrics of biotic impairment in both 2000 and 2001. An inverse relationship between biotic impairment with both current velocity and temperature was observed in 2001, although these factors were also linked to the effluent induced increase in discharge and settling pond induced increase in temperature. In 2000, SC was the only parameter significantly related to metrics of biotic impairment.

3.3.3 Habitat assessment

The RBP and QHEI habitat scores were significantly correlated ($r = 0.5722$, $p = 0.0325$) for LCW sites and data are provided in Table 3.3. These scores were not consistently significantly correlated ($\alpha = 0.05$) with the 3 metrics of biotic impairment in

Table 3.3. All impacted sites, except for LCS9, had scores at least as high as the reference site, LCRS2. Habitat scores for both assessments were noticeably depressed for the impacted sites, PR5 and LCS9.

3.3.4 Sediment toxicity tests

Mean *Daphnia* survivorship and reproduction were not statistically significantly different among LCW sites, with the exception of OR1, LCS4 and PR5. Mean survivorship at OR1 and LCS4 and mean reproduction for PR5 were substantially reduced compared to other study sites (Table 3.3). Overall, sediment toxicity was not observed for most impacted sites associated below Mine #31 (i.e., PR2, PR3, PR4, LCS6, LCS7, LCS9) and was not related to metrics of biotic impairment (Table 3.3).

3.3.5 Causality considerations

Each of the 12 causal considerations given in US EPA (2000) strengthened the argument for the mine effluent being the primary cause of the biotic impairment observed in LCW (Table 3.1), although complete field data sets were not available for temporality, consistency of association, analogy, consistency of evidence and coherence of evidence due to the lack of related studies. Overall, the potential stressors illustrated in Fig. 3.2 were much less likely candidates in terms of causing biotic impairment than the mine effluent.

3.3.6 Laboratory formulated solutions

The charge balance of the ion concentrations measured in the ME#31 resulted in 101.92 meq/L for cations and 98.79 meq/L for anions, translating to 3.07% more cations. The nominal concentrations of testing media were measured, and are provided in Table 3.4a. Based on percentage differences, the ion concentrations in the SE were representative of those in the ME#31, with SC (1.8%), hardness (6.5%), SO_4^{2-} (9.0%),

Na⁺ (2.6%), Cl⁻ (9.6%), Ca²⁺ (14.9%), Mg²⁺ (16.8%) and K⁺ (16.1%) all within 20% of the ME#31 concentrations. The measured concentrations for Al, Cu and Zn were higher while Fe and Mn were lower than in the ME#31. Except for Al, all reported metal concentrations were low enough to avoid concern in regard to both acute and chronic standards (EPA 1999). Aluminum concentrations in the ME#31 were above chronic WQC and greatly over-represented in the SE. For MS-I, the SO₄²⁻ concentration was higher because Na₂SO₄ was used to elevate the SC to that of the ME#31 and SE to compensate for excluded ions. The amount of Na₂SO₄ used for MS-II elevated the SO₄²⁻ concentration to that of the SE, and the Na⁺ concentration was calculated from the measured SO₄²⁻ concentration.

3.3.7 Water hardness study

Hardness was significantly logarithmically correlated with 48-h *Ceriodaphnia* LC₅₀ values using both Matrix I (r = 0.9603, p < 0.0001) and Matrix II (r = 0.9662, p < 0.0001), indicating hardness significantly ameliorated Na₂SO₄ toxicity (Fig. 3.4). The correlation of the aggregated data from both matrices was also significant for 48-h LC₅₀ values (r = 0.9574, p < 0.0001) and 48-h NOAEC endpoints (r = 0.7840, p < 0.0001) plotted against hardness, and the following equations were derived to predict Na₂SO₄ toxicity:

$$LC_{50} (\mu\text{S/cm}) = 1088.8 * \text{Ln} (\text{hardness}) + 198.3 \quad (2)$$

$$\text{Safe acute limit} (\mu\text{S/cm}) = 935.84 * \text{Ln} (\text{hardness}) + 228.06 \quad (3)$$

Using these equations, the LC₅₀ and conservative safe acute limits can be determined in terms of SC (μS/cm), using water hardness (mg/L as CaCO₃) as the independent variable. Using Matrix II due to its constant Na₂SO₄ concentration (6 g/L), the amount of toxicity

alleviated by hardness was observed to diminish considerably beyond a $\text{Ca}^{2+}:\text{Na}^+$ ratio of 0.05 (Table 3.2).

3.3.8 Water column toxicity testing

The mean 48-h *Ceriodaphnia* LC_{50} values were similar for the ME#31 and SE, which were predicted reasonably well by the Na_2SO_4 -hardness model (Table 3.2b). The model, however, under-predicted the actual ME#31 LC_{50} value by ~10%. Mean 7 d survivorship was consistently a much less sensitive and more variable endpoint than was 7 d reproduction in all testing-media, and thus was not reported. The 7 d NOAEC endpoints for mean reproduction obtained for the two tests in the ME#31 were 2910 ± 40 $\mu\text{S}/\text{cm}$ and 2553 ± 21 $\mu\text{S}/\text{cm}$ and the 7 d LOAEC endpoints were 3710 ± 46 $\mu\text{S}/\text{cm}$ and 3254 ± 26 $\mu\text{S}/\text{cm}$ (Table 3.4). These endpoints were similar to the NOAEC (2597 ± 31 $\mu\text{S}/\text{cm}$) and LOAEC (3331 ± 19 $\mu\text{S}/\text{cm}$) for the SE. The NOAEC and LOAEC endpoints for MS-I were 2641 ± 17 and 4269 ± 60 , respectively. Additionally, similar IC_{25} values were obtained for the tests with the ME#31 (3154 [1987 – 3578]; 3131 [2160 – 3877] $\mu\text{S}/\text{cm}$), SE (3177 [2194 – 3961] $\mu\text{S}/\text{cm}$) and MS-I (3254 [2988 – 3471] $\mu\text{S}/\text{cm}$).

Overall, the endpoints using MS-I were in accordance with those of the ME#31 and the SE. The aggregation of 7-d reproduction data from all these tests resulted in a significant negative correlation with SC ($r = -0.9719$, $p < 0.0001$). Separate correlation analysis of all four media (ME#31, SE, MS-I, MS-II) resulted in statistically similar linear equations and r-values (Fig. 3.5). The MS-II test (hardness = 88 mg/L as CaCO_3) resulted in substantially lower NOAEC (1084 ± 8 $\mu\text{S}/\text{cm}$), LOAEC (2103 ± 18 $\mu\text{S}/\text{cm}$) and IC_{25} (1355 $\mu\text{S}/\text{cm}$) endpoints for reproduction than observed in all other testing media (Fig.

3.5), with 0.4 ± 0.8 neonates produced at 3384 ± 31 $\mu\text{S}/\text{cm}$, and 0% survivorship at $\geq 4354 \pm 24$ $\mu\text{S}/\text{cm}$.

3.4 DISCUSSION

There are few published studies addressing the ecological impacts of high TDS discharges from active coal-mining and preparation facilities (Tietge *et al.* 1997, Chapman *et al.*, 2000; Goodfellow *et al.*, 2000; Kennedy *et al.*, in press) and no nationally recognized standards for $\text{Na}^+/\text{SO}_4^{2-}$ dominated TDS concentrations in freshwater systems. Although TDS toxicity depends on site-specific ionic composition (Mount *et al.*, 1997; Goodfellow *et al.*, 2000), the diversity of $\text{Na}^+/\text{SO}_4^{2-}$ -rich waters, including treated coal-mine effluents (Kennedy *et al.*, in press), saline lakes (Burnham and Peterka, 1975; Koel and Peterka, 1995), irrigation drainage water (Hart *et al.*, 1991; Ingersoll *et al.*, 1992; Dickerson *et al.*, 1996) and oil/gas extraction fluids (Goodfellow *et al.*, 2000), suggest a guidance for $\text{Na}^+/\text{SO}_4^{2-}$ dominated TDS is warranted.

The proximity of the biotic impairment described in Kennedy *et al.* (in press), causal considerations for strength of evidence analysis (Table 3.1), and linear correlation analyses between potential stressors and metrics (Table 3.3), indicated that the coal-mine effluent was the primary stressor in the receiving study-reach. Untreated AMD was not a concern due to the location of AML in the LCW (Cherry *et al.*, 1999), circum-neutral pH, and low WQC metals concentrations at study sites (Kennedy *et al.*, in press). Habitat scores and DO_2 levels at LCW impacted sites were comparable to reference sites and not significantly correlated with metrics of biotic impairment (Table 3.3). The combination of poor habitat scores and severe sediment deposition at PR5 and LCS9, however, likely contributed to reductions in EPT-H and %E indices. The habitat assessments differed

somewhat because RBP stressed the amount of water filling the channel and sediment deposition, while QHEI stressed maximum depth and site-gradient. Sediment toxicity endpoints and sediment depth measurements also were not related with metrics of biotic impairment. The increased current velocity and temperature conditions at impacted sites were induced by the effluent/settling pond, and thus co-varied with TDS and biotic impairment. These factors were from the same source and therefore did not confound linkage between impairment and the effluent. It is unlikely that current and temperature contributed significantly to the impairment; biotic impairment was not observed at LCRS1 and LPR1, where flow conditions were low, or at PR2, where temperature was among the highest of LCW sites ($24.0 \pm 3.5^{\circ}\text{C}$). Overall, using SC as a conservative tracer and the related acutely toxic nature of the effluent, validated by *Ceriodaphnia* testing, other stressors that may normally induce ecological degradation were at most secondary sources of impairment in this study.

The laboratory formulated SE and mock solutions were representative of the field-collected mine effluent in terms of relevant ionic composition, SC, acute toxicity (Table 3.2b) and chronic toxicity (Table 3.4). The concentrations of the most abundant ions were within 20% of the ME#31, with Na^+ and SO_4^{2-} concentrations as the primary constituents elevating TDS. The measured Al concentrations in the SE over-represented the Al in the ME#31, although the similar endpoints obtained in *Ceriodaphnia* testing in these media suggested that Al was not a primary contributor to the toxicity observed in the LCW.

Water hardness clearly ameliorated $\text{Na}^+/\text{SO}_4^{2-}$ dominated TDS toxicity. The amelioration of metal toxicity by hardness has been established, as described in Black *et*

al. (1980) and observed in other studies (Masnado *et al.*, 1995; Jackson *et al.*, 2000). These findings further diminished the probability that the relatively low concentrations of WQC metals caused biotic impairment at LCW sites. The effects of hardness on $\text{Na}^+/\text{SO}_4^{2-}$ dominated TDS toxicity, however, have not been as clearly documented. Our results indicated a strong inverse relationship between Na_2SO_4 toxicity and hardness ($r = 0.9574$, $p < 0.0001$), with substantially reduced acute toxicity observed at a hardness of 834 mg/L as CaCO_3 ($\text{LC}_{50} = 7535 \mu\text{S}/\text{cm}$) compared to 83 mg/L ($\text{LC}_{50} = 4827 \mu\text{S}/\text{cm}$). This alleviation of acute toxicity was not substantial beyond a $\text{Ca}^{2+}:\text{Na}^+$ ratio of $\sim 1:20$, although McWilliams (1983) suggested a ratio as high as 1:2.5. Mount *et al.* (1997) stated amelioration was not necessarily due to hardness alone, but the number and abundance of multiple cations in solution. Hardness also ameliorated chronic toxicity, illustrated by substantially lower 7-d *Ceriodaphnia* endpoints for MS-II (Fig. 3.5). Similarly, the lethal effects of 5 g NaCl to striped bass were ameliorated with 100 mg/L Ca^{2+} (Grizzle *et al.*, 1990), and a significant increase in *D. magna* and *C. dubia* fecundity was observed at a hardness of 170 ± 15 mg/L (Leblanc and Surprenant, 1984). Although this mechanism is poorly understood (Black, 1980; Grizzle *et al.*, 1990), the gills of some fish species become more permeable to Na^+ , Cl^- , H^+ and water in Ca^{2+} -poor solution (McWilliams and Potts, 1978; Pic and Maetz, 1981); divalent ions, such as Ca^{2+} , likely decrease membrane permeability (Potts and Fleming, 1970). Mechanisms for amelioration could be complexation of SO_4^{2-} in the form of CaSO_4 and/or competition between Ca^{2+} and Na^+ (Jackson *et al.*, 2000).

The majority of the acute toxicity of the mine effluent could be attributed to its two most abundant ions, Na^+ and SO_4^{2-} . Not only were all metals and Cl^- concentrations

in the Ca^{2+} -rich effluent below acute WQC, but also the Na_2SO_4 -hardness model was reasonably predictive of acute toxicity observed for the ME#31 and SE (Table 3.2b). Although the bulk of the ME#31 toxicity could be attributed to these TDS concentrations, its slightly lower LC_{50} may indicate some unaccounted for toxicity. This discrepancy was small, within the 95% confidence intervals of the other testing media and may have been the result of temporal drift in sensitivity of test organisms (Mount *et al.*, 1997). Burnham and Peterka (1995) and Goetsch and Palmer (1997) found Na_2SO_4 to be more toxic than NaCl to *Pimephales* and *Tricorythus*, respectively. These findings suggest SO_4^{2-} and Na^+ were responsible for much of the toxicity observed in the LCW. The single salt model developed by Mount *et al.* (1997) also predicted that the toxicity of Na^+ salts could be attributed to the associated anion (e.g. SO_4^{2-} , Cl^-), although Cl^- was more toxic to *C. dubia* than SO_4^{2-} . Meyer *et al.* (1985) found acute toxicity in *D. magna* caused by raw shale leachates when SC levels exceeded 7000 $\mu\text{S}/\text{cm}$. Although their study was conducted with a more tolerant test organism in a different solution, the similarity with our LC_{50} values (6713-7831 $\mu\text{S}/\text{cm}$) suggests that waters of high hardness (≥ 500 mg/L) are generally toxic at ~ 7000 $\mu\text{S}/\text{cm}$.

The majority of the chronic toxicity of the mine effluent can also be attributed to Na^+ and SO_4^{2-} . The slight discrepancy observed between testing media in acute testing was not apparent in chronic *Ceriodaphnia* testing, with very similar NOAEC (~ 2500 $\mu\text{S}/\text{cm}$), LOAEC (~ 3200 $\mu\text{S}/\text{cm}$) and IC_{25} (~ 3200 $\mu\text{S}/\text{cm}$) endpoints observed in all high-hardness media, suggesting $\text{Na}^+/\text{SO}_4^{2-}$ dominated TDS toxicity accounted for the longer-term impairment observed in the LCW (Table 3.4). This was supported by the strong linear correlation between SC and mean reproduction ($r = 0.9719$, $p < 0.0001$) and

strengthened by the integration of 7 d *Ceriodaphnia* data from Kennedy *et al.* (in review) in a different testing solution into this correlation analysis ($r = -0.9707$, $p < 0.0001$). Given MS-I produced similar endpoints to the ME#31, our data strongly indicate that Na^+ and SO_4^{2-} were the primary components of the chronic effluent toxicity in the LCW, at high hardness (≥ 790 mg/L). Additionally, 7 d *Ceriodaphnia* impairment was observed at the ~ 3200 $\mu\text{S}/\text{cm}$ treatment level in all media, where the already low metal concentrations were diluted by 40-50%. Thus, WQC metals and Cl^- toxicity were not major contributors to chronic impairment because (1) similar endpoints were found for all high-hardness testing media, (2) high water hardness ameliorates metals toxicity, and (3) over-represented Al concentrations in the SE still resulted in similar endpoints.

Overall, this study provided strong evidence that the coal-mine effluent significantly impaired sensitive aquatic organisms and that $\text{Na}^+/\text{SO}_4^{2-}$ dominated TDS was responsible for the majority of that toxicity. Such toxicity is most likely to be problematic in regions where relatively high levels of reduced sulfur are found. For discharges of high water hardness (≥ 790 mg/L as CaCO_3), potential acute toxicity to aquatic organisms is possible where dissolved solids levels in receiving streams exceed ~ 7000 $\mu\text{S}/\text{cm}$ (5167 mg/L), with potential chronic toxicity occurring at ~ 3200 $\mu\text{S}/\text{cm}$ (2342 mg/L). At a lower water hardness (88 mg/L), acute and chronic toxicity can occur at considerably lower levels, 5100 $\mu\text{S}/\text{cm}$ (3754 mg/L) and ~ 2100 $\mu\text{S}/\text{cm}$ (1523 mg/L), respectively.

These results were generated through study of a single treated coal-mine effluent and should not be interpreted to indicate that only coal-related facilities produce saline wastewaters (Goodfellow et al, 2000) or that all mine effluents are as saline/toxic. Given

treated coal-mining discharges are widespread, however, these results warrant special concern by that industry. Mines with discharges causing such conditions should consider limiting neutralizing reagent additions, prioritizing Ca^{2+} over Na^+ bases, to the minimum levels necessary to achieve regulatory compliance.

These findings also carry implications for agencies regulating coal-mining discharges. Although numerous acid-soluble cations are commonly present in untreated mining wastewaters, only Fe and Mn are regulated, with Mn generally most difficult to remove (Kleinmann, 1988). Mining firms faced with Mn discharge concerns in some cases add reagents to elevate pH beyond the 9.0 maximum to meet the 2.0 mg/L regulatory standard, requiring subsequent addition of acidic reagents to meet the WQC for pH (6.5 - 9.0), thus further increasing TDS. This regulatory practice occurs despite recognition that Mn is not generally recognized to be toxic, has no WQC and diminishes the incentive of mining firms to use constructed wetlands (not an effective treatment for Mn removal), a method of treatment capable of removing a broad suite of acid-soluble cations without elevating TDS (Zipper, 2000). In light of evidence for TDS toxicity, these policies should be reviewed. The critical concentration levels cited in this guidance are based on study of a single effluent in southeastern Ohio, and should be applied cautiously until verified by more general studies. Future work should focus on determining the sensitivities of other organisms in high TDS solutions.

ACKNOWLEDGEMENTS

The authors thank Dr. Rebecca Currie, Matthew Hull, T. Chad Merricks, Patrick Barry and Brian Denson for their suggestions and assistance in the laboratory and during

sometimes-intensive field excursions. We also thank Dr. David Soucek and Dr. Fred Benfield for their expert advice.

3.5 LITERATURE CITED

- American Public Health Association (APHA), American Water Works Association & Water Environment Federation. 1995. Standard methods for the examination of water and waste water, 19th ed. American Public Health Association, Wash, D.C.
- American Society for Testing and Materials (ASTM) Standard methods for measuring the toxicity of sediment-associated contaminants with freshwater macroinvertebrates. Designation: E 1706 – 95b.
- Black JA, Roberts RF, Johnson DM, Minicucci DD, Mancy KH, Allen HE. 1980. The Significance of Physiological Variables in Aquatic Bioassays of Heavy Metals. Water Quality Criteria for Freshwater Fish. Butterworth, London. 259-275.
- Burnham BL, Peterka JJ. 1975. Effects of saline water from North Dakota lakes on survival of Fathead Minnow (*Pimephales promelas*) embryos and sac fry. *J Fish Res Board Can* 32: 809-12.
- Chapman PM, Bailey H, Canaria E. 2000. Toxicity of total dissolved solids associated with two mine effluents to Chironomid larvae and early life stages of rainbow trout. *Environ Toxicol Chem* 19: 210-214.
- Cherry DS, Currie RJ, Latimer HA, Cairns J Jr, Diz HR, Johnson DM, Yeager MM 1999. The Leading Creek Improvement Plan: Southern Ohio Coal Company Meigs Mine No. 31. Final report to Southern Ohio Coal Company and American Electric Power, Columbus Ohio 43216.
- Cowgill UM, Milazzo DP. 1991. The sensitivity of two cladocerans to water

- Quality variables: salinity <467 mg NaCl/L and hardness <200 mg CaCO₃/L.
Arch Environ Contam Toxicol 21: 218-223.
- Currie RJ. 1999. Identification of ecosystem stressors in developing an enhancement plan for the Leading Creek Watershed, Meigs County, Ohio. Virginia Polytechnic Institute and State University, doctoral dissertation.
- Dickerson KK, Hubert WA, Berman HL. 1996. Toxicity assessment of water from lakes and wetlands receiving irrigation drain water. *Environ Toxicol Chem* 15: 1097-1101.
- Goetsch PA, Palmer CG. 1996. Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. *Arch. Environ Contam Toxicol* 32: 32-41.
- Goodfellow WL, Ausley LL, Burton DT, Denton DL, Dorn PB, Grothe DR, Heber MA, Norberg-King TJ, Rodgers JH Jr. 2000. Major ion toxicity in effluents: a review with permitting recommendations. *Environ Toxicol Chem* 19: 175-182.
- Grizzle, JM, Mauldin II AC, Young D, Henderson E. 1990. Effects of environmental calcium and sodium on postharvest survival of juvenile striped bass. *J Aquat Anim Health* 2: 104-108.
- Gulley DD. 1996. TOXSTAT[®]. Version 3.3. University of Wyoming Department of Zoology and Physiology, Laramie, WY.
- Hart BT, Bailey P, Edwards R, Hortle K, James K, McMahon A, Meredith C Swadling K. 1991. A review of the salt sensitivity of the Australian freshwater biota. *Hydrobiologia* 210: 105-144.

- Ingersoll CG, Dwyer FJ, Burch SA, Nelson MK, Buckler DR, Hunn JB. 1992. The use of freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drain water. *Environ Toxicol Chem* 11: 503-511.
- Jackson BP, Lasier PJ, Miller WP, Winger PW. 2000. Effects of calcium, magnesium, and sodium on alleviating cadmium toxicity to *Hyalella azteca*. *Bull Environ Contam Toxicol* 64: 279-286.
- Kennedy AJ, Cherry DS, Currie RJ. in press. Field and laboratory assessment of a coal] processing effluent in the Leading Creek Watershed, Meigs Co., Ohio. *Arch Environ Contam Toxicol*: in press.
- Kennedy AJ, Cherry DS, Currie RJ. In review. Assessment of a coal-processing Effluent Impacted Lotic System Using an Ecologically Relevant Test Organism, *Isonychia bicolor*. *Environ Monit Assessment*: in review.
- Kleinmann RLP, Watzlaf GR. 1988. Should the effluent limits for manganese be modified? Paper presented at the Mine Drainage reclamation Conference sponsored by the American Society for Surface Mining and Reclamation and the U.S. Department of Interior, April 17-22, 1988, Pittsburgh, PA.
- Koel TM, Peterka JJ. 1995. Survival to hatching of fishes in sulfate-saline waters, Devils Lake, North Dakota. *Can J Fish Aquat Sci* 52: 464-469.
- Latimer HA. 1999. An ecotoxicological evaluation of active coal mining, sedimentation and acid mine drainage in three tributaries of the Leading Creek Watershed, Meigs County, Ohio. Virginia Polytechnic Institute and State University, masters thesis.

- Leblanc GA, Surprenant DC. 1984. The influence of mineral salts on fecundity of the water flea (*Daphnia magna*) and the implications on toxicity testing of industrial wastewater. *Hydrobiologia* 108: 25-31.
- Masnado RG, Geis SW, Sonzogoni WC. 1995. Comparative acute toxicity of a synthetic mine effluent to *Ceriodaphnia dubia*, larval Fathead Minnow and the Freshwater mussel *Anodonta imbecilis*. *Environ Toxicol Chem* 14: 1913-1920.
- McWilliams PG, Potts WTW. 1978. The effects of pH and calcium concentrations on gill potentials in the brown trout, *Salmo trutta*. *J Comp Physiol* 126B: 277-286.
- McWilliams PG. 1983. An investigation of the loss of bound calcium from the gills of the brown trout, *Salmo trutta*, in acid media. *Comp Biochem Physiol* 74A: 107-116.
- Meyer JS, Sanchez DA, Brookman JA, McWhorter DB, Bergman HL. 1985. Chemistry and aquatic toxicity of raw oil shale leachates from Piceance Basin, Colorado. *Environ Toxicol Chem* 4: 559-572.
- Mount DR, Gulley DD, Hockett JR, Garrison TD, Evans JM. 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (Fathead Minnows). *Environ Toxicol Chem* 16: 2009-2019.
- Ohio Environmental Protection Agency. 1989. Biological criteria for the protection of aquatic life: Volume III. Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities. Ohio Environmental Protection Agency, Columbus, Ohio, USA (revision of 1987 reference).

- Pic P, Maetz J. 1981. Role of external calcium in sodium and chloride transport in the gills of seawater-adapted *Mugil capito*. *J Comp Physiol* 141: 511-521.
- Potts WTW, Fleming WR. 1970. The effects of prolactin and divalent ions on the permeability to water of *Fundulus kansae*. *J Exp Biol* 53: 317-327.
- Sall J, Lehman A. 1996. JMP start statistics. SAS Institute. Duxbury Press, Belmont, CA.
- Skousen J, Sextone A, Ziemkiewicz P. 2000. Acid mine drainage control and treatment. p. 131-168, In: R.I. Barnhisel, R.G. Darmody, and W.L. Daniels (eds). Reclamation of Drastically Disturbed Lands. American Society of Agronomy. Madison, WI. Monograph No. 41. 1082 pp.
- Suter II GW, Norton SB, Cormier SM. 2002. A methodology for inferring the causes of observed impairments in aquatic systems. *Environ Toxicol Chem* 21: 1101-1111.
- Tietge JE, Hockett JR, Evans JM. 1997. Major ion toxicity of six produced waters to three freshwater species: application of ion toxicity models and TIE procedures. *Environ Toxicol Chem* 16: 2002-2008.
- US EPA. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed. Washington D.C. EPA/600/4-90/027F, Cincinnati, OH.
- US EPA. 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms, 3rd ed. Washington D.C. EPA-600-4-91-002, Cincinnati, OH.
- US EPA. 1999. National Recommended water quality criteria-correction. EPA-822-Z-

99-001. Office of Water, Washington, D.C.

US EPA. 2000. Stressor identification guidance document. Office of Water,
Washington, D.C. 20460. EPA-822-B-00-025.

Zipper CE. 2000. Coal mine reclamation, acid mine drainage and the Clean Water Act. p.
169-191, in: R. Barnhisel, W. Daniels and R. Darmody (eds). Reclamation of
Drastically Disturbed Lands. American Society of Agronomy. Madison WI.
Monograph 41.

Table 3.1: Causal considerations for strength of evidence analysis from EPA 822-B-00-025 applied to the Leading Creek Watershed.

CAUSALITY CONSIDERATION	GENERAL DESCRIPTION	LEADING CREEK PROJECT APPLICATION
Spatial co-occurrence	Adverse impact observed downstream but not upstream of stressor?	Mayflies observed upstream of mine effluent but absent downstream (Fig. 3.5)
Temporality	Did the cause precede the effects?	History of the Leading Creek Watershed (Cherry <i>et al.</i> , 1999)
Biological gradient	Does the effect increase with increasing exposure?	Fig. 3.3 & 3.5; Table 3.3 (regression analysis)
Complete exposure pathway	Can stressor be traced from source to receptor?	Benthic macroinvertebrate surveys, <i>Corbicula</i> tests
Consistency of association	Does the impact occur in different places and times?	Similar salt toxicity results found: Mount <i>et al.</i> (1997), Goodfellow <i>et al.</i> (2000)
Experiment	How is the impact influenced by manipulation of the medium in the laboratory?	Toxicity testing using <i>C. dubia</i> in altered testing media (Tables 3.2, 3.4 & 3.5)
Plausibility	To what degree is cause & effect relationship expected? Is mechanism & stressor response plausible?	Stressor-response provided by laboratory toxicity testing; mechanism of toxicity is desiccation
Analogy	Is the given case similar to the cause and effect of well-established cases?	Radford & Graveland (1978), Mount <i>et al.</i> (1997)
Specificity	Is the proposed cause the only cause of the effect in question?	See flow chart (Fig. 3.2)
Predictive performance	Is magnitude of cause predictive of effect severity	Correlation between conductivity levels & biotic impairment (Table 3.3)
Consistency of evidence	Is hypothesized relationship between cause & effect consistent with evidence?	Kennedy <i>et al.</i> (in press)
Coherence of evidence	Can model explain inconsistencies along between cause & effect?	Linear regression models (this study)

Table 3.2: Matrices and associated hardness series and specific conductivity (SC) levels used in the hardness study to generate the Na₂SO₄-hardness model (a), and mean 48-h LC₅₀ values for the mine effluent, simulated effluent and mock solutions, with 95% confidence intervals provided in parentheses, and the predicted LC₅₀ generated by the Na₂SO₄-hardness model.

(a)

		Hardness Series									
		A	B	C	D	E	F	G	H	I	J
Matrix I	Hardness (mg/L)	83	110	140	206	318	506	600	688	752	834
	SC (μS/cm)	8813	8890	8900	8880	8920	8860	8950	8980	8930	8880
	LC ₅₀ (μS/cm)	4776 (4668-4887)	5760 (5463-6073)	6047 (5844-6258)	6190 (No limits)	6159 (6011-6310)	6829 (6416-7269)	7256 (6610-7964)	7527 (7083-7998)	7339 (6983-7715)	7535 (7208-7876)
Matrix II	Hardness (mg/L)	88	116	148	210	330	516	630	702	748	824
	SC (μS/cm)	8120	8170	8130	8240	8380	8550	8620	8730	8690	8770
	LC ₅₀ (μS/cm)	4569 (4351-4797)	5636 (No Limits)	5606 (5410-5810)	5828 (5457-6224)	6265 (5857-6701)	6728 (6319-7163)	7311 (6989-7649)	7095 (6644-7576)	7831 (7603-8066)	7504 (7026-8015)
	Nominal Ca ²⁺ :Na ⁺ Ratio	0.010	0.014	0.016	0.020	0.026	0.035	0.040	0.044	0.046	0.050

(b)

Medium	n	Mean Hardness (mg/L)	Mean LC ₅₀ (μS/cm)	
			Actual	Na ₂ SO ₄ Model
Mine effluent	3	776 ± 23	6713 (6441 - 6933)	7443
	3	835 ± 13	7109 (6721 - 7805)	7523
Simulated Effluent	3	185 ± 1	5784 (5350 - 6096)	5882

Table 3.3: Physiochemical parameters for Leading Creek Watershed sites and the Spearman correlation (r) between these parameters and metrics of biotic impairment from Kennedy *et al.* (in press). Bold r -values represent relationships significant at the $\alpha \leq 0.05$ level.

Site	Mean Conductivity ($\mu\text{S}/\text{cm}$)		DO ₂ (mg/L)	Temp (°C)	Habitat		Depth (m)	Sediment		Flow		
	2000	2001			RBP	QHEI		<i>Daphnia</i>		Current Velocity (m/s)	Discharge (MGD)	
								Survival	Fecundity			
LCRS1	553 ± 150	417 ± 99	6.9 ± 1.0	20.8 ± 4.9	108	72	0.10 ± 0.03	100 ± 0	20.3 ± 11.4	0.11	0.600	
OR1	4745 ± 559	2676 ± 1572	9.0 ± 1.0	21.6 ± 4.9	78	54	0.07 ± 0.01	50 ± 58	18.3 ± 11.4	0.33	1.257	
LCS4	1200 ± 594	600 ± 246	7.0 ± 1.1	21.8 ± 4.4	54	81	0.12 ± 0.06	63 ± 52	21.9 ± 5.9	0.05	5.273	
LCRS2	800 ± 250	406 ± 85	6.5 ± 0.8	20.5 ± 3.9	71	50	0.07 ± 0.02	88 ± 35	22.3 ± 6.8	0.42	11.359	
LCRS3	777 ± 244	381 ± 113	6.0 ± 1.5	20.9 ± 3.3	87	64	0.07 ± 0.01	88 ± 35	18.9 ± 11.6	0.33	11.032	
LPR1	n/a	399 ± 72	7.1 ± 3.1	20.9 ± 4.4	123	81	0.20 ± 0.06	100 ± 0	19.4 ± 3.5	0.00	0.022	
PR1	n/a	457 ± 172	6.3 ± 3.5	21.1 ± 3.7	103	74	n/a	88 ± 35	17.9 ± 4.7	0.09	0.124	
PR2	3433 ± 260	1890 ± 1080	11.9 ± 1.1	24.0 ± 3.5	110	71	0.06 ± 0.01	88 ± 35	20.6 ± 6.7	0.06	0.329	
PR3	8547 ± 235	7803 ± 115	7.7 ± 0.9	25.2 ± 3.8	113	60	0.06 ± 0.02	100 ± 0	20.3 ± 4.3	0.58	8.496	
PR4	8373 ± 283	7668 ± 186	8.0 ± 0.7	24.2 ± 3.6	103	69	0.08 ± 0.01	100 ± 0	22.0 ± 3.7	0.55	13.477	
PR5	8243 ± 369	7665 ± 361	7.7 ± 0.9	23.1 ± 3.3	71	55	0.14 ± 0.13	88 ± 35	13.0 ± 4.1	0.62	n/a	
LCS6	7373 ± 657	4913 ± 2415	7.2 ± 0.8	22.7 ± 4.0	90	64	0.07 ± 0.02	88 ± 35	36.0 ± 6.9	0.35	16.410	
LCS7	5367 ± 3557	4263 ± 2405	7.7 ± 0.5	22.6 ± 3.8	102	78	0.07 ± 0.05	86 ± 38	36.1 ± 10.1	0.28	18.302	
LCS9	4560 ± 2446	4118 ± 1721	9.0 ± 1.1	22.6 ± 3.8	34	45	0.32 ± 0.03	86 ± 38	25.1 ± 17.3	0.24	24.178	
Metric	Linear Regressions											
Percent Mayflies [‡]	r	-0.9303	-0.8315	-0.2646	-0.6217	-0.2134	0.3794	0.1734	-0.0077	-0.3088	-0.8139	-0.7869
	p	<0.0001	0.0002	0.3607	0.0176	0.4639	0.1810	0.5710	0.9790	0.2827	0.0004	0.0014
EPT-H Richness [‡]	r	-0.9129	-0.8321	-0.4493	-0.7916	0.1546	0.5043	0.1888	-0.0714	-0.2712	-0.8102	-0.6866
	p	<0.0001	0.0002	0.1070	0.0007	0.5977	0.0659	0.5368	0.8085	0.3483	0.0004	0.0095
<i>Corbicula</i> Growth [‡]	r	-0.4813	-0.6867	-0.1629	-0.5217	-0.2482	0.2129	-0.1082	-0.4612	-0.1063	-0.6468	-0.3449
	p	0.1339	0.0136	0.6130	0.0819	0.4367	0.5064	0.7378	0.1312	0.7424	0.0230	0.2989

[‡] Data are from Kennedy *et al.* (in press)

Table 3.4: Ion concentrations (a) and mean reproduction for 7-d *Ceriodaphnia* tests (b) for testing media. Each test was analyzed separately and boldface treatments with asterisks indicate a significant difference ($\alpha < 0.05$) from the control.

(a)

Parameter	Mine effluent	Simulated Effluent	Mock Solution I	Mock Solution II
Conductivity	8750	8910	8920	7150
TDS	~6468	~6587	~6595	~5279
Hardness	770	820	810	88
SO ₄	3672	3343	4596	3268
Na	1952	1901	2038	~1564
Cl	792	868	n/a	n/a
Ca	237.1	272.4	261.4	n/a
Mg	53.70	44.67	43.13	n/a
K	25.08	29.11	n/a	n/a
Fe	0.4394	0.2162	n/a	n/a
Mn	0.4074	0.3626	n/a	n/a
Al	0.2085	0.728	n/a	n/a
Cu	0.0033	0.0110	n/a	n/a
Zn	0.0045	0.0363	n/a	n/a

(b)

Mine effluent Test 1		Mine effluent Test 2		Simulated Effluent		Mock Solution I	
Conductivity ($\mu\text{S/cm}$)	Mean Neonates						
290 ± 11	34.1 ± 9.6	294 ± 8	40.4 ± 9.3	301 ± 11	33.2 ± 12.8	291 ± 7	35.9 ± 4.8
1780 ± 40	40.5 ± 13.0					1699 ± 50	37.4 ± 4.6
2300 ± 38	35.3 ± 2.9	2553 ± 21	33.6 ± 11.0	2597 ± 31	28.3 ± 4.9	2641 ± 17	32.2 ± 3.5
2910 ± 40	31.0 ± 7.8	3254 ± 26	29.6 ± 7.5*	3331 ± 19	24.0 ± 2.2*	3356 ± 25	26.7 ± 3.4*
3710 ± 46	22.3 ± 4.8*	4146 ± 35	24.2 ± 5.4*	4261 ± 25	17.5 ± 5.1*	4269 ± 60	17.8 ± 8.2*
4730 ± 45	5.5 ± 4.9*	5304 ± 44	9.2 ± 4.2*	5441 ± 20	7.8 ± 3.8*	5450 ± 29	3.2 ± 2.0*
6040 ± 62	0.3 ± 0.7*	6767 ± 32	0.0 ± 0.0*	6974 ± 24	0.2 ± 0.4*	6970 ± 14	0.0 ± 0.0*
7750 ± 44	0.0 ± 0.0*			8950 ± 25	0.0 ± 0.0*	8880 ± 0	0.0 ± 0.0*

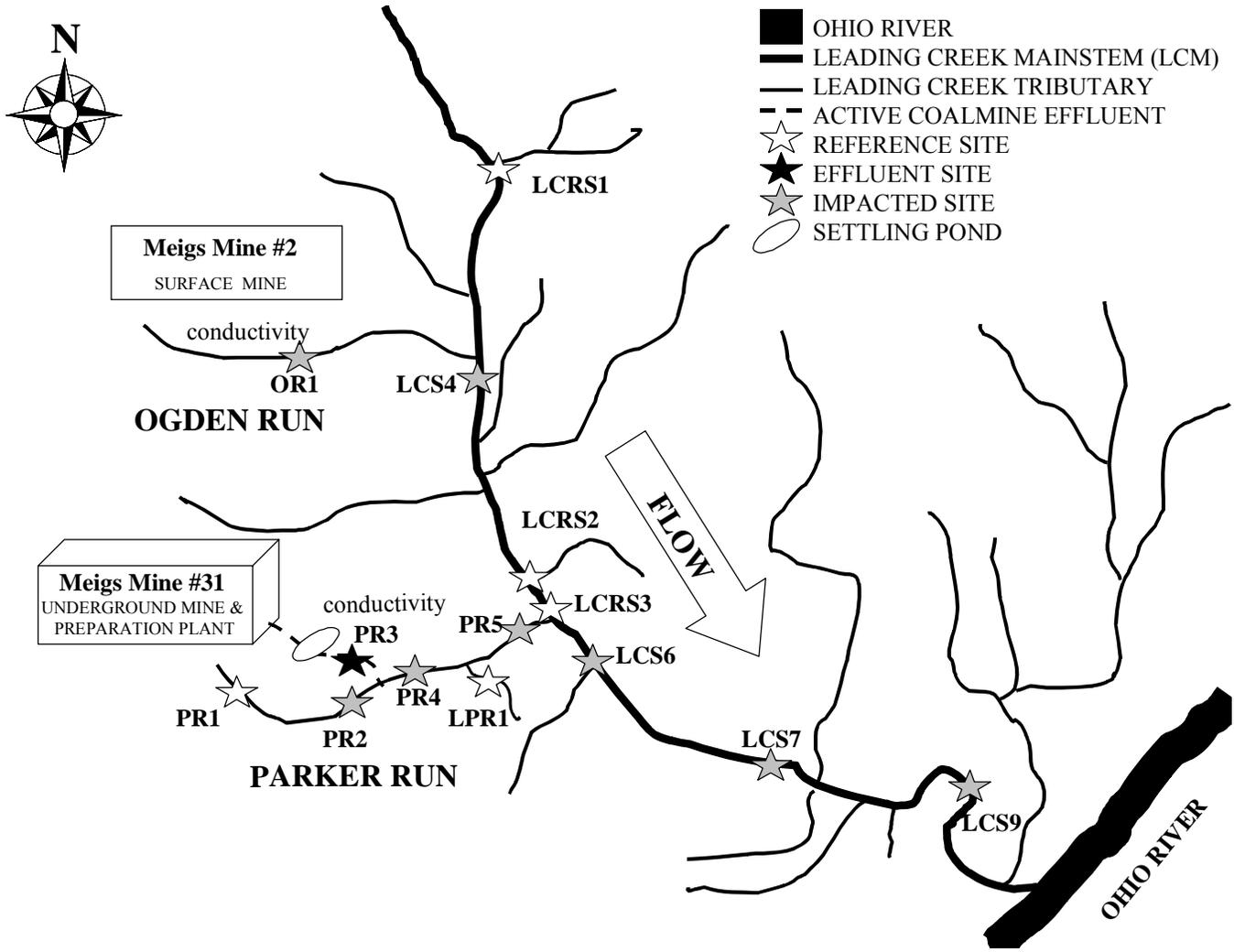


Fig. 3.1. Leading Creek Watershed study sites (not to scale).

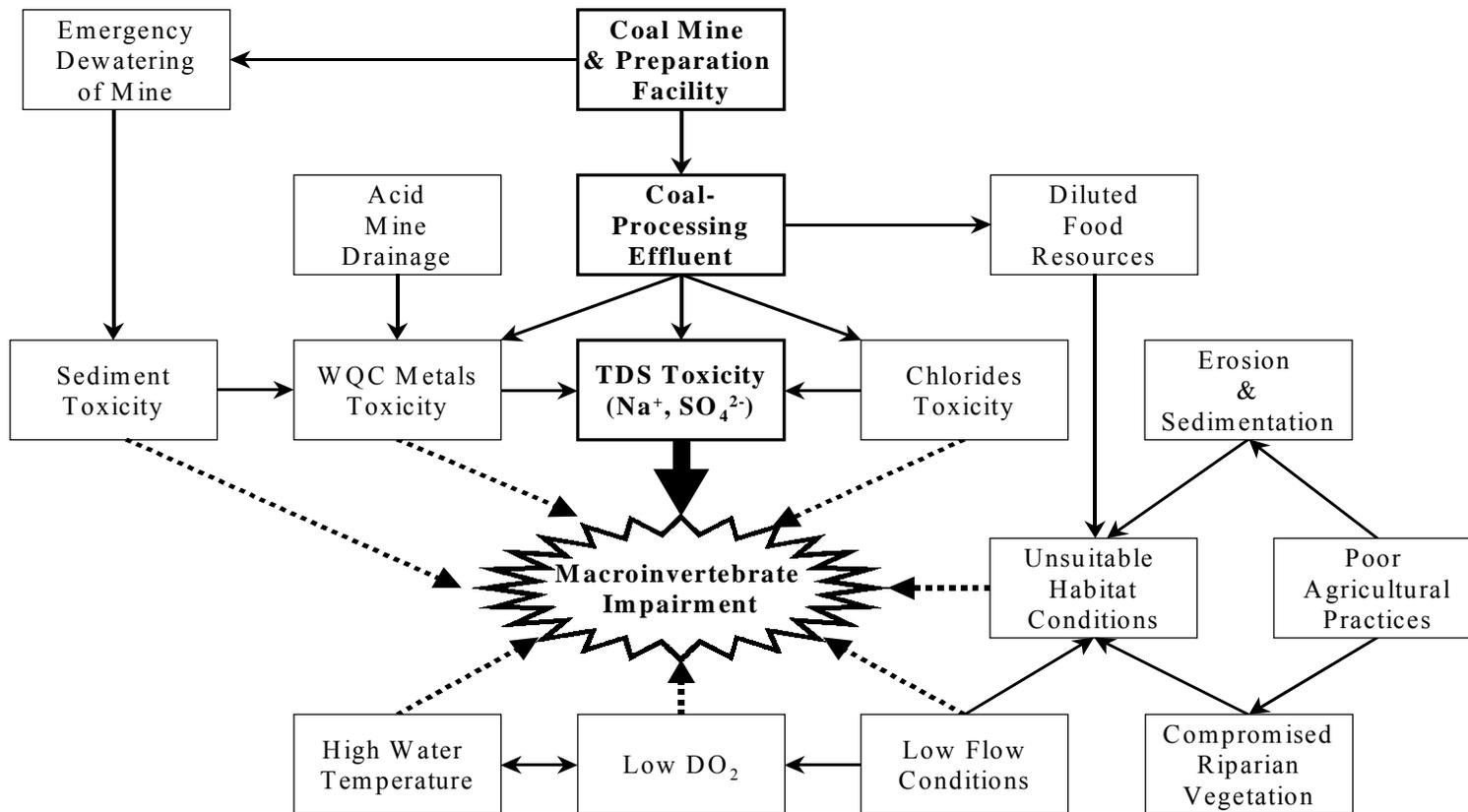
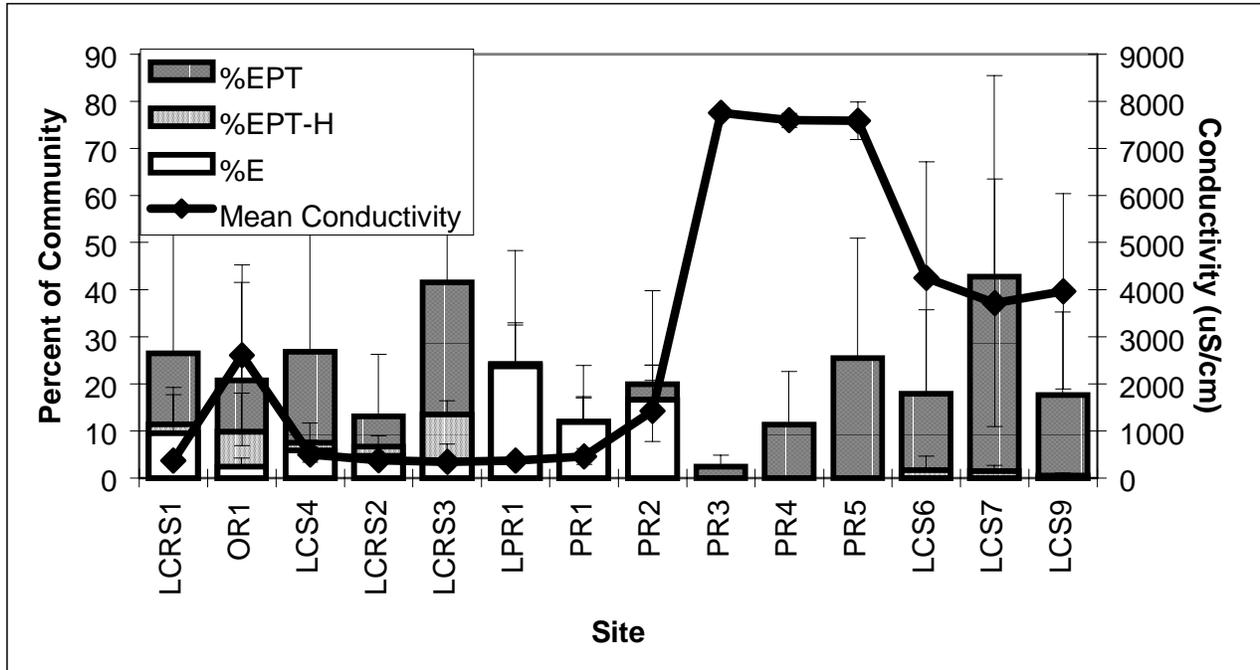
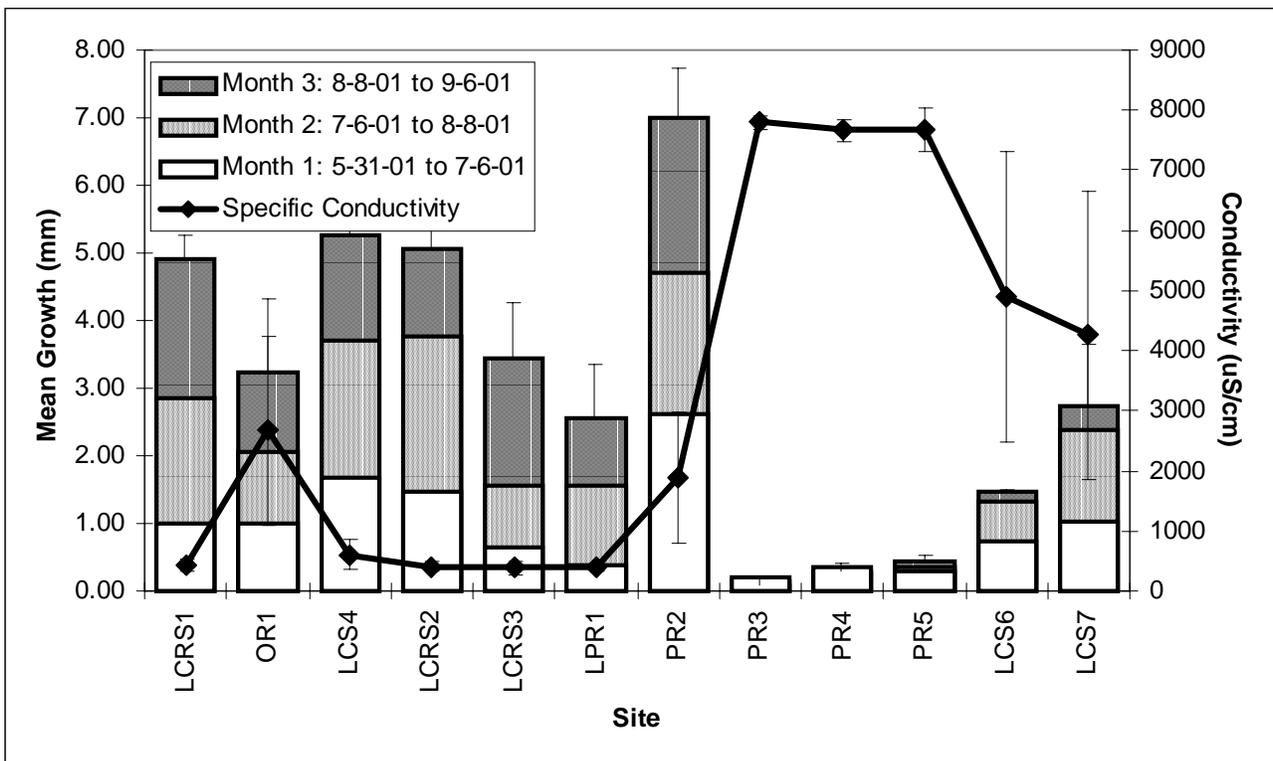


Fig. 3.2. Summary of potential stressors in the Leading Creek Watershed that confound establishment of a causal relationship between the observed biotic impairment and total dissolved solids (TDS) toxicity. Small arrows denote connections between potential causes of impairment (within the boxes), dashed arrows pointing to macroinvertebrate impairment indicate null hypotheses deemed as unlikely causal agents and the large solid arrow indicates the most plausible cause of impairment.



(a)



(b)

Fig. 3.3. Comparison between metrics of biotic impairment (columns) from Leading Creek Watershed sites and specific conductivity (line) for benthic macroinvertebrate surveys (a) and *in situ* *Corbicula fluminea* growth (b). Data are from Kennedy *et al.* (in press) and bars represent one standard deviation from the mean.

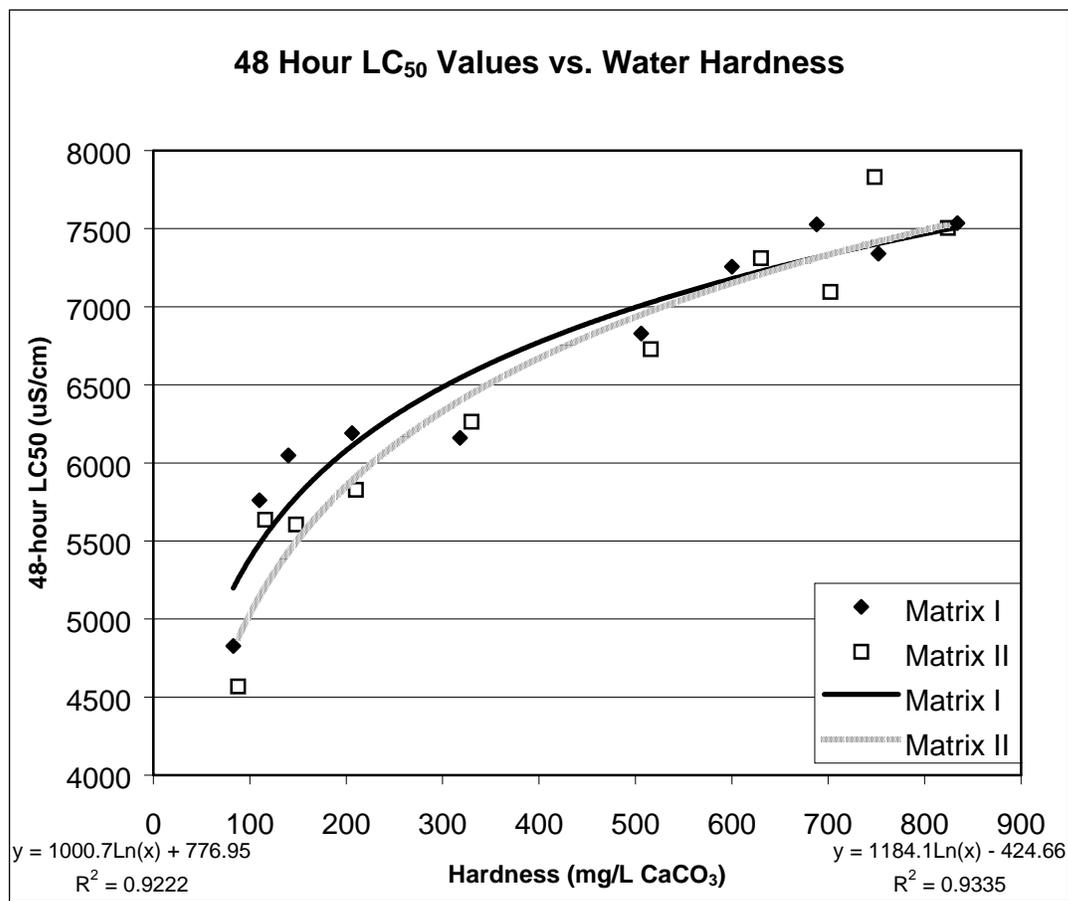
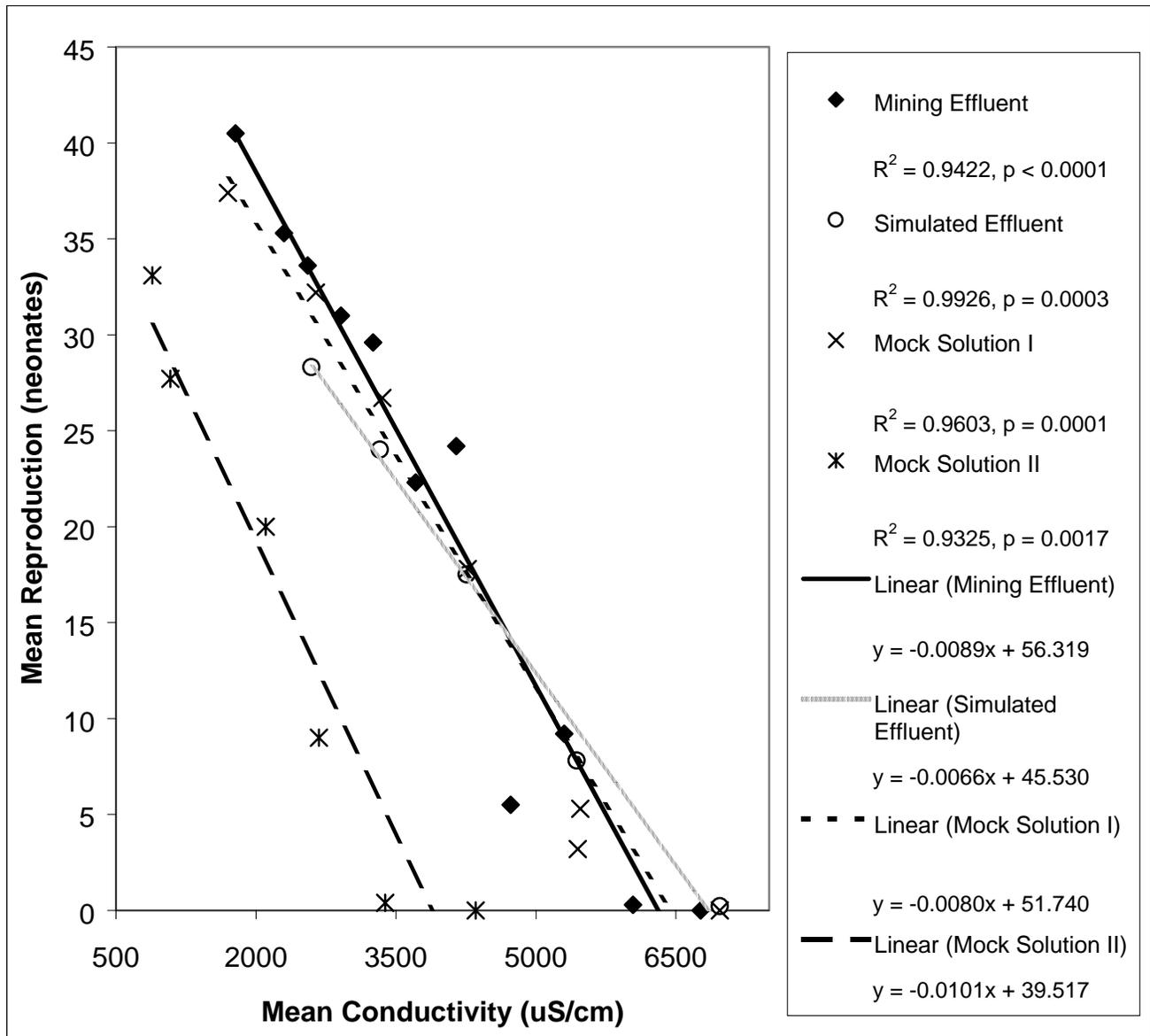


Fig. 3.4. Logarithmic correlation of water hardness and 48-hour LC₅₀ values (μS/cm) for *Ceriodaphnia dubia*. Filled diamonds represent data points derived by solutions in a series of set water hardness and specific conductivity adjusted to ~8900 μS/cm with Na₂SO₄ (Matrix I) while open squares represent data points derived using solutions of the same set hardness series but each with 6 g/L Na₂SO₄ (Matrix II).

Fig. 3.5. 7-d *Ceriodaphnia dubia* mean reproduction in the mine effluent, simulated effluent mock solution I and mock solution II. Equations for each linear regression are provided in the legend.



4 The Influence of Total Dissolved Solids on Aquatic Life

Abstract. In recognition of the deficiency in relevant toxicity endpoint databases, this paper addresses potential toxicity to aquatic life caused by high total dissolved solids (TDS) in freshwater systems receiving treated industrial effluents. Using the toxicity of a treated discharge from an active coal-mining facility in southeastern Ohio and a literature review of other research addressing TDS toxicity from other sources, several conclusions were made in terms of TDS toxicity and factors that influence it. Permit guidelines for high TDS industrial discharges should take into consideration that site-specific ionic composition, water hardness and temperature are important determining factors for how toxic a particular discharge will be to aquatic fauna in the receiving system. In addition, some research has reported that testing with indigenous benthic macroinvertebrates not only provides enhanced ecological relevance, but also more protective endpoints than standardized testing. Although these implications are intriguing, further study should be conducted to gauge the applicability of such testing to industrial permit testing.

4.1 INTRODUCTION

Ecotoxicological research has focused primarily on biotic impairment to aquatic fauna due to minutely toxic contaminants. However, concentrations of ordinarily benign ions, such sodium (Na^+) and sulfate (SO_4^{2-}), can reach levels in wastewater discharges that severely impair sensitive instream macroinvertebrates and laboratory test organisms (Kennedy *et al.*, in press; Kennedy *et al.*, in review; Kennedy *et al.*, in prep). Nationally recommended water quality criteria (WQC) for the protection of aquatic life do not exist for $\text{Na}^+/\text{SO}_4^{2-}$ dominated total dissolved solids (TDS). The following discussion was intended to elaborate on the review of major ion toxicity in effluents by Goodfellow *et al.* (2000), which provided an extensive summary of ion imbalance research for marine and

freshwater systems, with both hypertonic and hypotonic industrial discharge considerations. While Goodfellow *et al.* (2000) discussed a multitude of sources that can cause TDS toxicity (e.g., textile operations, desalination facilities, groundwater remediation systems, pharmaceutical wastes) and methodologies that can be applied to assess such toxicity (e.g., TIEs, synthetic effluents), this paper focuses specifically on the effects of a high TDS discharges into freshwater systems, with integration of protective endpoints using similar testing media obtained in previous study.

The majority of research on the ecological impacts of the coal-mining industry in the United States has focused on abandoned mined land (AML) and the associated acid mine drainage (AMD), low pH and metals concentrations; little attention has been allocated to the high TDS of treated mining discharges. Most studies that have discussed TDS toxicity were related to irrigation drainage water (Hart *et al.*, 1991; Ingersoll *et al.*, 1992; Dickerson *et al.*, 1996), inundation of seawater into freshwater systems (Williams and Williams, 1998; Chadwick *et al.*, 2000) or laboratory formulated salt solutions (Mount *et al.*, 1997). A large portion of this work was conducted in Australia, where salinity stress to aquatic fauna has been recognized and studied for a number of years (Hart *et al.*, 1991). Few studies have addressed the toxicity of active coal-mine effluents in terms of the resulting TDS toxicity (Tiegle *et al.*, 1997; Kennedy *et al.*, in press). With the exception of Chapman *et al.* (2000), no studies have clearly provided general safe standards for TDS concentrations and none have used a multiple species approach.

The objective of this paper was to compare endpoints obtained for TDS toxicity, with considerable focus allocated to a treated coal-mine effluent. The implications brought forth by the studies reviewed in this paper define the urgency to set a maximum

universal standard for TDS. Although researchers concur that site-specific ionic composition alters the toxicity of aqueous solutions such that regulatory standards cannot be based solely on SC or TDS endpoints (Mount et al, 1997, Goestch and Palmer, 1997; Goodfellow *et al.*, 2000), Na^+ and SO_4^{2-} are among the most benign ions from a toxicity standpoint (Mount *et al.*, 1997), providing an opportunity to suggest an approximate maximum biotic threshold.

4.2 BACKGROUND

4.2.1 Source of TDS levels

Anthropogenic environmental impacts in the Leading Creek Watershed (LCW), Meigs County Ohio, have been studied by a number of researchers (Cherry *et al.* 1999, Currie 1999, Latimer 1999, Kennedy *et al.* in press). The coal seams in Meigs County are characterized by relatively high sulfur content (3.4%). Meigs County Mine #31, an underground coal-mine and preparation facility, discharges into Parker Run, a tributary of Leading Creek Mainstem (LCM), following treatment in a settling pond. Groundwater seeping into Mine #31 becomes acidified due the oxidation of reduced sulfur in the coal seam and associated rock strata. The resulting low pH mobilizes acid soluble metals (e.g., Al, Cu, Fe, Zn). These concentrations must be removed from the mine water prior to discharge to comply with the Clean Water Act effluent limitations. Consequently, the wastewater is treated with alkaline reagents (e.g., NaOH, CaO) to increase the pH, in some cases to ~9.5, until the Mn concentration of the wastewater is reduced below 2 mg/L (Zipper, 2000). Finally, the pH is decreased within acceptable WQC (6.5 – 9.0). The result of this treatment is a circumneutral effluent discharge that is low in WQC metals but very high in TDS. These high TDS concentrations are elevated by cations

from the strong bases (Na^+ , Ca^{2+}) and anions from the byproducts of AMD reactions (SO_4^{2-} , Cl^-).

4.2.2 Mechanism of TDS toxicity

Detriments to aquatic life due to ionic stress/imbalance may occur in response to either deficiency or excess. Although hypotonic solutions are cause for concern in regard to deficits in required minerals and ion exchange channels, we focus solely on the toxic influences of ions in excess to freshwater life. The cause of mortality due to high TDS (i.e., hypertonic solution) is probably dehydration of gill and internal tissue, salt accumulation (Ingersoll *et al.*, 1992) and compromised osmoregulatory function of chloride cells (Wichard *et al.*, 1973; Peters *et al.*, 1985). A more detailed description was provided by other researchers (Wichard *et al.*; 1973; Ingersoll *et al.*, 1992; Peters *et al.*, 1995; Buchwalter *et al.*, 2002). Several factors, however, can dictate the extent of this toxicity. Obviously, the site-specific ionic composition of a discharge has a substantial influence on the resulting toxicity (Mount *et al.*, 1997; Goetsch and Palmer, 1997; Goodfellow *et al.*, 2000). Related to this consideration is the relative abundance of Ca^{2+} and Mg^{2+} ions, namely water hardness. Hardness, especially that elevated by Ca^{2+} (Leblanc and Surprenant, 1984; Jackson *et al.*, 2000; Welch *et al.*, 2000), has been shown to significantly alleviate toxicity due to metals (Black *et al.*, 1980; Masnado *et al.*, 1985; Jackson *et al.*, 2000) and Na^+ (McWilliams, 1983; Pic and Maetz, 1981; Kennedy *et al.*, in prep). Increases in temperature significantly raise the metabolic rates of ectotherms, thus making them more susceptible to TDS stress. This was addressed in a number of field and laboratory studies (Hart *et al.*, 1991; Williams and Williams, 1998; Chadwick *et al.*, 2000; Kennedy *et al.*, in review).

4.3 METHODS

4.3.1 Biotic response

Studies in this research series (Kennedy *et al.*, in press; Kennedy *et al.*, in review; Kennedy *et al.* in prep) have assessed impairment to sensitive aquatic fauna across multiple levels of biological response. Individual responses were measured using *in situ* *Corbicula fluminea* growth tests and *in vitro* toxicity testing with *Ceriodaphnia dubia*, *Daphnia magna*, *Pimephales promelas* and *Isonychia bicolor*. The population and community responses were measured by observing alterations to instream relative Ephemeroptera abundance (%E) and Ephemeroptera-Plecoptera-Trichoptera minus Hydropsychidae (EPT-H) metrics, respectively. These responses are listed in increasing order of ecological relevance but decreasing order of mechanistic elucidation, allowing discussion of not only severity and mechanism, but also causality. The primary stressor in LCW causing the observed biotic impairment was the mine effluent and the majority of its toxicity was attributed to its most abundant ions, Na⁺ and SO₄²⁻ (Kennedy *et al.*, in prep). Throughout these studies, specific conductivity (SC) was used as a surrogate of TDS levels due to strong positive correlations between the two (Kennedy *et al.*, in press) and the ease and cost efficiency of obtaining SC readings.

4.3.2 Toxicity endpoints

Data were taken from previous research and aggregated into Tables 4.1 and 4.2. Table 4.1 lists acute toxicity endpoints, while Table 4.2 provides chronic endpoints. For studies that provided no observable adverse effected concentration (NOAEC) and lowest observable adverse effected concentration (LOAEC) endpoints, the maximum allowable toxicant concentration (MATC) was calculated from the geometric mean of these endpoints; $MATC = (NOAEC * LOAEC)^{1/2}$. In cases in which the MATC could not be

calculated, the inhibition concentration at which laboratory/field metrics were reduced by 25% (IC₂₅) compared to the control/reference was determined (EPA 1994) and used as a vector of comparison between measures of impairment. This was performed because NOAEC and LOAEC endpoints were (1) difficult to determine and scientifically invalid for field metrics due to the inherent variability associated with uncontrolled studies and (2) too reliant on potentially arbitrary selection of treatment levels.

4.4 RESULTS & DISCUSSION

4.4.1 Field response

The most sensitive metrics of biotic impairment used during instream surveys in terms of the Na⁺/SO₄²⁻ dominated mine effluent were %E, EPT-H richness and 96 d *in situ* *Corbicula fluminea* growth (Kennedy *et al.*, in press). Short *et al.* (1991) also reported the tolerance of hydropsychids (*Cheumatopsyche*, *Hydropsyche*) to salinity stress in relation to other EPT taxa. The general consensus of previous researchers was that mayflies were the most adversely affected by salinity stress in field studies while Dipterans, Odonates (particularly *Ischnura spp.*), Coleopterans and shredding stoneflies are the most tolerant (Short *et al.*, 1991; Kennedy *et al.*, in press; Williams and Williams, 1998). Almost complete decimation of mayflies was observed above SC levels of ~3000 µS/cm (Kennedy *et al.*, in press; Kennedy *et al.*, in review). Hart *et al.* (1991) reported that mayflies and stoneflies (presumably predatory) were rarely found in waters exceeding 1000 mg/L.

4.4.2 Laboratory bioassay response

The impairment observed in the field metrics was strongly supported by testing in the laboratory. The standardized test organisms, *Pimephales promelas* and *Ceriodaphnia dubia*, were severely impaired by the field-collected mine effluent samples, with 10% and

0% survivorship observed in undiluted effluent, respectively (Kennedy *et al.*, in press). The sub-lethal endpoints for both organisms were much more sensitive than their survivorship. Chronic 7 d *Pimephales* tests resulted in NOAEC and LOAEC growth endpoints of 3800 ± 24 and 4843 ± 36 $\mu\text{S}/\text{cm}$. Chronic 7 d tests with *Ceriodaphnia*, however, were much more sensitive, with NOAEC and LOAEC reproduction endpoints of 2910 ± 40 and 3710 ± 46 $\mu\text{S}/\text{cm}$, respectively.

The sensitivity of test organisms to TDS toxicity varies considerably (Tables 4.1 & 4.2). It was been established in numerous studies that *C. dubia* is consistently more sensitive to salinity stress than is *D. magna*, which is in turn much more sensitive than *P. promelas* (Masnado *et al.*, 1995; Dickerson *et al.*, 1996; Mount *et al.*, 1997; Kennedy *et al.*, in press; Goodfellow *et al.*, 2000). Based on field distributions (Short *et al.*, 1991) and laboratory testing (Mount *et al.*, 1997; Kennedy *et al.*, in press), fish taxa should not be applied to protect the most sensitive macroinvertebrate assemblages from salinity stress due to their tolerance and instream avoidance behavior, although eggs and larvae are more sensitive than adults (Koel and Peterka, 1995). Most published studies have focused almost exclusively on acute TDS toxicity (Goetsch and Palmer, 1997; Mount *et al.*, 1997; Tietge *et al.*, 1997; Latimer, 1999; Chadwick *et al.*, 2000). Latimer (1999) concluded that SC values of 6250 $\mu\text{S}/\text{cm}$ and TDS concentrations of 6400 mg/L are acutely toxic to *C. dubia* regardless of ionic composition.

A technical report by the American Petroleum Institute (1998) suggested that SC levels exceeding 2000 $\mu\text{S}/\text{cm}$ impair freshwater aquatic life (Goodfellow *et al.*, 2000). Other researchers have implied that tests using some indigenous benthic macroinvertebrate taxa result in more sensitive endpoints than these standardized test

organisms to copper (Cherry *et al.*, 2002) and TDS (Chapman *et al.*, 2000; Kennedy *et al.*, in review). TDS guidelines of ~1000 mg/L were suggested in studies using the mayfly, *Isonychia* (Kennedy *et al.*, in review), and the midge, *Chironomus* (Chapman *et al.*, 2000). The states of Pennsylvania (1000 mg/L) and Alaska (1500 mg/L), among others, have suggested similar guidelines for TDS (personal communication with D.S. Cherry; Chapman *et al.*, 2000).

4.4.3 Field-lab comparisons

Suggesting safe limits for any toxicant should not be based on a single test organism or metric of biotic impairment (Cairns, 1986). Thus, a fairly comprehensive aggregation of endpoints obtained from previous studies was performed (Tables 4.1 and 4.2). Ranked acute (Table 4.1) and chronic (Table 4.2) toxicity endpoints are provided, based on testing of a variety of organisms in comparable Na₂SO₄ and/or NaCl dominated solutions. All endpoints were converted to SC. Compounding the wide range in organism sensitivities, hardness and temperature had an important influence on the testing endpoints obtained. For instance, *Ceriodaphnia* had a much lower LC₅₀ value at a hardness of 86 mg/L as CaCO₃ (5023 µS/cm) than at 776 mg/L as CaCO₃ (6713 µS/cm) and at 835 mg/L as CaCO₃ (7109 µS/cm). Temperature influenced the 96-h LC₅₀ values for the burrowing mayfly, *Hexagenia limbata*, at 18°C (6.3 ppt) and 28°C (2.4 ppt) in an aquarium salt solution (Chadwick *et al.*, 2000) and the 7-d LOAEC endpoints for the mayfly, *Isonychia bicolor*, at 12°C (4973 µS/cm) and 20°C (987 µS/cm) in a Na⁺/SO₄²⁻ dominated solution (Kennedy *et al.*, in review).

It may be of interest that three benthic macroinvertebrates, *Tricorythus*, *Paragnetina* and *Isonychia*, had lower endpoints than even the most sensitive

standardized test organism, *Ceriodaphnia*. However, because our knowledge of the heartiness of these organisms during *in vitro* testing is rudimentary compared to standardized test organisms, strong recommendations for Na⁺/SO₄²⁻ dominated TDS cannot be extrapolated from tests using field-collected benthic macroinvertebrates. This was not an issue for testing with *Ceriodaphnia*, and recommendations can be made based on testing at 25°C. The effects of hardness on TDS toxicity was addressed by Kennedy *et al.* (in prep) and the following equations were derived by the logarithmic regression of 20 data points obtained from testing with *Ceriodaphnia*:

$$LC_{50} (\mu\text{S/cm}) = 1088.8 * \text{Ln} (\text{hardness}) + 198.3$$

$$\text{Safe acute limit } (\mu\text{S/cm}) = 935.84 * \text{Ln} (\text{hardness}) + 228.06$$

Using these equations, predictions of acute Na⁺/SO₄²⁻ dominated TDS can be calculated using hardness as an independent variable. For example, at hardness levels of 80, 100, 500 and 800 mg/L as CaCO₃, acute toxicity would occur at 4969, 5212, 6965, and 7477 μS/cm, respectively; conservative safe levels occur at 4329, 4538, 6044, and 6483 μS/cm, respectively. Large ranges of hardness were provided because waters high in TDS are typically associated by high Ca²⁺ concentrations. A similar effect was observed in chronic testing with *Ceriodaphnia*, but the database was not complete enough to generate a model for chronic toxicity. Safe chronic levels, based on MATCs calculated from endpoints provided in Kennedy *et al.* (in prep) at hardness levels of 88 and 810 mg/L as CaCO₃ were 1510 and 2977 μS/cm, respectively (Table 4.2).

Elevated TDS levels have more far-reaching implications than only the persistence of aquatic fauna; researchers have suggested no use restrictions for conductivity (≤700 μS/cm), TDS (450 mg/L) and sodium (69 mg/L) for irrigation waters

drawn from streams in the Western U.S. (Hicks, 2000). These restrictions are lower than most TDS endpoints derived from ecotoxicological assessments because they are required to be protective of soils over a longer-term duration with consideration to absorption and accumulation of salts in the soil.

4.4.4 Implications to NPDES permit testing

When establishing permit guidelines for industrial discharges that are high in TDS, consideration should be allocated to the (1) site-specific ionic composition of the effluent (2) hardness of the effluent and receiving system, (3) ambient temperature of the effluent and receiving system, (4) indigenous aquatic fauna and (5) the ecological relevance and sensitivities/tolerances of standardized test organisms and testing protocol (Kennedy *et al.*, in press). In addition, testing with indigenous benthic macroinvertebrates can provide endpoints considerably more sensitive than *C. dubia* (Chapman *et al.*, 2000; Kennedy *et al.*, in review). Although such testing is intriguing in terms of its application to permit testing and its ecological relevance to receiving streams, it requires further study before it can be recommended. In determining the most appropriate metrics and test organisms to assess a discharge that is high in TDS from a regulatory standpoint, at least some consideration should be allocated to the potential of increased impairment due to TDS shock of non-acclimated test organisms (Wichard *et al.*, 1973; Hart *et al.*, 1991; Koel and Peterka, 1995). Future research should focus on compiling a large database of different testing organisms at relevant testing temperatures and hardness so that nationally recognized WQC for Na⁺/SO₄²⁻ dominated TDS may be derived. This research should involve the development of tests with indigenous benthic macroinvertebrates, including indexes to determine heartiness, as have been done for *Ceriodaphnia* (US EPA, 1994).

4.5 References

- Black JA, Roberts RF, Johnson DM, Minicucci DD, Mancy KH, Allen HE. 1980. The Significance of Physiological Variables in Aquatic Bioassays of Heavy Metals. Water Quality Criteria for Freshwater Fish. Butterworth, London. 259-275.
- Buchwalter DB, Jenkins JJ, Curtis LR. 2002. Respiratory strategy and temperature influences on contaminant and water uptake rates in aquatic insects: Implications for stressor-specific diagnostics. Annual Meeting of the North American Benthological Society, Pittsburgh, PA (USA), May 28-Jun 1, 2002 (World Meeting Number 000 5892).
- Chadwick MA, Feminella JW. 2001. Influence of salinity and temperature on the growth and production of a freshwater mayfly in the Lower Mobile River. *Limnol Oceanog.* 46: 532-542.
- Chapman PM, Bailey H, Canaria E. 2000. Toxicity of total dissolved solids associated with two mine effluents to *Chironomid* larvae and early life stages of rainbow trout. *Environ Toxicol Chem* 19: 210-214.
- Cairns, J. Jr. 1986. The myth of the most sensitive species. *BioScience* 36: 670-672.
- Cherry DS, Currie RJ, Latimer HA, Cairns J Jr, Diz HR, Johnson DM, Yeager MM. 1999. The Leading Creek Improvement Plan: Southern Ohio Coal Company Meigs Mine No. 31. Final report to Southern Ohio Coal Company and American Electric Power, Columbus Ohio 43216.
- Cherry DS, Van Hassel JH, Farris JL, Soucek DJ, Neves RJ. 2002. Site-specific derivation of the acute copper criteria for the Clinch River, Virginia. *Hum Ecol Risk Assess.* 8: 591-601.

- Currie RJ. 1999. Identification of ecosystem stressors in developing an enhancement plan for the Leading Creek Watershed, Meigs County, Ohio. Virginia Polytechnic Institute and State University, doctoral dissertation.
- Diamond JM, Winchester EL, Mackler DG, Gruber D. 1992. Use of the Mayfly *Stenonema modestrum* (Heptageniidae) in Subacute Toxicity Assessments. *Environ Toxicol Chem* 11: 415-425.
- Dickerson KK, Hubert WA, Berman HL. 1996. Toxicity assessment of water from lakes and wetlands receiving irrigation drain water. *Environ Toxicol Chem* 15: 1097-1101.
- Goetsch PA, Palmer CG. 1996. Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. *Arch Environ Contam Toxicol* 32: 32-41.
- Goodfellow WL, Ausley LL, Burton DT, Denton DL, Dorn PB, Grothe DR, Heber MA, Norberg-King TJ, Rodgers JH Jr. 2000. Major ion toxicity in effluents: a review with permitting recommendations. *Environ Toxicol Chem* 19: 175-182.
- Hart BT, Bailey P, Edwards R, Hortle K, James K, McMahon A, Meredith C Swadling K. 1991. A review of the salt sensitivity of the Australian freshwater biota. *Hydrobiologia* 210: 105-144.
- Hicks M. 2000. Establishing surface water quality criteria for the protection of irrigation water supplies. Washington State Department of Ecology, draft discussion paper, December 2000. Publication Number 00-10-073.
- Ingersoll CG, Dwyer FJ, Burch SA, Nelson MK, Buckler DR, Hunn JB. 1992. The use of

- freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drain water. *Environ Toxicol Chem* 11: 503-511.
- Jackson BP, Lasier PJ, Miller WP, Winger PW. 2000. Effects of calcium, magnesium, and sodium on alleviating cadmium toxicity to *Hyalella azteca*. *Bull Environ Contam Toxicol* 64: 279-286.
- Kapoor NN. 1979. Osmotic regulation and salinity tolerance of the stonefly nymph, *Paragnetina media*. *Insect Physiol.* 25: 17-20.
- Kennedy AJ, Cherry DS, Currie RJ. in press. Field and laboratory assessment of a coal] processing effluent in the Leading Creek Watershed, Meigs Co., Ohio. *Arch Environ Contam Toxicol*: in press.
- Kennedy AJ, Cherry DS, Currie RJ. In review. Assessment of a coal-processing Effluent Impacted Lotic System Using an Ecologically Relevant Test Organism, *Isonychia bicolor*. *Environ Monit Assessment*: in review.
- Kennedy AJ, DS Cherry, Currie RJ. In prep. Stressor Identification and Evaluation of the Relative Ionic Contribution of a Coal-mine effluent to Biotic Impairment. *Environ. Toxicol Chem*: in prep.
- Koel TM, Peterka JJ. 1995. Survival to hatching of fishes in sulfate-saline waters, Devils Lake, North Dakota. *Can J Fish Aquat Sci* 52: 464-469.
- Latimer HA. 1999. An ecotoxicological evaluation of active coal mining, sedimentation and acid mine drainage in three tributaries of the Leading Creek Watershed, Meigs County, Ohio. Virginia Polytechnic Institute and State University, masters thesis.

- Leblanc GA, Surprenant DC. 1984. The influence of mineral salts on fecundity of the water flea (*Daphnia magna*) and the implications on toxicity testing of industrial wastewater. *Hydrobiologia* 108: 25-31.
- Masnado RG, Geis SW, Sonzogogni WC. 1995. Comparative acute toxicity of a synthetic mine effluent to *Ceriodaphnia dubia*, larval Fathead Minnow and the Freshwater mussel *Anodonta imbecilis*. *Environ Toxicol Chem* 14: 1913-1920.
- McWilliams PG. 1983. An investigation of the loss of bound calcium from the gills of the brown trout, *Salmo trutta*, in acid media. *Comp Biochem Physiol* 74A: 107-116.
- Meyer JS, Sanchez DA, Brookman JA, McWhorter DB, Bergman HL. 1985. Chemistry and aquatic toxicity of raw oil shale leachates from Piceance Basin, Colorado. *Environ Toxicol Chem* 4: 559-572.
- Mount DR, Gulley DD, Hockett JR, Garrison TD, Evans JM. 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (Fathead Minnows). *Environ Toxicol Chem* 16: 2009-2019.
- Peters GT, Cherry DS, Cairns JC Jr. 1985. Responses of *Isonychia bicolor* to alkaline pH: an evaluation of survival, oxygen consumption, and chloride cell ultrastructure. *Can J Fish Aquat. Sci* 42: 1088-1095.
- Pic P, Maetz J. 1981. Role of external calcium in sodium and chloride transport in the gills of seawater-adapted *Mugil capito*. *J Comp Physiol* 141: 511-521.
- Short TM, Black JA, Birge WJ. 1991. Ecology of a saline stream: community responses to spatial gradients of environmental conditions. *Hydrobiologia* 226: 167-178.

- Tietge JE, Hockett JR, Evans JM. 1997. Major ion toxicity of six produced waters to three freshwater species: application of ion toxicity models and TIE procedures. *Environ Toxicol Chem* 16: 2002-2008.
- US EPA. 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms, 3rd ed. Washington D.C. EPA-600-4-91-002, Cincinnati, OH.
- Welch PG, Lipton J, Chapman GA, Podrabsky TL. 2000. Relative importance of calcium and magnesium in hardness-based modification of copper toxicity. *Environ Toxicol Chem* 19: 1624-1631.
- Wichard W, Tsui PTP, Komnick H. 1973. Effect of different salinities on the coniform chloride cells of mayfly larvae. *J Insect Physiol* 19: 1825-1835.
- Williams DD, Williams N.E. 1998. Aquatic insects in an estuarine environment: densities, distribution and salinity tolerance. *Freshwater Biol.* 39: 411-421.
- Zipper CE. 2000. Coal mine reclamation, acid mine drainage and the Clean Water Act. p. 169-191, in: R. Barnhisel, W. Daniels and R. Darmody (eds). Reclamation of Drastically Disturbed Lands. American Society of Agronomy. Madison WI. Monograph 41.

Table 4.1: Acute testing endpoints in high salinity solution. Table includes test organism, testing temperature (Temp), water hardness testing duration, testing endpoints in the original units reported by the researchers and endpoints converted into conductivity. All endpoints were based on survivorship of the test organism.

Organism	Testing Solution	Temp (°C)	Hardness (mg/L)	Duration (hours)	Original Units	Conductivity (µS/cm) [†]	Study
<i>Tricorythus sp.</i>	Na ₂ SO ₄	10.0 – 16.0	n/a	96	50 mS/m [‡] 100 mS/m [§]	500 [‡] 1000 [§]	Goetsch & Palmer (1997)
<i>Paragnetina media</i>	NaCl	20.0	n/a	72	0.95 ppt [‡] 1.1 ppt [§]	2400 [‡] 2600 [§]	Kapoor (1979)
<i>Ceriodaphnia dubia</i>	Na ₂ SO ₄	25.0	n/a	48	3080 mg/L [#]	4195 [#]	Mount <i>et al.</i> (1997)
<i>Ceriodaphnia dubia</i>	Na ₂ SO ₄	25.0	86	48	5023 µS/cm [#]	4698 [#]	Kennedy <i>et al.</i> (in prep)
<i>Hexagenia limbata</i>	Aquarium Salt	28.0	n/a	96	2.4 ppt [#]	4800 [#]	Chadwick <i>et al.</i> (2000)
<i>Daphnia magna</i>	NaCl	20.0	563	48	6500 mg/L [#]	5790 [#]	Meyer <i>et al.</i> (1985)
<i>Ceriodaphnia dubia</i>	Coal Mine effluent	25.0	776	48	6713 µS/cm [#]	6713 [#]	Kennedy <i>et al.</i> (in press)
<i>Ceriodaphnia dubia</i>	Simulated Mine effluent	25.0	835	48	7109 µS/cm [#]	7109 [#]	Kennedy <i>et al.</i> (in prep)
<i>Daphnia magna</i>	Na ₂ SO ₄	20.0	563	48	8600 mg/L [#]	11411 [#]	Meyer <i>et al.</i> (1985)
<i>Daphnia magna</i>	Na ₂ SO ₄	20.0	n/a	48	4580 mg/L [#]	6156 [#]	Mount <i>et al.</i> (1997)
<i>Hexagenia limbata</i>	Aquarium Salt	18.0	n/a	96	6.3 ppt [#]	9500 [#]	Chadwick <i>et al.</i> (2000)

<i>Pimephales promelas</i>	NaCl	17.0	563	96	11400 mg/L [#]	15071 [#]	Meyer <i>et al.</i> (1985)
<i>Pimephales promelas</i>	Na ₂ SO ₄	17.0	563	96	15200 mg/L [#]	20039 [#]	Meyer <i>et al.</i> (1985)
<i>Pimephales promelas</i>	Na ₂ SO ₄	25.0	n/a	96	7960 mg/L [#]	10574 [#]	Mount <i>et al.</i> (1997)

† Converted, if necessary, according to Standard Methods (1995) for salinity and Kennedy *et al.* (in review) for TDS

‡ No observable adverse effected concentration (NOAEC)

§ Lowest observable adverse effected concentration (LOAEC)

Lethal concentration at which a 50% mortality is observed

Table 4.2: Chronic testing endpoints in high salinity solution. Table includes test organism, testing temperature (Temp), water hardness testing duration, testing endpoints in the original units reported by the researchers and endpoints converted into conductivity. The following abbreviations were used: Survival (S), Reproduction (R), Growth (G) and Survival to Hatching (SH).

Organism	Testing Solution	Temp (°C)	Hardness (mg/L)	Duration (days)		Original Units	SC (µS/cm) [†]	MATC (µS/cm) [†]	Study
<i>Isonychia bicolor</i>	Synthetic Effluent	20.0	659	7	S	550 µS/cm [‡] 987 µS/cm [§]	550 [‡] 987 [§]	737	Kennedy <i>et al.</i> (in review)
<i>Isonychia bicolor</i>	Synthetic Effluent	20.0	659	7	S	619 µS/cm [‡] 966 µS/cm [§]	619 [‡] 966 [§]	773	Kennedy <i>et al.</i> (in review)
Relative Ephemeroptera Abundance	Coal-mine effluent	24.6	774	n/a	S	1492 µS/cm [#]	1492 [#]	n/a	Kennedy <i>et al.</i> (in press)
<i>Ceriodaphnia dubia</i>	Na ₂ SO ₄	25.0	88	7	R	1084 µS/cm [‡] 2103 µS/cm [§]	1084 [‡] 2103 [§]	1510	Kennedy <i>et al.</i> (in prep)
<i>Chironomus tentans</i>	Synthetic Effluent	23.0	~1144	10	S	1220 mg/L [‡] 1750 mg/L [§]	1763 [‡] 2457 [§]	2081	Chapman <i>et al.</i> (2000)
<i>Chironomus tentans</i>	Synthetic Effluent	23.0	~1413	10	G	1134 mg/L [‡] 2089 mg/L [§]	1652 [‡] 2900 [§]	2189	Chapman <i>et al.</i> (2000)
<i>Stizostedion vitreum</i>	Na ₂ SO ₄ rich lake	11.5	n/a	Various	SH	1150 mg/L [‡] 2400 mg/L [§]	1812 [‡] 3301 [§]	2446	Koel & Peterka 1995
<i>Esox lucius</i>	Na ₂ SO ₄ rich lake	19.9	n/a	Various	SH	1150 mg/L [‡] 2400 mg/L [§]	1812 [‡] 3301 [§]	2446	Koel & Peterka 1995
<i>Corbicula fluminea</i>	Coal-mine effluent	25.2	774	96	G	2521 µS/cm [#]	2521 [#]	n/a	Kennedy <i>et al.</i> (in press)
<i>Ceriodaphnia dubia</i>	Coal-mine effluent	25.0	834	7	R	2553 µS/cm [‡] 3254 µS/cm [§]	2553 [‡] 3254 [§]	2882	Kennedy <i>et al.</i> (in prep)
<i>Ceriodaphnia dubia</i>	Synthetic Effluent	25.0	810	7	R	2597 µS/cm [‡] 3331 µS/cm [§]	2597 [‡] 3331 [§]	2941	Kennedy <i>et al.</i> (in prep)

<i>Ceriodaphnia dubia</i>	Na ₂ SO ₄ CaSO ₄ MgSO ₄	25.0	820	7	R	2641 μ S/cm [†] 3356 μ S/cm [§]	2641 [‡] 3356 [§]	2977	Kennedy <i>et al.</i> (in prep)
<i>Stenonema modestrum</i>	NaCl	12.0	n/a	14	G	2.0 ppt [†] 2.7 ppt [§]	2850 [‡] 3700 [§]	3247	Diamond <i>et al.</i> (1992)
<i>Ceriodaphnia dubia</i>	Coal-mine effluent	25.0	730	7	R	2910 μ S/cm [†] 3710 μ S/cm [§]	2910 [‡] 3710 [§]	3286	Kennedy <i>et al.</i> (in prep)
<i>Isonychia bicolor</i>	Synthetic Effluent	12.0	659	7	S	2734 μ S/cm [†] 4973 μ S/cm [§]	2734 [‡] 4973 [§]	3687	Kennedy <i>et al.</i> (in review)
<i>Stenonema modestrum</i>	NaCl	12.0	n/a	14	S	2.7 ppt [†] 3.5 ppt [§]	3700 [‡] 4800 [§]	4214	Diamond <i>et al.</i> (1992)
<i>Pimephales promelas</i>	Coal-mine effluent	25.0	818	7	G	3800 μ S/cm [†] 4843 μ S/cm [§]	3800 [‡] 4843 [§]	4290	Kennedy <i>et al.</i> (in press)
<i>Pimephales promelas</i>	Coal-mine effluent	25.0	818	7	S	4843 μ S/cm [†] 6180 μ S/cm [§]	4843 [‡] 6180 [§]	5471	Kennedy <i>et al.</i> (in press)

[†] Converted, if necessary, according to Standard Methods (1995) for salinity and Kennedy *et al.* (in review) for TDS

[‡] No observable adverse effected concentration (NOAEC)

[§] Lowest observable adverse effected concentration (LOAEC)

[#] Inhibition concentration at which a 25% reduction is observed

Alan J. Kennedy

--Environmental Toxicologist--

Curriculum Vita

Last Modification: November 14, 2002

Contact Information:

[home] 409 Lee Street

Blacksburg, VA 24060

Cell phone: (540) 230-9053

Home phone: (540) 961-6993

[work] Virginia Tech

Department of Biology, 2119 Derring Hall

Blacksburg, VA 24061

Lab phone: (540) 231-9071

email: alkennel@vt.edu

URL: http://128.173.184.249/cherry/Al's_tapage/Al's_homepage.htm

Date & place of birth: October 19, 1976 (Midland, Michigan)

OBJECTIVE:

To obtain an entry to mid-level environmental scientist/technician position with a technical laboratory and/or field setting in the private, academic or state/federal regulatory sectors.

EDUCATION:

M.S. student, Virginia Tech, Department of Biology, Aquatic Ecotoxicology (graduating December 2002). Thesis involved a myriad of risk assessment methodologies to gauge biotic impairment caused by the total dissolved solids (TDS) toxicity of a treated coal-mine effluent in southeastern Ohio. [GPA: 3.83/4.00]

Objectives/Findings:

- ✓ Established toxicity of a treated coal-mine effluent in terms of TDS
- ✓ Established causal relationship between the mine effluent & biotic impairment
 - Toxicity Identification Evaluation (TIE) of mine effluent
 - Isolated $\text{Na}^+/\text{SO}_4^{2-}$ dominated TDS toxicity
- ✓ Designed 7 day bioassay for the mayfly, *Isonychia bicolor*

B.S. Environmental Biology/Zoology, Michigan State University, Department of Zoology, with high honors (May 1999). [GPA: 3.82/4.00]

RELEVANT COURSEWORK:

Aquatic Toxicology

Aquatic Entomology*

Cells & Molecules*

Chemistry (inorganic)*

Chemistry (organic I & II)*

Crop & Landscape Science

Ecology*

Environmental Chemistry

Environmental Physiology

Environmental Toxicology

Evolution

Fish & Wildlife Management

Genetics

Invertebrate Biology*

Limnology

Organisms & Populations*

Ornithology*

People & the Environment

Plant Ecology

Plant Systematics*

Resource Ecology

Statistics in Research I

Stable Isotopes Ecology

Stream Ecology

*Laboratory included

RELEVANT EXPERIENCE:

Graduate Research Assistant (June 2000 – present), Virginia Tech, Department of Biology, Blacksburg, VA. Performed toxicity tests for industries with National Pollutant Discharge & Elimination System (NPDES) permits.

Graduate Teaching Assistant (August 2000 – May 2001), Virginia Tech, Department of Biology, Blacksburg, VA. Served as laboratory instructor for general & principles of biology.

Quality Control Technician (January 2000 – June 2000) Nalco Finishing Technologies Group, Jackson, MI. Conducted tests to assure industrial coolants met specified parameters.

Fisheries Research Technician (August 1998 – December 1999), Michigan State University, Department of Fisheries & Wildlife, East Lansing, MI. Involved in projects analyzing the influences of impoundments on stream biota, including field & laboratory work on fish & macroinvertebrates.

Tropical Ecology & Management Study Abroad (May 1999), San Salvador Island, Bahamas (Michigan State University).

Camp Fishing Instructor (June 1998 – August 1998), Michigan DNR & Lansing Parks & Recreation, Urban Fishing Project, Lansing, MI. Educated children on such topics as environmental ethics, fish identification & angling.

FIELD & LABORATORY EXPERIENCE:

Field:

- *In situ* Asian Clam (*Corbicula fluminea*) studies
- Macroinvertebrate collection & identification (Qualitative/Quantitative)
- Electroshocking fish
- Habitat assessment (RBP, QHEI)
- Physicochemical parameters

Laboratory:

- Aging trout scales
- Fish gut content analysis
- Microbalances to measure dry weight of aquatic organisms
- Centrifuge to separate oils from industrial coolants
- Total dissolved solids, total suspended solids & dirt-load analysis
- Titrations

Toxicology:

- National Pollutant Discharge & Elimination System (NPDES) permit testing
- Culturing *Ceriodaphnia dubia* & *Daphnia magna* (all aspects)
- Formulation of EPA¹⁰⁰ moderately-hard reconstituted water
- Chronic sediment toxicity testing using *Chironomus tentans*, *D. magna* & *Hyalella azteca*
- Acute & chronic water column testing utilizing *C. dubia*, *D. magna* & *Pimephales promelas*

Other Skills:

- Microsoft Office & Frontpage
- Toxstat
- Jumpin
- SAS
- Delorme's Topo USA (version 3.0)
- First aide & CPR training
- SCUBA certified
- GPS

ORGANIZATIONS & HONORS:

- Society of Environmental Toxicology & Chemistry
- North American Benthological Society
- Phi Kappa Phi National Honor Society
- Golden Key National Honor Society
- Deans List, 8 semesters (Michigan State University)
- Study Abroad Scholarship (1999)
- Adray Scholarship (1995)

PUBLICATIONS & MANUSCRIPTS:

A.J. Kennedy, D.S. Cherry and R.J. Currie. In press. Field and laboratory assessment of a coal-processing effluent in the Leading Creek Watershed, Meigs Co., Ohio. Archives of Environmental Contamination and Toxicology, in press.

A.J. Kennedy, D.S. Cherry and R.J. Currie. In review. Evaluation of Ecologically Relevant Bioassays for a Lotic System impacted by a Coal-mining effluent, using *Isonychia bicolor*. In review: Environmental Monitoring and Assessment, in review.

A.J. Kennedy, D.S. Cherry and C.E. Zipper. Stressor identification and evaluation of relative ionic contribution of a coal mine effluent to biotic impairment. In prep: Environmental Toxicology and Chemistry.

PRESENTATIONS:

A.J. Kennedy, D.S. Cherry and R.J. Currie. Assessment of active coal mine effluent toxicity to sensitive freshwater macroinvertebrates. Poster presented at the national meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD, November 11-15, 2001.

A.J. Kennedy, D.S. Cherry and R.J. Currie. Laboratory assessment of an impacted lotic system using an ecologically relevant test organism. Poster presented at the national meeting of the North American Benthological Society, Pittsburgh, PA, May 28 – June 1, 2002.

A.J. Kennedy, D.S. Cherry, R.J. Currie and C.E. Zipper. Relative ionic contribution of a coal-processing effluent to biotic impairment. Platform presentation at the national meeting

of the Society of Environmental Toxicology and Chemistry, Salt Lake City, UT, November 16-20, 2002.

INVITED LECTURES:

Stressors in Aquatic Systems. Biomonitoring techniques lecture given to a biology class in the Environmental Science Department at Roanoke College, Salem, VA, November 30, 2001.

TECHNICAL REPORTS:

Quarterly NPDES permit reports:

International Paper
Celanese Acetate
Brush Wellman

IBM
Special Reports:
American Electric Power

[e.g.]

- Cherry, D.S., R.J. Currie, D.J. Soucek, T.S. Schmidt, M.S. Hull, A.J. Kennedy. 2000. Toxicity Test Laboratory Performance Evaluation (DMR-QA Study 20) for Acute and/or Chronic Tests. US EPA Round Robin Test Evaluations.
- Cherry, D.S., R.J. Currie, D.J. Soucek, T.S. Schmidt, M.S. Hull, A.J. Kennedy, Merricks, T.C., 2001. Quarterly Chronic Biomonitoring Results of a Water Flea (*Ceriodaphnia dubia*) and Fathead Minnow (*Pimephales promelas*) To Pine Bluff Mill Effluent, International Paper Company, Pine Bluff, Arkansas.
- Cherry, D.S., R.J. Currie, T.S. Schmidt, A.J. Kennedy, Merricks, T.C., 2001. Sediment Toxicity Testing of Stream and River Sites Near American Electric Power (AEP) Facilities in Ohio, West Virginia, Virginia, Kentucky, and Indiana. Group 3 (round 2, single species tests): Conesville, Muskingham and Pickway Plants. Submitted to AEP, Columbus, Ohio.
- Cherry, D.S., R.J. Currie, M.S. Hull, A.J. Kennedy and T.C. Merricks. July 2001. Evaluation of Potential Acute Toxicity of 003 Effluent from International Business Machines (IBM), Poughkeepsie New York.

PROFESSIONAL REFERENCES:

Dr. Donald S. Cherry
Biology Department
Virginia Tech
(540) 231-6766
dcherry@vt.edu

Dr. Carl E. Zipper
Department of Soil Science
Virginia Tech
(540) 231-9782
czip@vt.edu

Dr. E. Fred Benfield
Biology Department
Virginia Tech
(540) 231-5802
benfield@vt.edu

Dr. David Soucek
IL Natural History Survey
Illinois DNR
(217) 265-5489
d-soucek@inhs.uiuc.edu

Dr. Daniel Hayes
Department of Fisheries
Michigan State University
(517) 432-3781
hayesdan@msu.edu

Dr. John Schwartz
Michigan Sea Grant
Michigan State University
(517) 353-9568
schwar18@msu.edu