Ozark Cavefish Distribution Related to Mining and Water Quality in the Tri-State Mining District of Jasper and Newton Counties, Missouri

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Summary

Mining activities in the Tri-State Mining District (TSMD) have contaminated groundwaters with metals, chemicals, and altered environmental conditions. Obligate subterranean aquatic organisms (stygobites) including federally-listed Threatened Ozark Cavefish *Amblyopsis rosae* potentially occupy shallow aquifers that were impacted. Our goal was to describe relationships between mining related contamination and the distribution of Ozark Cavefish and other stygobites to inform ongoing Natural Resource Damage Assessment (NRDA) and enhance conservation and recovery efforts.

We identified strong spatial patterns in the distribution of groundwater organisms, mining-associated contaminants, and other water quality characteristics in relation to designated mining-impacted areas. We performed surveys for aquatic life and collected water and sediment samples in 67 locations including 25 sites inside mined areas and 42 outside. Stygobites were observed in two sites (8%) inside mined areas and 24 sites (57%) outside. Ozark Cavefish were detected in 19% of sites including one site inside mined areas and 12 sites outside with four sites new locations for the species. Bristly Cave Crayfish *Cambarus setosus* were found in 37% of sites (one site inside and 24 outside) including six new sites. Aquatic organisms infrequently found inside mined areas were epigean species that are relatively tolerant of degraded habitat and water quality conditions (e.g. Bluegill and Ringed Crayfish).

Sites inside mined areas were marked by high concentrations of metals (dissolved and in sediments) and sulfate. Other major forms of water quality impairment in sites inside mined areas included a lack of nutrients (low nitrate+nitrite), low dissolved oxygen, elevated ammonia, and high temperatures. Additional pollutants (e.g. herbicides, pesticides, PAHs, phthalates) were present at only low concentrations, detected rarely and inconsistently, or more frequent in sites outside mined areas.

Ordination of sites using non-metric multidimensional scaling (NMDS) showed a clear grouping of sites along gradients in metals and environmental variables according to location inside vs. outside mined areas and to the presence of stygobites. The pattern included positive associations between the occurrence of stygobites and both nitrate+nitrite and dissolved oxygen and negative associations with total Phosphorous (P), ammonia, and mining-derived contaminants.

Logistic regression models indicated water quality variables were the best predictors of stygobite distribution (nitrate+nitrite and total P) compared to mining-related variables (sulfate and lead). This was in part due to several sites outside mined areas with low metals concentrations where stygobites were not found. The scarcity of sites inhabited by stygobites inside mined areas precluded a focused comparison of the strength of mining vs. environmental variables for predicting stygobite presence in just those sites. However, even if environmental water quality factors were optimal it is unlikely stygobites could persist at most sites inside mined areas. When groundwater criteria for mining-related contaminants (cadmium, lead, manganese, sulfate, zinc) are considered together, 64% of sites inside mined areas exceeded

chronic thresholds for at least one mining-related contaminant compared to 19% outside. The positive relationship with nitrate+nitrite may reflect benefits to cave fauna from moderate nutrient enrichment and a correlation with landuse in agricultural and urban areas.

Overlap of the Springfield Plateau's karst geology and associated groundwater fauna with historical and ongoing contamination related to mining in the TSMD threatens the survival of unique organisms such as the Ozark Cavefish and may already have eliminated them from some locations. Our results showed that stygobites were unlikely to be present in sites sampled inside designated mining-impacted areas with high concentrations of metals and sulfate, a dearth of nutrients (nitrogen), and low dissolved oxygen the main sources of water quality impairment. In particular, our study illustrates how mining impacts are superimposed on a template of water quality determinants that broadly structure stygobite distributions.

Introduction

Parts of Jasper and Newton counties in Missouri fall within the Tri-State Mining District (TSMD), an area encompassing southwest Missouri, southeast Kansas, and northeast Oklahoma that was mined extensively for lead and zinc from c.1850 to 1960 (IEC 2008). As a result of mining and related activities, large amounts of metals were released into surrounding above- and below-ground environments (Stratus Consulting Inc. 2006). In subterranean ecosystems, groundwaters and sediments were contaminated by cadmium, lead, and zinc (which are known to be toxic at sufficiently high concentrations to a wide variety of plants and animals (Kapustka et al. 2004). Given the unique habitats and species communities characteristic of groundwater environments, there is a high risk for ecological damage and loss of species (Elliott 2000, Romero 2009).

A diverse array of uniquely-adapted organisms potentially occupy the shallow groundwater aquifers that are impacted by historical mining (Noltie and Wicks 2001, Elliott 2007, Graening et al. 2010). Of particular concern is the Ozark Cavefish *Amblyopsis rosae*, a federally-listed Threatened and Missouri-listed Endangered species that is known from relatively few locations associated with the Springfield Plateau in southwestern Missouri, Arkansas, and Oklahoma (USFWS 1989) (Figure 1). There are 21 locations in Missouri considered to be currently occupied by the species and accessible for monitoring with a total of fewer than 50 locations occupied throughout the species' range (Pflieger 1997). The only known habitat for the Ozark Cavefish is the groundwater of the shallow Springfield Plateau aquifer that exists within a few hundred feet of the surface. Other obligate cave-dwelling, aquatic organisms (stygobites) also occur in the region and include the Bristly Cave Crayfish *Cambarus setosus* that is regarded as a Species of Conservation Concern (SOCC) by the Missouri Department of Conservation (MDC)(Pflieger 1996, Graening et al. 2006, MDC 2011) as well as other small, invertebrate amphipods and isopods (e.g. the Ozark Cave Amphipod *Stygobromus ozarkensis*; Elliot 2003).

Limited information is available to describe the toxicity of metals and other mining-related chemicals to stygobites or the role that chronic contamination of underground habitat associated with the TSMD has played in shaping species' distributions. However, detrimental effects of metals to epigean aquatic organisms, in particular fish, are well-known. Excessive concentrations of cadmium, lead, and zinc may cause injury or mortality by disrupting physiological processes, decreasing growth and reproduction, altering behavior, and restricting available habitat (extensive literature reviews can be found in Eisler 1985, 1988, 1993; Sorenson 1991; Kaputska et al. 2004; a detailed review also is available in a preliminary extent of injury assessment in the TSMD done by Stratus Consulting Inc. 2006). Exposure to elevated cadmium is associated with damage to gill tissue leading to respiratory and ionoregulatory failure in addition to immunological depression and muscular dysfunction (EPA 1976, Eisler 1985, Sorensen 1991, Stratus Consulting Inc. 2006). Elevated lead concentrations can lead to muscular and neurological damage including scoliosis and paralysis, hematological disorders, and excess mucous on gill membranes causing asphyxiation (EPA 1976, Eisler 1988, Sorensen 1991, Stratus Consulting Inc. 2006). Elevated zinc also damages gill membranes, suppresses immune response, and may disrupt enzyme pathways linked to the biosynthesis of DNA and

RNA (Sorensen 1991, Eisler 1993, Stratus Consulting Inc. 2006). Sub-lethal effects of exposure to elevated metals include avoidance behavior that may restrict organisms from meeting life history requirements (Atchison et al. 1987). Cutthroat Trout *Oncorhynchus clarkii* avoidance of dissolved zinc was related to declines in natural populations associated with mining contamination in Idaho (Woodward et al. 1997). Metals contamination may also have negative, indirect effects on the ecologies of organisms by reducing or eliminating sensitive prey species thereby affecting the behavior and health of predators (Clements 1991, Clements and Rees 1997). Cadmium, lead, and zinc released by mining in the TSMD are believed to limit fish populations in the Spring River system of Jasper County, in particular the Neosho Madtom *Noturus placidus* which is a federally-listed Threatened species (Wildhaber et al. 2000).

In comparison to epigean species, troglobites are regarded as highly sensitive to pollution and disturbance (Elliott 2000, Wynne and Pleytez 2005). This is primarily a result of the potential for bioaccumulation of contaminants in tissues associated with the lengthy lifespans of troglobites, reduced physiological resistance imparted by low metabolic rates, and adaptation by many troglobites to relatively static environments (Dickson et al. 1979, Romero 2009). Therefore it is reasonable to expect that stygobites in groundwaters associated with the TSMD would be vulnerable and particularly responsive to the acute and chronic effects of mining-related contaminants.

This project is designed to provide information that will enhance species recovery efforts and help meet the goals of an ongoing Natural Resource Damage Assessment (NRDA; http://www.dnr.mo.gov/env/hwp/sfund/nrda.htm) to determine the extent of injury to natural resources as a result of contamination by mining wastes in the TSMD. Both federal and state plans describe degraded water quality as a primary threat to Ozark Cavefish. The federal recovery plan (USFWS 1989) specifically describes water quality in the western portion of the species' range as threatened by toxic metals in the area of old lead and zinc mines of the TSMD and calls for water quality monitoring of heavy metals and population surveys in potential Ozark Cavefish sites. The state action plan (MDC 1999) states that groundwater pollution may be the single greatest threat to the continued existence of the Ozark Cavefish with mine leachates at the top of a list of examples. Water quality monitoring, including assessments for metals contamination, is a primary objective cited for species conservation and recovery.

Our overall goal was to describe relationships between contamination of groundwater and the distribution of Missouri populations of Ozark Cavefish and other stygobites in the region affected by the TSMD to inform the NRDA process and enhance conservation of unique and vulnerable subterranean ecosystems.

Project objectives included:

1. Perform surveys of known (active), unconfirmed, and potential Ozark Cavefish sites inside and outside designated TSMD affected areas to document occurrence of aquatic life and in particular stygobites, including Ozark Cavefish and Bristly Cave Crayfish.

2. Collect water and sediment samples to measure baseline chemical conditions and test for concentrations of metals associated with mining.

3. Describe patterns in the occurrence of aquatic life and in water quality variables in relation to the TSMD.

We expected that sites in proximity to mining-impacted areas would be characterized by higher concentrations of dissolved and deposited metals in addition to other mining-related chemicals and hypothesized that this would have a negative relationship with the presence of aquatic groundwater organisms. In addition, we expected environmental water quality characteristics, such as nutrient concentrations and suites of chemicals typically identified with agricultural and industrial activities, to be highest in rural and urban areas but with limited relation to mining activities. We also hypothesized a negative relationship between the presence of aquatic groundwater life and high concentrations of these water quality variables.

In this report, we present the results of our surveys for aquatic species and analyses of water and sediment samples collected from across a region including Jasper and Newton counties in MO, Ottawa County, OK, and Cherokee County, KS; discuss the evidence for spatial patterns in the association of groundwater contaminants with aquatic life; and compare the strength of mining related contamination with other forms of environmental pollution as agents structuring the distribution of stygobites relative to the TSMD.

Methods

Study Area and Selection of Sample Sites

Sample sites were initially selected from a list of groundwater access locations classified as active, unconfirmed, or potential sites for Ozark Cavefish mostly within Newton and Jasper Counties, MO (Figure 1; Figure 2). Four additional sites that were outside of this area were also sampled including two sites in Lawrence County, MO; one site in Cherokee County, KS; and one site in Ottawa County, OK. All sites were within the geologic boundaries of the Springfield Plateau (for a description of the geographic, geologic, and environmental setting of the region: http://www.dnr.mo.gov/env/wrc/groundwater/education/provinces/springfieldplatprovince.htm). The sites in Lawrence County (R17, R36) were known to be inhabited by Ozark Cavefish. The site in Kansas (M19) was in a mining-impacted area, whereas the site in Oklahoma (R29) was outside of mined areas. Active sites were those sites where Ozark Cavefish had been positively identified and monitored in the past, unconfirmed sites had unsubstantiated reports of Ozark Cavefish, and potential sites were those that did not have reported sightings of Ozark Cavefish but had habitat that appeared suitable. In addition, sites were described as geographically located inside designated mining-impacted areas and labeled with “M##” or outside mined areas and labeled “R##” based on information provided by the U.S. Fish and Wildlife Service (USFWS), onsite observations, and reference to topographic maps for sites near the boundaries of mined areas. This provided a preliminary guide to identifying sampling sites and partitioning effort to help ensure that a range of conditions might be encountered in sites with the potential to harbor Ozark Cavefish and other groundwater organisms.

At each site we attempted to collect three types of data: a visual survey for the presence of larger-sized aquatic species, onsite measurements of water chemistry, water samples for laboratory analyses of dissolved constituents, and a sediment sample for laboratory analysis for metals. We surveyed a diversity of sample sites including cave streams, shallow wells, mine features, and springs. Any natural opening in the bedrock was considered a cave with interior water classified visibly as having no, slow, medium, or fast flow based on turbulence and movement of sediment. Surveyed wells appeared to have been dug for the purpose of accessing the shallow groundwater aquifer for drinking water. They were presumably dug by hand, lined with flat stones and occasionally intersected voids in the karst thereby facilitating access to the well by groundwater organisms. Mine features were water-filled, open shafts of varying diameter, shape, and depth. These differed from wells in that the original purpose for their creation was to access mineral ore deposits in near-surface bedrock (< 100 m) and they were usually deeper than hand-dug wells. The mine voids often intersect karst features. Much of the ore that has been extracted existed in the same aquifer that contains habitat for Ozark Cavefish and other stygobites. Spring sites were natural groundwater discharge points that had been in several instances altered to retain water in a catchment area by way of a spring box or spring house.

Surveys for Aquatic Life

Our visual surveys for larger-sized aquatic species in caves, spring boxes, and spring houses included a search of all reasonably accessible aquatic habitats at the time of the survey using powerful hand-lights and head lamps. Surveys in wells and mine shafts included use of a high-powered spot-light (15 million candle power) to observe potential habitat. Depth of visibility into wells and mine shafts varied greatly in relation to turbidity and flow rate, but in general a 20 foot well with 15 feet of water and low turbidity and flow could be effectively surveyed. Some wells, mines, and springs were further surveyed with the aid of a SideWinder Color Underwater Video Camera with 100-foot cable. The camera's handheld controller allowed for toggling between a down-looking, fixed wide angle view and a horizontal view that could be panned 300 degrees. An internal LED lighting pod illuminated the camera's field of view. Visibility when using the LED lighting was limited to within 4 feet of the camera and was best within 2 feet. The camera was connected to a portable LCD screen that allowed onsite viewing and a digital video recorder (DVR). In some highly secure locations, we were able to conduct underwater video surveillance for approximately 24 hour time periods using a motion-triggered, auto-on feature. For all sites we recorded the amount of time spent in observation and identified as precisely as possible the aquatic species that were seen. Though survey methods and effort differed among sites due to a wide diversity in habitat and logistical features, we believe it did not differ materially between the location factors we were interested in and provide evidence for this assumption. We therefore chose not to adjust results for search effort as the intent in each site was to provide a thorough survey. Indeed, with the variety of approaches required we are not aware of a single standardization (e.g. time, distance, or area searched) that would not introduce additional unwanted bias. Replicate surveys in all sites might have allowed us to incorporate search methods and effort into estimates of detection probability (MacKenzie et al. 2002); however, that degree of sampling intensity was beyond the scope of this project. Ozark Cavefish, Bristly Cave Crayfish, and other vertebrates visible to the unaided eye were readily identified. However, smaller invertebrates could only be tentatively identified as they were usually beyond capture though cave-adapted forms were as a group distinctive (troglobitic isopods and amphipods). For this reason we were unable to verify the identification of amphipods and isopods as stygobites (obligate groundwater-adapted species) as opposed to stygophiles (species that may complete their life-cycles in aquatic environments above or below ground). We did not attempt to screen water or sediment for the presence of microscopic organisms.

Onsite Measurements of Water Quality

We determined water temperature, dissolved oxygen concentration, specific conductance (conductivity), pH, and turbidity onsite using portable field instruments at the time of sample collection. The instruments we used included a YSI 550 Dissolved Oxygen and Temperature Meter, an Oakton EcoTestr EC Low Conductivity Pocket Tester, an Oakton EcoTestr pH2 Waterproof Pocket Tester, and a Hach 2100p Portable Turbidimeter. Instruments were routinely checked to chemical standards and field calibrated following manufacturer's instructions.

Water Samples

We collected water samples into pre-cleaned collection bottles following protocols established by Missouri Department of Natural Resources (MDNR). We ensured that sites were sampled under comparable hydrologic conditions by avoiding sample collection following significant precipitation. In caves, samples were collected from locations as far from the entrance as practical to ensure that the sample was an accurate reflection of the water that was surveyed for aquatic life. In shallow habitats, water was collected directly into sample bottles. In wells and mine shafts, an Aquatic Research Instruments Horizontal Point PVC Water Sampler was used to collect samples at depths < 10 m. Samples were immediately placed on ice and shipped to the MDNR Environmental Services Program (ESP) laboratory in Jefferson City, MO, for analyses within 48 hours in accordance with established chain-of-custody record procedures. Analyses included concentrations of dissolved metals, numerous specific water chemistry parameters, and a qualitative analysis of organic compounds (QOA) (Appendix A). The QOA identified organic compounds that were within the analytical thresholds for detection but did not determine the amounts of each substance. Analysis methods used by ESP laboratory followed EPA-approved standard procedures (http://www.dnr.mo.gov/env/esp/cas/cas-analyticalcapabilities.htm).

Sediment Samples

We collected composite grab samples of wetted sediments to measure cadmium, lead, and zinc concentrations associated with deposited fine materials. In shallow habitats, sediment was collected with multiple scoops of a wooden spoon and transferred into plastic bags. In deep-water habitats (mine shafts and wells), a 23-cm Eckman dredge was employed. The samples were dried completely at room temperature and analyzed for metal concentrations using a Thermo Scientific Niton XL3t x-ray fluorescence (XRF) Analyzer at the USFWS Ecological Services Field Office in Columbia, MO. Samples were unattainable in some sites that were assessed for dissolved metals because fine materials were not present in sufficient quantity or because obtaining sediment was not feasible (e.g. extreme depth).

Statistical Analyses

The dataset that we developed for analyses was composed of sites with a single water sample for each site and a slightly smaller subset of sites with matching sediment samples. Water samples initially collected near the entrance in two cave sites (R28, M19) were replaced with samples later collected from interior locations to provide more relevant results. Water samples were collected from both a cave and an intersecting well in each of two sites (R18, R19); however, sediment could only be collected from within the caves. Consequently, we elected to retain only the cave-derived water samples for those two sites for statistical analyses. Values for metals and chemicals below Method Detection Limits (< MDLs) were replaced with 50% of the MDL for all analyses (Allert et al. 2011). Statistical analyses were performed using the software R (version 2.13.1 Copyright (C) 2011 The R Foundation for Statistical Computing).

We investigated overall patterns in the relationships between a reduced set of mining-associated variables (dissolved metals, sulfate) and environmental variables (nutrients, dissolved oxygen) with regard to both site location category (inside vs. outside of mining-impacted areas) and the presence of aquatic life using Non-metric Multidimensional Scaling (NMDS) ordination techniques (McCune and Grace 2002). Spearman Rank correlations (Zar 1984), prevalence of substances in samples, and our specific information needs helped guide selection of the variables included in the ordination. We used the Vegan package in R to perform the NMDS analysis. The variables were scaled and an optimal distance metric selected using the rankindex function. The metaMDS function was used to configure the sample sites in two dimensions. The ordination was then displayed with sites symbolized by location category and also with sites symbolized by the type of aquatic life that was observed. Vectors showing the strength and direction of gradients in variables were fit to graphs using the envfit function.

Guided by the outcome of the NMDS analysis, we used logistic regression techniques in an information theoretic (IT) modeling approach (Burnham and Anderson 2002) to investigate the relative importance of water quality variables at predicting the presence of stygobites in sample sites. We first modeled mining-related variables and environmental water quality variables separately, consistent with the groupings observed in the ordination. Prior to modeling, variables were transformed to improve approximation of a normal error distribution (Zar 1984).

For most variables, a log-transformation was used; however, dissolved oxygen (mg/L) was squared and a square root transformation was applied to nitrate+nitrite N (mg/L). Logistic regression models were then created using the glm function in R with a binomial error distribution (Crawley 2007). We used the second order Akaike's Information Criterion (AICc) and associated statistics calculated using the R package AICcmodavg (aictab function) as the basis for comparing models (Anderson 2008). In a second step, we combined the mining-related and environmental variables that appeared in the top model (lowest AICc) from each initial analysis into a new model set in order to compare the relative predictive ability of the "best" model parameters. We used multi-model inference involving model averaging parameter estimates across all models (modavg function) and ranking the relative importance of predictor variables to reach the best estimate of each parameter's effect on presence/absence of stygobites and suggest how to focus future research. Overall fit of the global model containing all mining and environmental variables was assessed using a Pearson chi-square comparison testing the null hypothesis that fit is adequate (Anderson 2008). We also used simple linear models and an IT approach to evaluate the evidence that a two-level location factor (inside vs. outside mined areas) explained differences in key mining-related and environmental variables.

We adhered to a descriptive evaluation of the QOA results given the relatively small number of compounds that were detected in multiple sites. Counts of sites in which each compound was detected were summarized by location category and presence/absence of stygobites.

We used logistic regression (glm with binomial error distribution) to model the response of stygobite presence/absence to log-transformed sediment metal concentrations. We also modeled the effect of the two-level location factor on log-transformed sediment metal concentrations with a simple linear model. Information theoretic methods (Burnham and Anderson 2002) were employed to evaluate the evidence for each full model relative to the corresponding null model.

Results

Surveys and collection of water and sediment samples were completed between November 18, 2009, and May 6, 2011. A total of 80 sites were surveyed with the intent of collecting samples; however, some sites were dry and appeared to contain significant water only following heavy precipitation or during chronically wet time periods. Consequently, we were successful at collecting water samples from 67 of the sites and sediment samples from 50 sites (75%; Table 1). Qualitative analysis of organic compounds was completed for 59 sites (88%). Of the sites where water samples were collected, 42% were wells, 30% caves, 16% mine shafts, and 12% springs. Overall, 37% of the sites where water samples were collected were within mined areas compared to 63% that were outside.

Surveys for Aquatic Life

Aquatic life was observed in several sites and at a higher frequency outside of mined areas. Overall, we found aquatic life in 41 sites (61%) including 44% of sites that were inside mined areas compared to 71% that were outside (Table 2; Appendix B). Stygobites were discovered in two sites (8%) inside mined areas and 24 sites (57%) outside. Surveys revealed that 13 sites were inhabited by Ozark Cavefish, 19% of the total searched. This included four sites that were previously not known to be occupied by the species: R19, R22, and R37 located in and just north of Neosho and R36 located in Lawrence County east of Monett (Figure 2). Also included as occupied were three sites where the species is regarded as extant by Missouri Department of Conservation (MDC) but was not seen during the surveys performed for this study (there were no historical Ozark Cavefish sites included in this study). We found Bristly Cave Crayfish in 25 sites. Included were six sites that were not previously known to be occupied: R19, R22, R25, and R39 which were all near Neosho; R27 northeast of Sarcoxie and near the border of Jasper County; and R41 northeast of Joplin (Figure 2). In addition, we counted as occupied five sites where the species was regarded by MDC as extant but was not detected during our surveys. The two species co-occurred in 12 sites, with only one site inhabited by Ozark Cavefish in the apparent absence of Bristly Cave Crayfish. Smaller invertebrate stygobites (amphipods and/or isopods that could possibly have been stygophiles) were discovered in three sites. With the exception of site M17 in southwest Newton County, Ozark Cavefish and Bristly Cave Crayfish were found exclusively in sites outside of designated mining-impacted areas. Only one site inside of mined areas, M19, was inhabited by a small invertebrate stygobite (Table 2).

We documented epigean aquatic organisms in 36% of sites inside mined areas and 14% of sites outside mined areas (Table 2). A limited assortment of fish and invertebrates were identified. Bluegill *Lepomis macrochirus*, were seen in two sites (one site, M06, inside mined areas), and Ringed Crayfish *Orconectes neglectus* and unidentified crayfish (likely Ringed Crayfish or Northern Crayfish *O. virilis*) were seen in six sites (two inside mined areas). Other fish included Channel Catfish *Ictalurus punctatus* and Largemouth Bass *Micropterus salmoides* (seen in one site inside mined areas), and Western Mosquitofish *Gambusia affinis* (seen in one site outside mined areas). Unidentified fish were observed in three sites (one inside mined areas) and unidentified aquatic macroinvertebrates were seen in four sites (two inside mined areas).

Effort allocated to surveys varied by method (direct visual vs. camera) and site type (caves, mines, springs, and wells) due to interactions between site characteristics and the logistical constraints imposed on gear and investigators. However, differences in the mean durations of observations between site location categories for each survey method were relatively minor. The overall mean duration of search effort by direct visual observations was lower than the mean duration of observations by use of the underwater video camera (1.6 and 2.1 hr/site, respectively). Direct visual observation was the predominant method used in caves where use of the camera was not feasible (mean = 2.4 hr/site); however, the camera was utilized more in wells (mean = 4.5 hr/site) where the ability to view horizontally into crevices between rock slabs and at depth was valuable. Average time spent in direct visual observation ranged from 1.3 hr/site in mines, springs, and wells to 2.0 hr/site in caves. Time spent in direct visual observation was similar between sites inside (mean = 1.4 hr/site) and outside of mined areas (mean = 1.6 hr/site). The camera was used for a total of 140.5 hours in 22 sites. This included installation for extended and overnight motion-activated recording in seven sites for periods of 6.5 to 20 hr (mean = 16.4 hr) and more limited use (< 3 hr) to enhance the thoroughness of searches in 15 additional sites (mean search time = 1.5 hr). Out of the 67 sites, we employed the camera in 36% that were inside mining-impacted areas and 31% that were outside. The mean duration of observations by using the camera was slightly higher in sites inside mined areas (2.4 hr/site) compared to sites outside (1.9 hr/site). Stygobites were discovered by use of the camera in 45% of the sites in which it was used compared to 36% of sites in which it was not used.

Onsite Measurements of Water Quality

Measurements of water quality made onsite provided snapshots of prevailing seasonal temperature and flow conditions at the time surveys were performed (Table 3; Appendix B); however, there were differences corresponding to location (Table 4) and the presence of stygobites (Table 5). Temperatures measured in sample sites ranged from 9 to 29^{o}C with the eight warmest measurements (> 21^{o}C) recorded inside mined areas including five mine shafts, two wells, and a cave. The warmest temperature measured in a site inhabited by Ozark Cavefish during this study was 15^{o}C (site M17). Conductivity measured in sample sites ranged from 110 to 900 µS/cm, though only four measurements were > 600 µS/cm. These four sites all were inside designated mining-impacted areas and included three mine shafts (M02, M20, M21) and one cave (M05). Ozark Cavefish were found in a site with conductivity = 530 µS/cm. We found that pH exhibited a limited range of from 6.5 to 8.7, values defined by state water quality criteria (WQC; Code of State Regulations 10 CSR 20-7) as acceptable. By contrast, dissolved oxygen varied widely in sample sites, ranging from 0.2 to 10.2 mg/L. We found that 13 of 21 sites with dissolved oxygen below the applicable WQC of 6 mg/L were inside mined areas with the four lowest measurements (< 1 mg/L) in three mine shafts (M02, M06, M15) and a well (M08). Ozark Cavefish were not observed in sites with dissolved oxygen < 7.4 mg/L. Turbidity measured in sample sites ranged from 0.1 to 891 NTU, though only measurements made at two well sites, one inside mined areas (M10) and one outside (R01), exceeded 100 NTU and were marked by exceptional sediment deposition or visible flow. The highest turbidity measurement associated with Ozark Cavefish in this study was 7.3 NTU.

Water Samples

Chemicals and metals detected in water samples were summarized across sites and considered for inclusion in subsequent analyses (Table 3; Appendix B). Inspection of the data showed that three chemicals, atrazine, dieldrin, and methoxychlor, were found infrequently and could be excluded from additional analyses. The herbicide atrazine was detected in four sites (three outside, one inside mining-impacted areas) with WQC (3 µg/L) exceeded in site R35 (9.2 µg/L) that was outside of mining-impacted areas. Bristly Cave Crayfish were observed in site R35 during this and other studies. Dieldrin, an organochlorine pesticide, was detected in one site outside mining-impacted areas, site M10, at a concentration (0.96 µg/L) that exceeded WQC (0.00014 µg/L). Aquatic life was not observed there. The pesticide methoxychlor was detected in site R31 at a concentration (0.12 µg/L) that exceeded WQC (0.001 µg/L). Site R31 was outside designated mining-impacted areas and inhabited by surface crayfish though stygobites were not found.

Concentrations of dissolved metals and sulfate were highest in sites associated with mining activities and in several instances exceeded WQC (Table 3; Table 4; Appendix B). By contrast, concentrations were markedly lower in sites inhabited by stygobites (Table 5) that were rarely found where state criteria were exceeded. Cadmium was detected in 21 sites (max = 30.6 µg/L, hardness = 258 mg/L) including 15 sites inside mined areas. Acute WQC for cadmium were exceeded in four sites (three inside mined areas), and chronic criteria exceeded in 11 sites (eight inside mined areas). Lead was detected in 22 sites (max = 25.6 µg/L, hardness = 258 mg/L) including 14 inside mined areas. Chronic WQC for lead were exceeded in five sites, all inside mined areas. Zinc was detected in all but one site (max = 3,780 µg/L, hardness = 437 mg/L) with acute and chronic WQC exceeded in 12 sites, 11 inside mined areas. Copper was detected in all but two sites, with concentrations exceeding acute WQC in two sites, one inside and one outside mined areas, and chronic WQC exceeded in one site outside mined areas. Nickel concentrations did not exceed WQC, though ten of 11 sites with the highest concentrations (> 3 µg/L) all were inside mined areas. Manganese concentrations exceeded WQC in 12 sites including eight that were inside mined areas, and iron concentrations exceeded acute WQC in two sites (one inside mined areas) and chronic WQC in one site (inside mined areas). Sulfate was measured at concentrations that exceeded WQC in four sites with the 15 sites with the highest concentrations (19.2 to 398 mg/L) all inside mined areas. In addition, hardness and alkalinity ranged higher in sites associated with mining activity. The seven sites with highest values for hardness (258 to 530 mg/L) and six of eight sites with the highest values for alkalinity (186 to 349 mg/L) all were inside mined areas.

In contrast to the mining-related contaminants, nutrient concentrations were generally not related to location category or were higher in sites that were outside designated mining-impacted areas (Table 4). There were few instances of values exceeding WQC (Table 3; Appendix B) though there were differences related to the presence of stygobites (Table 5). Ammonia nitrogen (N) was detected in 18 sites with a maximum concentration = 1.15 mg N/L (temperature = 15.8^{o}C, pH = 7.5) measured in site R01. Six sites (four inside mined areas, two outside) had ammonia concentrations ≥ 0.1 mg N/L; however, the estimated concentration of un-ionized ammonia, the form most harmful to aquatic organisms, would not have exceeded 0.013 mg/L in any sample (based on equations in Emerson et al. 1975). The concentration of nitrate+nitrite (mg N/L) did not exceed WQC for nitrate (10 mg N/L) with ten of 11 sites with the highest measurements (3.5 to 5.1 mg N/L) outside of mined areas. Total phosphorus (P) concentration > 1 mg/L occurred twice, both in sites that were outside mined areas and of the nine sites with concentrations > 0.20 mg/L four were outside of mined areas.

Ordination of Sites Based on Water Sample Characteristics

We based the NMDS ordination of sites on a combination of mining-related and environmental variables including dissolved metals (cadmium, lead, zinc), sulfate, ammonia, dissolved oxygen, nitrate+nitrite, and total P. Cadmium was retained in the analysis despite a strong correlation (0.73) with zinc because it was a metal we were specifically interested in (Table 6). Other metals were not included because they were strongly, positively correlated with the four variables already selected or because they were detected at concentrations generally below levels of concern. Environmental variables were included due to their well-known roles in regulating aquatic community dynamics (nutrient concentrations, dissolved oxygen) and potential toxicity to organisms (ammonia, nitrate-nitrite). Correlations among these variables were relatively low (Table 7). We did not include temperature or turbidity in the ordination because as described above some measurements may not have adequately represented sites and infrequently exceeded levels of concern. Turbidity also was positively correlated with total P (0.62).

We found distinctive patterns in the grouping of sites along the two ordination axes (NMDS1 and NMDS2) from the perspective of proximity to mining-impacted areas and the occurrence of aquatic organisms. The ordination process employed the Kulczinski distance metric and reached two convergent solutions after 38 tries with final stress of the best solution = 0.14. Gradients in metals and sulfate aligned closely in a negative direction along NMDS1 with zinc and sulfate having the strongest influence (Figure 3). Vectors for ammonia and total P were also oriented in similar, negative directions along NMDS2 with comparable strengths. Gradients in nitrate+nitrite and dissolved oxygen were not as tightly associated but had a positive influence along NMDS1. The grouping of sites in relation to location category showed that sites outside of mined areas were associated with lower concentrations of dissolved metals and sulfate (opposite the direction of gradients indicated by vectors), and higher concentrations of dissolved oxygen and nitrate+nitrite (Figure 3). Though there were some sites inside mined areas associated with high dissolved oxygen and nitrate+nitrite, sites outside mined areas were not associated with high concentrations of metals with the exception of site R11. This site was a well located in south-central Jasper County near the southeastern boundary of mined areas (Figure 2) with elevated cadmium (6.6 µg/L), lead (1.8 µg/L), and zinc (233 µg/L). The ordination of sites in relation to aquatic life showed a tight grouping with sites where stygobites were present associated with high dissolved oxygen and nitrate+nitrite and low concentrations of metals, sulfate, ammonia, and total P (positive scores on NMDS1; Figure 4). The grouping was particularly distinct for sites inhabited by Ozark Cavefish.

Multimodel Inference Using Mining-Related and Environmental Variables

We developed logistic regression models to predict the probability of occurrence of stygobites as a function of the mining-related and environmental variables used in the NMDS ordination. Initial model selection procedures treated each group of variables separately and involved a comparison of models incorporating all possible combinations of explanatory variables in each group (not including interaction terms). This amounted to 16 models utilizing environmental variables (combinations of ammonia, dissolved oxygen, nitrate+nitrite, and total P) and the same number using mining-related variables (combinations of dissolved cadmium, lead, sulfate, and zinc). The highest ranking model with environmental variables included nitrate+nitrite and total P and had a weight of 0.45 (Table 8). In fact, the top four models all included those terms with the addition of dissolved oxygen and ammonia also producing plausible models but with minimal increase in model probability (evidence ratio, E, of model 1 to global model 4 = 4.7). Models 5 to 16 received minimal support compared to model 1 (E > 153) though any model that incorporated one or a combination of environmental variables offered a substantially better fit to the data than the null model. Logistic regression models for individual factors (models 8, 11, 14, and 15) identified negative relationships between the probability of stygobite presence and both ammonia and total P concentrations, and positive relationships with dissolved oxygen and nitrate+nitrite concentrations (Figure 5).

The highest ranking model among the set that incorporated mining-related variables included lead and sulfate and had a model weight of 0.21 (Table 9). There was high to moderate evidence for models 2 through 14 as well (E < 15), but minimal support (E > 126) for the null model and the model including only cadmium. Sulfate appeared in the top eight models, lead in three of the top five. Logistic regression models for individual factors (models 2, 9, 11, and 16) indicated negative relationships between the probability of stygobite presence and concentrations of each mining-related variable (Figure 6).

Based on the outcome of the separate model selection procedures, we combined terms in the top models (nitrate+nitrite, total P, lead, sulfate) and developed a new model set including all possible combinations of those four variables. For comparison, we also included an overall global model composed of all eight environmental and mining-related variables (9 parameters; Table 10). As there was no evidence for lack of fit of the global model ($^{2}_{67-9=57}$ = 46.1, *p* = 0.85), lower-order models were assumed to have adequate fit. The top-ranked model for predicting the probability that stygobites were present included nitrate+nitrite and total P as explanatory variables (model weight = 0.45); however, model 2 added sulfate (model weight = 0.36) and model 3 added lead (model weight = 0.18) as terms and received substantial support compared to model 1 with evidence ratios < 2.6 (Table 10). There was minimal evidence for models ranked 4th and higher (E > 158). There was considerable evidence in support of the three best models and collectively they included all four of the predictor variables. In light of this, we ranked each variable by its relative importance, arrived at by summing up the model weights for each model associated with a particular variable. We then used model averaging to estimate the value of the model parameters across all models. Nitrate+nitrite and total P were the most important variables at predicting stygobite presence in the sites we studied, with sums of model weights near 1 (Table 11). The parameter estimate and associated confidence interval for nitrate+nitrite were positive suggesting that stygobites were more likely to occupy sites with higher concentrations. The parameter estimate and confidence interval for total P was, by contrast, negative, indicating that stygobites were more likely to be found where concentrations of this nutrient were low. In comparison, sulfate and lead ranked substantially lower with parameter estimates that were negative confirming that increased lead and sulfate were associated with a reduced probability of observing stygobites; however, confidence intervals on the parameter estimates included zero, indicating that across the range of sites included in this study lead and sulfate were weak predictors of stygobite presence as there were numerous sites outside of mining-impacted areas with low concentrations where stygobites were not found.

The spatial relationships between concentrations of nitrate+nitrite, total P, presence of stygobites, and proximity to mining-impacted areas are illustrated in Figure 7, with dissolved oxygen and ammonia in Figure 8. Nitrate+nitrite concentrations were at their highest in sites where stygobites were found toward the south and east portion of the study area and generally not associated with mining-impacted areas. Perhaps more notably, stygobites were not found in sites with particularly low concentrations of nitrate+nitrite. A linear model predicting nitrate+nitrite concentration using the two-level location factor was strongly-supported (AICc model weight = 1.000, LL = -52.9, E > 1000, slope ± SE = -0.62 ± 0.14) over a null model (delta AICc = 15.4, LL = -61.7), confirmation that sites inside mined areas had lower concentrations of nitrate+nitrite. In addition, there was significant evidence in support of the logistic regression model predicting a positive effect on stygobite presence in response to nitrate+nitrite concentrations compared to the null model (E > 1000; Table 8). Concentrations of total P were highest in the west and south portions of the study area with stygobites present only in sites with low concentrations. Though several sites in mining-impacted areas had moderate to high concentrations of total P, there was no appreciable evidence for a model that included a location effect (full model: AICc model weight = 0.53, LL = -109.2, E = 1.1; null model: delta AICc = 0.2, LL = -110.4). The logistic regression model predicting a negative effect of total P on stygobite presence was, however, strongly supported over the null model (E = 96; Table 8). Dissolved oxygen concentrations were negatively related to locations categorized as inside mined areas (full model: AICc model weight = 0.996, LL = -314.3, E = 230, slope ± SE = -26.9 ± 7.2; null model: delta AICc = 10.9, LL = -307.7). The most obvious spatial pattern (Figure 8) was that several sites inside mined areas had very low concentrations (< 5.4 mg/L) of DO. There was moderate evidence that the probability of stygobites inhabiting a site was positively related with dissolved oxygen concentration compared to the null model (E = 13; Table 8). We found a moderately strong, positive relationship between concentrations of ammonia and location inside mined areas (full model: AICc model weight = 0.954, LL = -85.5, E = 21, slope ± SE = 0.65 ± 0.22; null model: delta AICc = 6.0, LL = -89.6); however, the evidence that stygobite presence was negatively related to ammonia concentration was moderately weak when compared to the null model (E = 11; Table 8).

Spatial relationships for sulfate and metals concentrations, stygobite presence, and mining impacts are portrayed in Figure 9 and Figure 10. High concentrations of sulfate and metals occurred in northwestern portions of the study area where there was a high density of mining-associated sites. There was very strong support for linear models predicting sulfate and lead concentration that included a two-level location factor (sulfate full model: AICc model weight = 1.000, LL = -89.7, E > 1000; sulfate null model: delta AICc = 26.2, LL = -104.0; lead full model: AICc model weight = 1.000, LL = -101.6, E > 1000; lead null model: delta AICc = 14.1, LL = -109.8). There also was strong to moderate evidence for negative effects of sulfate (E = 109) and lead (E = 26) concentrations on the probability of stygobite presence based on the logistic regression models compared to the null model (Table 9). Zinc and cadmium concentrations also were higher inside mined areas (zinc full model: AICc model weight = 1.000, LL = -139.8, E > 1000, slope ± SE = 3.25 ± 0.50; zinc null model: delta AICc = 26.2, LL = -104.0; cadmium full model: AICc model weight = 0.979, LL = -130.9, E = 47, slope ± SE = 1.44 ± 0.44; cadmium null model: delta AICc = 7.7, LL = -135.8). The evidence for a negative effect on stygobite presence was moderately strong for zinc (E = 16), but weak for cadmium (E = 1.0).

Qualitative Organic Analysis

Qualitative analysis of organic compounds in water samples detected 54 different chemicals in 46 of 59 (78%) samples (Table 12; Appendix B). Most samples contained from one to five compounds, though samples from four sites including three outside mined areas yielded from six to ten. A variety of pollutants were represented; the most frequently detected included phthalate plasticizers and industrial hydrocarbons. We speculated that most of the compounds that were detected would have negative effects on aquatic life if they occurred at high concentrations; therefore, we considered counts of compounds in each site as an indicator of the general level of organic pollution. We found that sites inside mined areas were generally characterized by fewer organic compounds (mean ± SE = 1.6 ± 0.3) compared to sites that were outside (3.0 ± 0.4). We further discovered that counts of organic compounds were higher in sites inhabited by stygobites (3.3 ± 0.5) compared to sites where stygobites were not observed (2.0 ± 0.3).

Most organic compounds identified by QOA were detected in fewer than three sites; however, five sites had eight or more detections (Table 12; Appendix B). The compound 2-sec-Butyl-3-methyl-1-pentene was found in 18 sites, including 15 outside of mined areas and 13 where stygobites were present. Dibutyl phthalate (DBP) was found in 13 sites including 10 outside mined areas and four where stygobites were present. A similar compound, bis (2-ethylhexyl) phthalate, was found in eight sites including five outside mined areas and four where stygobites were present. The compound 1,1,2,2-Tetrachloroethane was detected in nine sites including four outside mined areas and two sites where stygobites were present. Trans-2,4-Dimethylthiane, S,S-dioxide was detected in seven sites, all outside mined areas including six sites where stygobites were present.

Sediment Samples

Lead and zinc concentrations were detected in all sediment samples and generally followed patterns observed for the dissolved equivalents in water samples (Table 3; Table 4; Table 5; Appendix B). Concentrations of lead and zinc in sediments were highly correlated with each other (Spearman Rank correlation, $r_s = 0.81$) and with dissolved concentrations (lead: $r_s = 0.63$; zinc: $r_s = 0.82$). Lead in sediment was measured at a concentration as high as 1,421 mg/kg with the eight highest concentrations (> 300 mg/kg) all from sites inside mining-impacted areas. Stygobites were not observed in those sites though epigean aquatic species were present in four sites. The linear model that included a two-level location factor was strongly-supported (AICc model weight = 1.000, LL = -63.4, E > 1000, slope ± SE = 1.49 ± 0.27) over a null model (delta AICc = 22.9, LL = -76.0), confirming that sites inside mined areas had higher lead concentrations in sediment. In addition, logistic regression provided moderate evidence that stygobites had a lower probability of occurring in sites with higher lead concentrations in sediment (full model: AICc model weight = 0.927, LL = -30.4, E = 13, slope ± SE = -0.82 ± 0.35; null model: delta AICc = 5.1, LL = -34.0; Table 5). Zinc in sediment ranged as high as 190,356 mg/kg with the nine highest concentrations (> 2400 mg/kg) all from sites inside mined areas. Again, stygobites were not observed in those sites though epigean aquatic species were present in four sites. The linear model with a location effect was strongly-supported (AICc model weight = 1.000, LL = -80.4, E > 1000, slope ± SE = 1.96 ± 0.37) over a null model (delta AICc = 20.4, LL = -91.8) and indicated higher sediment zinc concentrations in sites inside mined areas. Logistic regression, however, supplied only weak evidence that the probability of stygobite presence declined with increasing zinc concentration (full model: AICc model weight = 0.796, LL = -31.6, E = 3.9, slope ± SE = -0.49 ± 0.25; null model: delta AICc = 2.7, LL = -34.0). By contrast, cadmium was detected in sediment in only six sites including four inside mined areas and two outside. The maximum concentration of cadmium, 1,587 mg/kg , was measured in mine shaft M14. Measurements in other sites ranged from 12 mg/kg in site R37, a well inhabited by Ozark Cavefish, to 31 mg/kg in site M01, another mine shaft.

Discussion

Our results emphasize that mining impacts are superimposed on a template of water quality determinants that broadly structure stygobite distributions. We found strong spatial patterns in the distribution of groundwater organisms, mining-associated contaminants, and other water quality characteristics in relation to designated mining-impacted areas in the TSMD. Samples from sites inside mined areas were marked by high concentrations of cadmium, lead, and zinc, dissolved and in sediments, as well as sulfate with a corresponding absence of stygobites (Table 13). Other major forms of impairment in sites inside mined areas included a lack of nutrients (low nitrate+nitrite), low dissolved oxygen, elevated ammonia, and high temperatures relative to sites outside mined areas. Considering the broad range of contaminants that samples were tested for, metals contamination was the major source of groundwater pollution inside mined areas. We detected relatively few organic chemicals, and other pollutants were generally in low concentration or inconsistent. There was a striking absence of stygobites in sites inside mined areas, with just one site inhabited by Ozark Cavefish (M17, a hand-dug well) and another by an unidentified isopod (M19, a cave stream). Groundwater samples from these two sites had low concentrations of dissolved metals and therefore may have experienced minimal influence from historical mining activities. Each site was relatively close to the edge of mining-impacted areas in southwestern Newton County (M17) and southeastern Cherokee County, KS (M19)(Figure 2). Overall, stygobites were more likely to occupy sites in which nitrate+nitrite and to a lesser extent dissolved oxygen were higher, and where P, sulfate, lead, zinc, and ammonia were reduced (Table 13).

The NMDS ordination illustrated the distinct grouping of sites inside mined areas that were contaminated by dissolved metals and sulfate and the contrasting grouping of sites outside mined areas in which stygobites were observed and water quality characteristics were apparently within tolerable ranges. Based on the ordination and subsequent statistical analyses we inferred positive associations between the occurrence of stygobites and both nitrate+nitrite and dissolved oxygen. Negative associations were indicated for total P, ammonia, and the mining-derived variables. Modeling indicated that water quality variables that were the best predictors of stygobite distribution (nitrate+nitrite and total P) were overall better predictors than the top mining-related variables (sulfate and lead). One reason metals were comparatively weak predictors is because there were several sites outside mined areas with low metals concentrations where stygobites were not found. The scarcity of sites inhabited by stygobites inside mined areas precludes a spatially restricted comparison of the strength of metals concentrations vs. other water quality variables for predicting stygobite presence in just those sites. Therefore, using a modeling approach we are unable to differentiate between two competing hypotheses (or their combination) that address the question of why stygobites were absent from mined areas during our surveys: 1) nitrate+nitrite deficiency and impairment due to other water quality characteristics such as low dissolved oxygen, 2) elevated metals and sulfate. However, given the probable toxicity of metals to aquatic organisms at the concentrations we measured, there is compelling evidence that even if other water quality factors were optimal it is unlikely stygobites could survive at many sites inside mined areas.

We detected cadmium, lead, and zinc in water and sediment samples collected primarily inside mined areas and at concentrations known to be harmful to a wide variety of aquatic life. Other recent studies in the TSMD and New Lead Belt of southeast Missouri have documented negative relationships between mining-derived metals in water and sediment and epigean stream organisms including fish (Wildhaber et al. 2000, McKee et al. 2010) and crayfish (Allert et al. 2008, Allert et al. 2011). There are no data that describe the specific tolerances of Ozark Cavefish to water quality conditions or to concentrations of contaminants. However, Species Sensitivity Distributions (SSDs) and species-specific Chronic Metals Exposure Response Curves compiled by EPA (2010) allow prediction of the concentrations of metals that would have adverse effects on sensitive aquatic organisms and imply that concentrations we measured in many sites inside mined areas could harm stygobites. We estimated chronic and lethal effects concentrations for Ozark Cavefish based on a coarse assessment of both EPA databases using data for fishes. Acute effects were predicted for the metal concentration resulting in mortality of 50% of test fishes (LC50) over 3 to 30 day exposures and water hardness > 60 mg/L. In some instances, the effect concentrations derived from EPA data were less stringent than the MDNR criteria recommended for protection of aquatic life that more directly incorporated water hardness (WQC; Code of State Regulations 10 CSR 20-7). For example, cadmium was detrimental to salmonid reproduction at concentrations ≥ 1 µg/l (interpolated effect concentration at 5% response level for Brook Trout *Salvelinus fontinalis*, chronic metals plot 33; Benoit et al. 1976) and the LC50 is predicted to be approximately 2 µg/l for the 5% most sensitive species (temperatures ≤ 15^{o}C, SSD plot 39). However, WQC for cadmium were somewhat lower with 0.3 µg/l the minimum concentration calculated to meet criteria for chronic effects in our data. Based on a combination of the WQC chronic effect and EPA acute effect levels, cadmium in 11 of 25 sites (44%) inside mined areas could have chronic effects on Ozark Cavefish and acute effects in six sites (24%). This is compared to chronic effect concentrations of cadmium in 3 of 42 sites (7%) and acute effects in two sites (5%) outside of mined areas. Lead had chronic, negative effects on survival of Fathead Minnow *Pimephales promelas* (chronic metals plot 11; Davies et al. 1976) and hematology of Rainbow Trout *Oncorhynchus mykiss* (Hodson et al. 1978) at concentrations ≥ 13 µg/l. However, WQC indicated that chronic effects could occur at concentrations as low as 1.7 µg/l in our data. The LC50 for the 5% most sensitive species was 15 µg/l (temperatures > 15^{o}C, SSD plot 124). Considering both WQC and EPA data, Ozark Cavefish could be at risk for chronic effects of lead in 5 of 25 sites (20%) inside mined areas compared to 1 of 42 sites (2%) outside. Lead concentrations did not exceed acute effects criteria in any site. Chronic, negative effects of zinc on reproduction of Fathead Minnows occurred at concentrations ≥ 54 µg/l (chronic metals plot 85; Benoit and Holcombe 1978). The LC50 for zinc was 110 µg/l for the 5% most sensitive species (temperatures > 15^{o}C, SSD plot 204). Both sets of EPA data suggested lower effect concentrations for zinc than WQC. Ozark Cavefish could therefore experience chronic effects of elevated zinc in 14 of 25 sites (56%) and acute effects in 12 of 25 sites (48%) inside mined areas compared to chronic effects in 4 of 42 sites (10%) and acute effects in 1 of 42 sites (2%) outside. Other metals, such as manganese and nickel, were also at higher concentrations inside mined areas sampled during our study. Manganese exceeded WQC in 8 of 25 sites (32%) inside mined areas but none outside; nickel concentrations did not exceed WQC. When criteria for metals (cadmium, lead, manganese, zinc) and sulfate are considered together, 16 of 25 sites (64%) inside mined areas exceeded chronic thresholds for at least one contaminant compared to 8 of 42 sites (19%) outside. When dissolved oxygen is added to the group of criteria, 18 sites (72%) inside mined areas exceed chronic thresholds compared to 10 sites (24%) outside.

Given the longevity of many stygobites and the increased potential for accumulation of metals in tissues, even exceedingly low concentrations may ultimately prove harmful (Dickson et al. 1979). Chronic, cumulative, and synergistic effects on aquatic life of contaminants that are at low concentration are poorly understood but may present a unique threat in groundwater ecosystems with dynamics characterized by slow turn-over rates and organisms that may have increased sensitivities to pollution (Wynne and Pleytez 2005). Sources alternative to mining can contribute toxic concentrations of metals to sites inhabited by Ozark Cavefish including application of municipal sludge and poultry waste as fertilizers to pastures and croplands (Graening and Brown 2003). However, our results suggest that metals contamination was rare in sites outside of mining-impacted areas that were dominated by rural land uses. Water samples collected in five sites inhabited by Ozark Cavefish not sampled during this study and far removed from significant mining activity failed to detect dissolved cadmium or lead, and zinc only at low concentrations (2.9 to 9.7 µg/l); D. Novinger unpublished data).

Sulfate was identified by our model selection procedures as the strongest mining-derived predictor of stygobite presence. The potential toxicity of sulfate and other dissolved metals is greatly modified by water hardness, with increased hardness reducing the degree of toxicity to aquatic organisms (Elphick et al. 2011). Considering the values for sulfate we measured and relatively high water hardness, particularly in sites inside mined areas, it is unlikely that sulfate concentrations were high enough to be directly deleterious to aquatic organisms. Instead, it seems probable that sulfate was a more general indicator of mining activity and may index the combined influence of other mining-derived contaminants. Moderate to strong correlations between sulfate and lead (r_s = 0.50), zinc (0.58), and nickel (0.70) support this interpretation.

We were intrigued by the strong support model selection statistics provided for the association of nitrate+nitrite with stygobite presence and the corresponding lack of this nutrient in sites inside mined areas. Excessive nutrient enrichment is generally known to disrupt cave ecosystem function by altering community structure and favoring invasion by epigean species (Graening and Brown 2003). However, low levels of enrichment may have positive effects on Ozark Cavefish and other stygobites by enhancing food supplies in energy-poor environments (Brown and Johnson 2001). The positive relationship between stygobites and the low to moderate concentrations of nitrate+nitrite that we measured could therefore reflect benefits from energy enhancement such as improved growth and reproductive potential. The range in concentrations that we measured was within the range of reference conditions reported for this ecoregion and unlikely to be harmful to most aquatic organisms (EPA 2000). At concentrations beyond some critical threshold, we would expect the relationship to shift from positive to negative (Graening and Brown 2003). For comparison, ongoing monitoring of water quality in sites inhabited by Ozark Cavefish using onsite test kits and continuous instrument recording has documented median nitrate concentrations of 4.0 and 5.9 mg N/L, respectively (D. Novinger unpublished data). The pattern we observed can likely be attributed to correlation between site locations and landuse in groundwater recharge areas such as application of fertilizers to croplands and lawns, animal waste in runoff from livestock operations, and sewage treatment facilities. Internal sources of nitrogen, e.g. bat guano, are likely inconsequential in the sites we sampled as few of them offered habitat for bats or supported significant bat populations and guano piles were not observed. It is now understood that most locations inhabited by Ozark Cavefish do not possess appreciable bat resources (Graening et al. 2010).

Stygobite presence was negatively related with total P in a pattern largely independent of mining influence. It is unlikely that P was directly toxic to aquatic organisms as the concentrations measured in our study were not excessive with only two sites > 1 mg/L and six sites > 0.3 mg/L. However, P concentrations exceeded EPA criteria (EPA 2010) in many sites and may indicate pollution problems (http://www.epa.gov/nutrientpollution/index.html). Ongoing water quality monitoring in Ozark Cavefish sites using onsite test kits found concentrations of orthophosphate as high as 1.9 mg/L with a median of 0.3 mg/L (D. Novinger unpublished data). Perhaps total P is a correlate with other unmeasured organic pollution that limited the suitability of sites for stygobites. The role of N and P in the eutrophication of surface waters is well known and in a few sites the ratio of total N: total P may have been too low (< 7, i.e. N limited) to support a food base for larger aquatic organisms (Horne and Goldman 1994).

Though it did not rank as one of the top water quality predictors of stygobite presence, dissolved oxygen concentrations were notably low in a high percentage of sites inside mined areas. Organisms adapted to groundwater ecosystems may possess tolerance for low dissolved oxygen by virtue of reduced metabolic rates or other unique physiological traits (Poulson 1963, Gannon et al. 1999). Optimum dissolved oxygen concentrations would be substantially higher and vary in accordance with environmental factors, physiological status, and an organism's ability to escape hypoxic conditions (Adams and Johnson 2001). Hill (1968) discovered that Spring Cavefish *Forbesichthys agassizii*, a species found in southeast Missouri that is less cave-adapted compared to Ozark Cavefish (Poulson 1963), preferred dissolved oxygen > 6 mg/L. In our study, the lowest measurement of dissolved oxygen in a site occupied by stygobites (amphipods and isopods) was 2.3 mg/L, and we only observed Ozark Cavefish where dissolved oxygen exceeded 7 mg/L. However, Poulson (1963) found that Ozark Cavefish had markedly lower metabolic rates than other amblyopsids including *F. agassizii*, implying tolerance for low oxygen conditions. Data collected from monitoring populations over several years (D. Novinger unpublished) show that dissolved oxygen in sites occupied by Ozark Cavefish may decline to < 2 mg/L, though median values were closer to 8 mg/L. Whereas Ozark Cavefish may tolerate exceptionally low dissolved oxygen, chronically hypoxic conditions are likely avoided.

Temperature conditions also could have excluded stygobites from some sample sites, in particular eight sites that were inside mined areas where temperatures exceeded 21^{o}C. However, we were constrained to measure temperature relatively close to the surface (< 3 m) and our estimates may not accurately reflect temperature conditions throughout deep mine shafts that may have demonstrated strong thermal stratification. Several mine shaft sites were sampled during July and August when air temperatures were high; therefore temperatures measured near the surface may have been strongly influenced by air temperature whereas temperatures at depth would be more likely to approach ambient groundwater temperatures. Our ability to visually survey sites using the video camera and spotlights frequently exceeded the depth at which temperature was measured. The warmest temperature measured during this study in a site where stygobites were present (Bristly Cave Crayfish) was 20.2^{o}C, with the warmest temperature coinciding with presence of Ozark Cavefish being 15^{o}C. During population monitoring in other sites inhabited by Ozark Cavefish (D. Novinger unpublished), temperatures as high as 20.4^{o}C have been measured with median values near 14^{o}C.

The QOA identified numerous organic chemicals in water samples with sites outside of mined areas, several inhabited by stygobites, contributing the most. Unfortunately, such contaminants have become a ubiquitous component of shallow groundwaters in many regions and may threaten aquatic life through a variety of often poorly-understood mechanisms. Quantitative analyses would be required to determine if chemical concentrations exceeded established criteria (e.g. WQC). However, despite the overall number of compounds detected there were few that occurred in more than a small number of sites. We were unable to find information about likely sources or uses for the most commonly detected hydrocarbon, 2-sec-Butyl-3-methyl-1-pentene (18 sites). Dibutyl phthalate (DBP) was also broadly detected (13 sites) and is used as a plasticizer including as an additive to nail polish, adhesives, printing ink, insecticides, and insect repellants. The compound is regulated by MDNR (WQC) and may interfere with fetal development and disrupt endocrine function (for more information: http://www.atsdr.cdc.gov/tfacts135.pdf). The compound 1,1,2,2-Tetrachloroethane is used as a solvent, degreaser, and in fuels and is described as toxic to aquatic life (for more information: http://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=800&tid=156). It also is regulated by MDNR. It seems likely that in this study the number of organic compounds detected was a more general measure of non-point source pollution correlated with landuse in the rural and urban settings that characterized the region outside mined areas. Most importantly, our data highlight the close surface connection, vulnerability, and potential extent of contamination of shallow groundwater in these karst systems (Graening and Brown 2003) and is a topic that deserves additional research.

If not for a small number of epigean aquatic species, we would have documented waters in most sites in mined areas as lifeless. The species that were most common in those sites are regarded as moderately to generally tolerant of degraded water quality and habitat conditions. We found Bluegill, a fish already considered metal tolerant in the TSMD based on calculated acute toxicity thresholds (Stratus Consulting Inc. 2006), in one mine shaft. It also is a species considered moderately tolerant of poor stream environmental conditions (Jester et al. 1992). Ringed Crayfish and/or Northern Crayfish were discovered in two wells inside mined areas, are species with broad regional distributions, and are regarded as moderately tolerant of degraded conditions including elevated silt and turbidity (Pflieger 1996).

Certain aspects of our approach limit the scope of our conclusions. For example, we were unable to select sites from inside and outside mined areas using random sampling and also were unable to visit sites in a random order. Because of this, we cannot generalize beyond the assemblage of sites that were sampled and were cognizant of the potential for confounding effects caused by nuisance covariates, e.g. seasonal changes in temperature, water levels, and correlated water quality characteristics. We applied sampling protocols to help ensure comparable conditions including avoiding sample collection following significant precipitation and also assessed data for correlations. Consequently, we do not believe unwanted covariation significantly undermines our main conclusions. Finally, concluding absence of stygobites in our study sites means assuming perfect detection probability, or at least the same probability in all sites. Considering the diversity of physical and chemical characteristics and the unique sampling logistics each site presented, this assumption is surely invalid though we made every attempt to survey each site thoroughly. A sampling approach incorporating multiple re-surveys of each site could overcome this deficiency (MacKenzie et al. 2002) but would be costly of resources and would have required substantial reductions in sample sizes to complete the study within the desired time. We elected to maximize the number of different sites that were sampled at the risk of underestimating the presence of stygobites, a discrepancy that we believed would be largely independent of whether sites were inside or outside mined areas. Our methods were sufficient to discover Ozark Cavefish in four sites and Bristly Cave Crayfish in six sites where they were previously unknown.

Overlap of the Springfield Plateau's karst geology and associated groundwater fauna with historical and ongoing contamination related to mining in the TSMD threatens the survival of unique organisms such as the Ozark Cavefish and may already have eliminated them from some locations. Our research showed that stygobites were unlikely to be present in sites sampled inside designated mining-impacted areas with high concentrations of metals and sulfate, a dearth of nutrients (nitrogen), and low dissolved oxygen sources of water quality impairment. Conservation and recovery of threatened species and habitat in karst ecosystems of the Springfield Plateau would greatly benefit from additional research to describe the physiological tolerances of Ozark Cavefish and other stygobites to contaminants and water quality characteristics, as well as the optimal ranges of environmental conditions for these species. Continued surveys in unconfirmed and potential Ozark Cavefish sites and delineation of recharge zones are needed to better define the distribution of stygobites and identify the sources of pollution that threaten them throughout the region.

Conclusions

- We found strong spatial patterns in the distribution of groundwater organisms, mining-associated contaminants, and other water quality characteristics in relation to designated mining-impacted areas in the TSMD in Jasper and Newton counties, MO. Ozark Cavefish were found in four new sites, and Bristly Cave Crayfish in six new sites.

- There was a distinct absence of stygobites, in particular Ozark Cavefish and Bristly Cave Crayfish, in groundwater sample sites inside mined areas. The two sites occupied by stygobites within but near the edge of mined areas had low concentrations of metals in water and sediment samples and may have been minimally impacted by mining.

- Samples from sites inside mined areas were marked by high concentrations of cadmium, lead, and zinc, dissolved and in sediments, as well as sulfate. Other major forms of water quality impairment in sites inside mined areas included a lack of nutrients (low nitrate+nitrite), low dissolved oxygen, elevated ammonia, and high temperatures relative to sites outside mined areas. However, even if environmental water quality factors were optimal it is unlikely stygobites could persist at most sites inside mined areas. When criteria for mining-related contaminants are considered together, 64% of sites inside mined areas exceeded chronic thresholds for at least one mining-related contaminant compared to 19% outside.

- Nutrient concentrations, including nitrate+nitrite and total P, were the best range-wide predictors of stygobite presence in the sites sampled for this study. The positive relationship with nitrate+nitrite may reflect benefits to cave fauna from moderate nutrient enrichment and a correlation with landuse in agricultural and urban areas.

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Table 1. Number of sites where water samples (W), sediment samples (S), and QOA tests (Q) were completed by site type and location category.

Site Type	Inside Mined Areas			Outside Mined Areas			Total		
	W	S	Q	W	S	Q	W	S	Q
Cave	4	3	3	16	15	15	20	18	18
Mine	11	8	11	0	0	0	11	8	11
Spring	0	0	0	8	8	7	8	8	7
Well	10	5	8	18	11	15	28	16	23
Total	25	16	22	42	34	37	67	50	59

Table 2. Counts of sites by type of aquatic life observed and location category.

Aquatic Life Category	Inside Mined Areas	Outside Mined Areas	Total
Aquatic life absent	14	12	26
Epigean aquatic life	9	6	15
(Stygobite absent Sub-total)	(23)	(18)	(41)
Stygobite invertebrates	1	2	3
Bristly Cave Crayfish	0	10	10
Ozark Cavefish	1	12	13
(Stygobite present Sub-total)	(2)	(24)	(26)
(Aquatic life present Sub-total)	(11)	(30)	(41)
Total	25	42	67

Table 3. Descriptive statistical summaries of sample site water quality characteristics determined by onsite measurements, water sample analyses, and sediment sample analyses. Precision is reported for instruments used for onsite measurements and Minimum Detection Limit (MDL) is reported for water and sediment sample tests. Applicable State of Missouri water quality criteria (WQC; MDNR 10 CSR 20-7 v.5/31/2012) and EPA nutrient criteria for rivers and streams in Ecoregion XI (*; EPA 2000) are included as a minimum (dissolved oxygen), maximum, or range if established.

Variable	Precision/ MDL	n	Mean	SD	Min	Q1	Median	Q3	Max	WQC
Onsite Measurements										
Temperature ^{o}C	0.2	66	15.6	4.4	8.7	13.2	14.4	17.1	29.2	20[1]
Specific conductance µS/cm	1%	64	345	152	110	250	350	383	900	NA
pH	0.1	66	7.5	0.5	6.5	7.2	7.4	7.6	8.7	6.5-9.0
Dissolved oxygen mg/L	0.3	65	6.8	2.7	0.2	5.3	7.9	8.6	10.2	6[1]
Dissolved oxygen %	2	65	67.0	25.7	2.4	54.4	77.0	84.6	99.2	NA
Turbidity NTU	2%	66	24.8	110.4	0.1	1.2	2.8	10.9	891.0	2.30*
Water Samples										
Atrazine µg/L	0.25	67	0.30	1.12	0.13	0.13	0.13	0.13	9.20	3[2]
Dieldrin µg/L	0.1	67	0.06	0.11	0.05	0.05	0.05	0.05	0.96	0.00014[2]
Methoxychlor µg/L	0.1	67	0.05	0.01	0.05	0.05	0.05	0.05	0.12	40[2]/0.03[3]
Cadmium µg/L	0.2	67	1.5	4.6	0.0	0.1	0.1	0.3	30.6	5[2,4]
Copper µg/L	0.01	67	1.6	2.7	0.3	0.5	0.8	1.6	16.1	1,300[2,4]
Iron µg/L	1.0	67	67.1	228.4	0.5	3.7	8.9	21.8	1,360.0	300[2]
Lead µg/L	0.25	67	1.0	3.4	0.1	0.1	0.1	0.4	25.6	15[2,4]
Magnesium mg/L	0.01	67	3.0	1.8	0.8	1.7	2.5	3.3	8.7	NA
Manganese µg/L	0.25	67	92.9	340.9	0.1	1.0	2.8	14.0	2,570.0	50[2]
Nickel µg/L	0.2	67	2.2	4.0	0.1	0.7	1.0	1.6	20.2	100[2,4]
Sulfate mg/L	0.01	67	34.2	79.2	3.1	5.9	7.3	19.2	398.0	250[4,5]
Zinc µg/L	0.25	67	305.1	834.4	0.3	2.3	5.8	54.2	3,780.0	5,000[2,4]
Chloride mg/L	0.01	67	9.4	8.9	1.5	4.2	6.7	10.3	42.4	250[6]
Calcium mg/L	0.01	67	67.8	38.2	14.4	46.5	63.9	75.6	203.0	NA
Hardness mg/L	0.01	67	181.5	100.2	42.5	125.0	170.8	202.0	530.0	NA
Alkalinity mg/L	5.0	67	136.5	51.2	37.0	105.5	140.0	164.0	349.0	NA
Ammonia N mg/L	0.03	67	0.06	0.16	0.02	0.02	0.02	0.03	1.15	acute 1.4-32.6 chronic 0.24-6.66[7]
Nitrate+nitrite N mg/L	0.01	66	1.81	1.44	0.01	0.54	1.71	2.85	5.08	10[2,8]
Total nitrogen mg/L	0.01	66	2.07	1.45	0.06	0.80	1.95	3.13	5.42	0.31*
Total phosphorous mg/L	0.01	66	0.13	0.34	0.01	0.02	0.03	0.10	1.96	0.01*
QOA # detected	NA	59	2.5	2.2	0	1	2	4	10	NA

Variable	Precision/ MDL	n	Mean	SD	Min	Q1	Median	Q3	Max	WQC
Sediment Samples										
Cadmium mg/kg	9.7	51	37.9	221.3	4.9	4.9	4.9	4.9	1587.0	NA
Lead mg/kg	8.1	50	164.5	264.0	13.0	34.5	53.0	113.2	1421.0	NA
Zinc mg/kg	13.5	50	4,749	26,819	50	183	347	1,046	190,356	NA

[1] Criteria for protection of aquatic life (AQL) in cold-waters
[2] Criteria for groundwater protection (GRW)
[3] Criteria for AQL
[4] Criteria for AQL are hardness-dependent
[5] Criteria for drinking water protection (DWS)
[6] Chronic criteria for DWS
[7] Acute criteria for total ammonia N for cold-water fisheries are pH-dependent. The range based on min and max pH measured is shown; chronic criteria assume early life-stages of aquatic life are present and are pH and temperature-dependent. The range based on min and max pH and temperature measured is shown
[8] Criteria are for nitrate-N

Table 4. Mean, standard deviation (SD), median, interquartile range (Q1-Q3), and sample size (n) for variables measured in water and sediment samples summarized by sample site location category (inside vs. outside designated mining impacted areas).

	Inside Mined Areas					Outside Mined Areas				
Variable	Mean	SD	Median	IQR	n	Mean	SD	Median	IQR	n
Temperature ^{o}C	18.6	5.3	16.9	14.7-21.4	25	13.8	2.3	13.6	12.5-14.7	41
Conductivity µS/cm	401	213	350	253-470	22	316	99	340	253-380	42
pH	7.4	0.5	7.3	7.1-7.6	25	7.5	0.5	7.4	7.2-7.7	41
Dissolved oxygen mg/L	5.2	3.1	5.4	3.2-8.1	24	7.7	2.0	8.2	7.2-8.8	41
Turbidity NTU	19.2	29.8	4.7	2.0-20.7	24	28.0	137.0	2.1	1.1-7.3	42
Cadmium µg/L	3.36	7.02	0.26	0.10-1.01	25	0.33	1.09	0.10	0.08-0.10	42
Copper µg/L	1.91	2.96	1.01	0.69-1.94	25	1.47	2.64	0.64	0.49-1.18	42
Iron µg/L	88.4	260.0	8.3	4.4-21.3	25	54.4	209.7	9.2	3.6-21.3	42
Lead µg/L	2.4	5.4	0.4	0.1-1.6	25	0.22	0.28	0.13	0.13-0.13	42
Magnesium mg/L	3.8	2.1	3.0	2.1-4.8	25	2.5	1.4	2.2	1.6-3.1	42
Manganese µg/L	211.5	530.2	8.0	2.1-97.4	25	22.3	94.1	1.6	0.8-4.6	42
Nickel µg/L	4.52	5.86	1.75	1.00-3.95	25	0.89	0.84	0.87	0.36-1.06	42
Sulfate mg/L	77.7	118.7	26.2	7.2-81.2	25	8.3	4.6	6.4	5.3-10.1	42
Zinc µg/L	790.3	1233.3	54.5	15.6-1060.0	25	16.3	39.1	3.4	1.2-11.0	42
Chloride mg/L	8.8	9.1	5.5	3.1-8.9	25	9.8	8.9	7.3	4.4-10.7	42
Calcium mg/L	87.9	51.9	72.0	60.1-95.5	25	56	19.5	57	44-71	42
Alkalinity mg/L	153	59	150	131-170	25	127	44	128	97-160	42
Hardness mg/L	235	136	196	159-258	25	146	501	157	116-185	42
Ammonia N mg/L	0.07	0.12	0.03	0.02-0.06	25	0.05	0.18	0.02	0.02-0.02	42
Nitrate+nitrite N mg/L	1.03	1.24	0.41	0.06-2.01	24	2.3	1.36	2.1	1.2-3.1	42
Total nitrogen mg/L	1.41	1.3	0.8	0.3-2.2	24	2.4	1.4	2.4	1.2-3.3	42
Total phosphorous mg/L	0.13	0.18	0.05	0.02-0.15	24	0.14	0.40	0.03	0.02-0.06	42
Cadmium (sediment) mg/kg	108.1	394	4.9	4.9-9.4	16	5.8	4.4	4.9	4.9-4.9	34
Lead (sediment) mg/kg	381	383	265	95-598	16	63	59	44	31-71	34
Zinc (sediment) mg/kg	13989	47066	2584	458-3326	16	400	431	217	130-537	34

Table 5. Mean, standard deviation (SD), median, interquartile range (Q1-Q3), and sample size (n) for variables measured in water and sediment samples summarized by presence/absence of stygobites in sample sites.

	Stygobites Absent					Stygobites Present				
Variable	Mean	SD	Median	IQR	n	Mean	SD	Median	IQR	n
Temperature ^{o}C	16.7	5.2	15.2	13.1-19.5	40	14.0	2.0	13.7	13.2-14.7	26
Conductivity µS/cm	344	181	330	250-370	38	347	99	370	305-400	26
pH	7.5	0.5	7.4	7.2-7.6	40	7.4	0.4	7.5	7.2-7.6	26
Dissolved oxygen mg/L	6.1	3.1	6.9	3.9-8.6	39	7.8	1.6	8.3	7.2-8.6	26
Turbidity NTU	36.0	138.9	4.7	1.3-14.0	41	6.5	15.7	1.8	1.0-4.2	25
Cadmium µg/L	2.25	5.70	0.10	0.05-0.69	41	0.22	0.59	0.10	0.10-0.10	26
Copper µg/L	1.74	2.55	0.97	0.54-1.94	41	1.47	3.09	0.64	0.54-0.96	26
Iron µg/L	70.0	205.5	9.6	4.4-47.7	41	62.6	264.8	8.2	3.5-14.4	26
Lead µg/L	1.59	4.34	0.13	0.13-0.82	41	0.18	0.13	0.13	0.13-0.13	26
Magnesium mg/L	3.2	2.1	2.6	1.9-4.2	41	2.6	1.1	2.4	1.7-3.1	26
Manganese µg/L	145.7	429.1	3.0	1.2-33.1	41	9.7	22.9	2.6	1.0-4.9	26
Nickel µg/L	3.18	4.91	1.12	0.74-3.17	41	0.77	0.45	0.90	0.36-0.99	26
Sulfate mg/L	50.5	98.2	11.3	6.2-37.6	41	8.6	6.0	6.6	5.2-8.4	26
Zinc µg/L	491	1028	21	3-233	41	12	20	4	2-11	26
Chloride mg/L	8.3	8.6	5.5	3.1-8.9	41	11.1	9.1	8.4	5.1-11.8	26
Calcium mg/L	71.6	46.7	62.8	46.8-77.9	41	61.8	17.5	64.1	48.3-72.8	26
Alkalinity mg/L	137	57	140	107-162	41	135	41	140	106-163	26
Hardness mg/L	192	122	174	122-205	41	165	45	169	131-194	26
Ammonia N mg/L	0.08	0.20	0.02	0.02-0.05	41	0.02	0.02	0.02	0.02-0.02	26
Nitrate+nitrite N mg/L	1.27	1.28	0.68	0.22-1.96	41	2.71	1.23	2.77	1.72-3.66	25
Total nitrogen mg/L	1.59	1.37	1.15	0.48-2.41	41	2.86	1.24	2.65	1.93-3.94	25
Total phosphorous mg/L	0.19	0.42	0.05	0.02-0.15	41	0.04	0.05	0.03	0.01-0.03	25
Cadmium (sediment) mg/kg	63	293	5	5-5	29	5	2	5	5-5	21
Lead (sediment) mg/kg	240	325	101	36-310	29	60	56	43	34-55	21
Zinc (sediment) mg/kg	7904	35129	604	200-2685	29	392	408	220	182-465	21

Table 6. Spearman rank correlations among dissolved metals and sulfate measured in water samples.

	Cadmium	Copper	Iron	Lead	Manganese	Nickel	Sulfate	Zinc
Cadmium	1.00	0.22	-0.07	0.45	0.36	0.49	0.39	0.73
Copper	0.22	1.00	0.50	0.44	0.44	0.43	0.38	0.52
Iron	-0.07	0.50	1.00	0.28	0.52	0.22	0.18	0.19
Lead	0.45	0.44	0.28	1.00	0.39	0.54	0.50	0.62
Manganese	0.36	0.44	0.52	0.39	1.00	0.44	0.44	0.58
Nickel	0.49	0.43	0.22	0.54	0.44	1.00	0.70	0.65
Sulfate	0.39	0.38	0.18	0.50	0.44	0.70	1.00	0.58
Zinc	0.73	0.52	0.19	0.62	0.58	0.65	0.58	1.00

Table 7. Spearman rank correlations among environmental variables measured onsite and in water samples.

	Ammonia	Dissolved oxygen	Nitrate+nitrite	Temperature	Total P	Turbidity
Ammonia	1.00	-0.28	-0.30	0.44	0.48	0.46
Dissolved oxygen	-0.28	1.00	0.39	-0.44	-0.19	-0.08
Nitrate+nitrite	-0.30	0.39	1.00	-0.26	0.01	-0.12
Temperature	0.44	-0.44	-0.26	1.00	0.17	0.29
Total P	0.48	-0.19	0.01	0.17	1.00	0.62
Turbidity	0.46	-0.08	-0.12	0.29	0.62	1.00

Table 8. Model selection statistics for 16 models of environmental water quality parameters used to predict presence/absence of stygobites including ammonia N (am), dissolved oxygen (do), nitrate+nitrite N (nn), total P (tp). Shown are the number of estimated parameters (K), second-order Akaike's information criterion (AICc), difference in AICc relative to the best model (Delta_AICc), model likelihood (ModelLik), model probability or weight (AICcWt), log-likelihood (LL), and cumulative model probabilities (Cum.Wt).

Model	K	AICc	Delta_AICc	ModelLik	AICcWt	LL	Cum.Wt
1. nn+tp	3	61.737	0.000	1.000	0.448	-27.675	0.448
2. do+nn+tp	4	62.851	1.114	0.573	0.257	-27.086	0.705
3. am+nn+tp	4	63.450	1.713	0.425	0.190	-27.397	0.895
4. am+do+nn+tp	5	64.839	3.102	0.212	0.095	-26.902	0.990
5. am+do+nn	4	71.799	10.062	0.007	0.003	-31.561	0.993
6. am+nn	3	72.013	10.275	0.006	0.003	-32.813	0.995
7. do+nn	3	72.144	10.407	0.005	0.002	-32.872	0.998
8. nn	2	72.495	10.758	0.005	0.002	-34.152	1.000
9. do+tp	3	79.777	18.040	0.000	0.000	-36.688	1.000
10. am+do+tp	4	80.830	19.093	0.000	0.000	-36.076	1.000
11. tp	2	82.430	20.693	0.000	0.000	-39.120	1.000
12. am+tp	3	83.022	21.285	0.000	0.000	-38.317	1.000
13. am+do	3	83.609	21.872	0.000	0.000	-38.608	1.000
14. do	2	86.439	24.702	0.000	0.000	-41.123	1.000
15. am	2	86.721	24.984	0.000	0.000	-41.267	1.000
16. .	1	91.556	29.819	0.000	0.000	-44.747	1.000

Table 9. Model selection statistics for 16 models of mining-related water quality parameters including dissolved cadmium (cd), lead (pb), sulfate (so), and zinc (zn). Description of headings follows Table 8.

Model	K	AICc	Delta_AICc	ModelLik	AICcWt	LL	Cum.Wt
1. pb+so	3	81.881	0.000	1.000	0.213	-37.750	0.213
2. so	2	82.167	0.286	0.867	0.185	-38.990	0.398
3. cd+pb+so	4	82.972	1.091	0.580	0.123	-37.164	0.521
4. so+zn	3	83.651	1.769	0.413	0.088	-38.635	0.609
5. pb+so+zn	4	84.131	2.250	0.325	0.069	-37.743	0.678
6. cd+so	3	84.227	2.346	0.309	0.066	-38.923	0.744
7. cd+so+zn	4	84.535	2.654	0.265	0.057	-37.945	0.801
8. cd+pb+so+zn	5	84.979	3.098	0.212	0.045	-36.998	0.846
9. pb	2	85.036	3.154	0.207	0.044	-40.424	0.890
10. pb+zn	3	85.916	4.034	0.133	0.028	-39.767	0.918
11. zn	2	85.973	4.092	0.129	0.028	-40.893	0.946
12. cd+pb+zn	4	86.699	4.817	0.090	0.019	-39.027	0.965
13. cd+zn	3	86.945	5.063	0.080	0.017	-40.282	0.982
14. cd+pb	3	87.198	5.317	0.070	0.015	-40.409	0.997
15. .	1	91.556	9.675	0.008	0.002	-44.747	0.998
16. cd	2	91.599	9.718	0.008	0.002	-43.706	1.000

Table 10. Model selection statistics for 17 models that combined the parameters in the top-ranked environmental and mining-related water quality models (see Table 8 and Table 9). Description of headings follows Table 8.

Modnames	K	AICc	Delta_AICc	ModelLik	AICcWt	LL	Cum.Wt
1. nn+tp	3	61.737	0.000	1.000	0.405	-27.675	0.405
2. nn+so+tp	4	62.197	0.460	0.795	0.322	-26.771	0.727
3. nn+pb+tp	4	63.606	1.869	0.393	0.159	-27.475	0.887
4. nn+pb+so+tp	5	64.541	2.804	0.246	0.100	-26.771	0.986
5. nn+so	3	71.010	9.273	0.010	0.004	-32.312	0.990
6. am+do+nn+tp+cd+pb+so+zn	9	71.874	10.137	0.006	0.003	-25.270	0.993
7. nn+pb	3	71.884	10.147	0.006	0.003	-32.749	0.995
8. nn+pb+so	4	72.434	10.697	0.005	0.002	-31.889	0.997
9. nn	2	72.495	10.758	0.005	0.002	-34.152	0.999
10. so+tp	3	75.190	13.453	0.001	0.000	-34.401	1.000
11. pb+so+tp	4	76.848	15.111	0.001	0.000	-34.096	1.000
12. pb+tp	3	79.402	17.665	0.000	0.000	-36.507	1.000
13. pb+so	3	81.881	20.144	0.000	0.000	-37.750	1.000
14. so	2	82.167	20.430	0.000	0.000	-38.990	1.000
15. tp	2	82.430	20.693	0.000	0.000	-39.120	1.000
16. pb	2	85.036	23.299	0.000	0.000	-40.424	1.000
17. .	1	91.556	29.819	0.000	0.000	-44.747	1.000

Table 11. Results of model averaging parameters in the combined environmental and mining-related water quality models (Table 10). Shown is the sum of model probabilities for models incorporating each parameter, the model-averaged parameter estimate, and associated unconditional standard error and 95% confidence interval.

	AICcWt	Model-averaged estimates		
Parameter	Sum	Coefficient	SE	95% CI
Nitrate+nitrite N	0.999	2.76	0.83	(1.12 , 4.39)
Total P	0.991	-1.26	0.49	(-2.21 , -0.31)
Sulfate	0.431	-0.61	0.49	(-1.56 , 0.35)
Lead	0.268	-0.15	0.41	(-0.95 , 0.66)

Table 12. Counts of sites in which organic chemical compounds were detected by QOA including for each compound the total number of sites, totals by location category (inside vs. outside mined areas), and totals by stygobite absence (A) or presence (P).

	Sites	Location		Stygobites	
Organic Chemical Compound	#	in	out	A	P
2,3,3-Trimethyl-2-hexene	1		1		1
(2,6,6-Trimethylcyclohex-1-enylmethane sulfonyl) Benzene	1		1		1
1-(1-Ethyl-2,3-dimethyl-cyclopent-2-enyl) ethanone	5	2	3	2	3
1-(1-Methyl-cyclohexyl)-ethanone	1		1		1
1-(1-methylethyl) Cyclopentanone	1		1	1	
1,1,2,2-Tetrachlorethane	9	4	5	7	2
1,1,2-Trimethyl-3-(2-methylpropyl) cyclopropane	2		2		2
1,1,4-Trimethylcyclohexane	1		1		1
1,1-Dimethyl propyl cyclohexane	1		1	1	
1,2,3,4,5-Pentamethyl cyclopentane	4		4	2	2
1-Dotriacontanol	1	1		1	
1-Ethyl-4-methyl trans-cyclohexane	1		1	1	
1-Isopropyl-1-methyl cyclohexane	6	1	5	2	4
1-Isopropyl-3-methyl cyclohexane	1		1	1	
1-Methyl-2-propyl cyclohexane	1		1	1	
1-Methyl-5-(1-methylethenyl)-(R)-cyclohexene	1		1	1	
2-(1,1-Dimethylethyl)-4-(1-methyl-1-phenylethyl) Phenol	4		4		4
2-(1-methylpropyl) Cyclopentanone	3		3	1	2
2,2,3-Trimethyl-1-hexene	1		1		1
2,2-Dimethyl-3-octene	3	1	2	1	2
2,3,3-Trimethyl-1-hexene	2		2		2
2,3,4,5-Tetramethyl-1, 4-hexadiene	4	1	3		4
2,3-Epoxy cholstane (2.alpha., 3.alpha., 5.alpha.)	1	1		1	
2,4-Di-t-butyl-6-nitrophenol	1	1		1	
2,5,5-Trimethyl-2-hexane	1		1		1
2,5,8,11 - Tetraoxadodecane	4		4	3	1
2-Hexyl-1-octanol	1	1		1	
2-Methyl propanoic acid, 2-ethyl-3-hydroxyhexyl ester	3		3	2	1
2-Methyl propanoic acid, 3-hydroxy-2,4,4-trimethylpentyl ester	6	1	5	4	2
2-Methyl-2,2-diemethyl-1-(2-hydroxy-1-methylethyl) propyl ester propanoic acid	1	1		1	
2-Methyl-2-ethyl-3-hydroxyhexyl ester propanoic acid	1	1		1	
2-Methyl-3-butyn-2-ol	1		1		1
2-Methyl-propanoic acid, 3-hydroxy-2,4,4-trimethylpentyl ester	1		1		1
2-sec-Butyl-3-methyl-1-pentene	18	3	15	5	13
3,5,9-Trimethyl-deca-2,4,8-trien-1-ol	1		1		1
3-Ethyl-2-methyl-1-pentene	4	1	3	1	3

Table 12 continued.

Organic Chemical Compound	Sites #	Location in	Location out	Stygobites A	Stygobites P
3-Methyl propanoic acid, 3 hydoxy-2,4,4-trimethylpentyl ester	1		1	1	
3-Methyl-(E)-2-Nonene	1		1		1
3-Methyl-1-penten-4-yn-3-ol	1	1		1	
Bis (2-ethylhexyl) phthalate	8	3	5	5	3
Cholesta-3,5-diene	1	1		1	
Cholesterol	1	1		1	
Cyclic octaatomic sulfur	2	2		2	
Dibutyl phtalate	13	3	10	9	4
Diethyl toluamide	4	2	2	2	2
Ethyl benzene	1		1		1
Methoxy-phenol oxime	3		3	2	1
Mono (2-ethylhexyl) ester Hexanedioic acid	1	1		1	
Monocylohexyl ester phthalic acid	1		1		1
Phthalic acid, diisooctyl ester	1	1		1	
Phthalic anhydride	2		2	1	1
p-tert-amyl phenoxy ethanol	1	1		1	
p-Xylene	3		3	2	1
trans-2,4-Dimethylthiane, S,S-dioxide	8		8	1	7

Table 13. Summary of general relationships described during this study between environmental and mining-related variables and 1) site location, and 2) presence of stygobites.

Variable	Relationship to location (inside vs. outside mined areas)	Relationship to stygobites (present vs. absent)
Nitrate+nitrite	Lower inside	Higher where present
Total P	Similar inside and outside	Lower where present
Dissolved oxygen	Lower inside	Somewhat higher where present
Ammonia	Higher inside	Somewhat lower where present
Sulfate	Higher inside	Lower where present
Lead	Higher inside	Lower where present
Zinc	Higher inside	Lower where present
Cadmium	Higher inside	Similar where present and absent

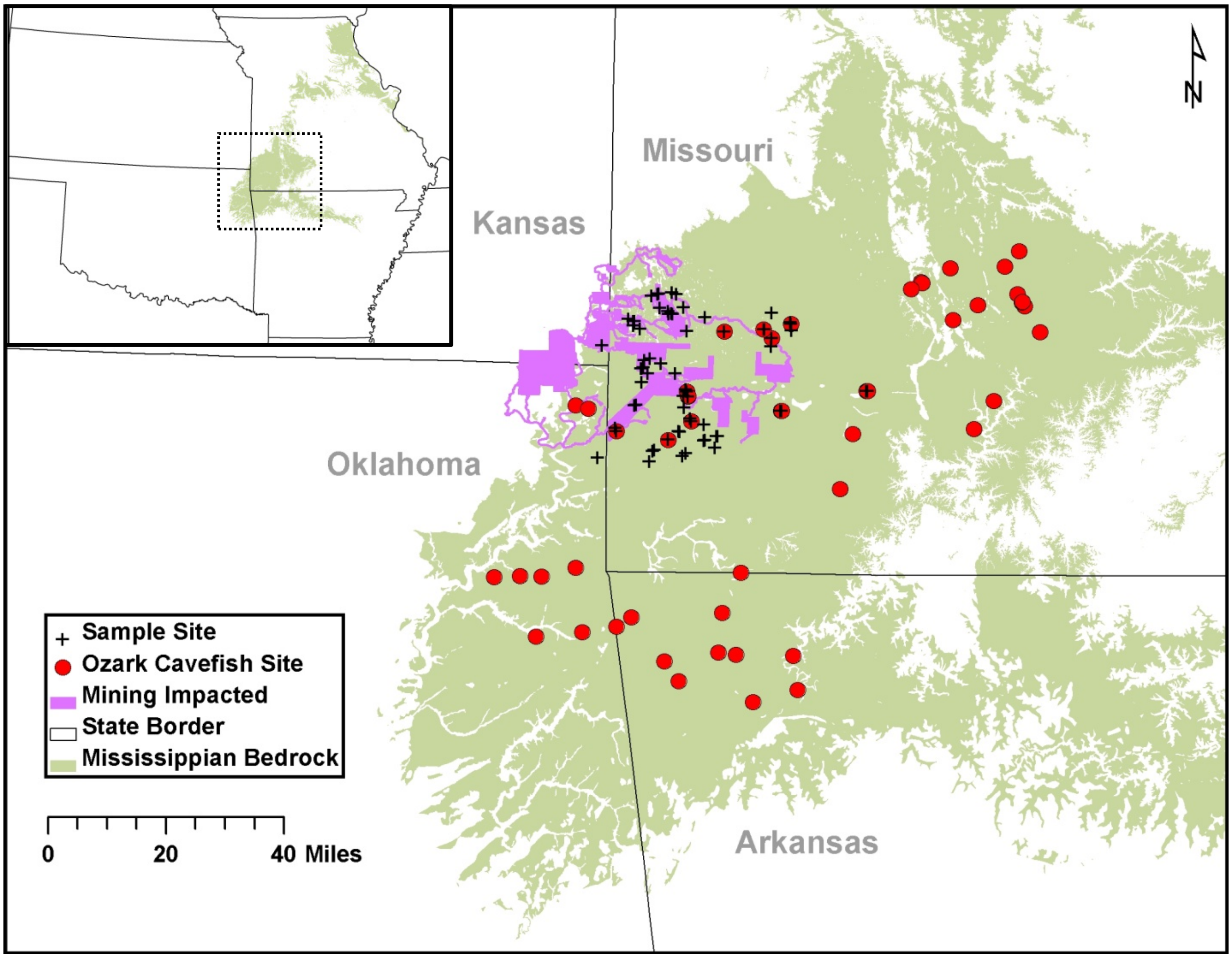


Figure 1. Ozark Cavefish distribution (current and historical sites) in relation to the Mississippian bedrock geology of the Springfield Plateau, areas impacted by mining in the Tri-State Mining District, and sites sampled during this study (adapted from Graening et al. 2010).

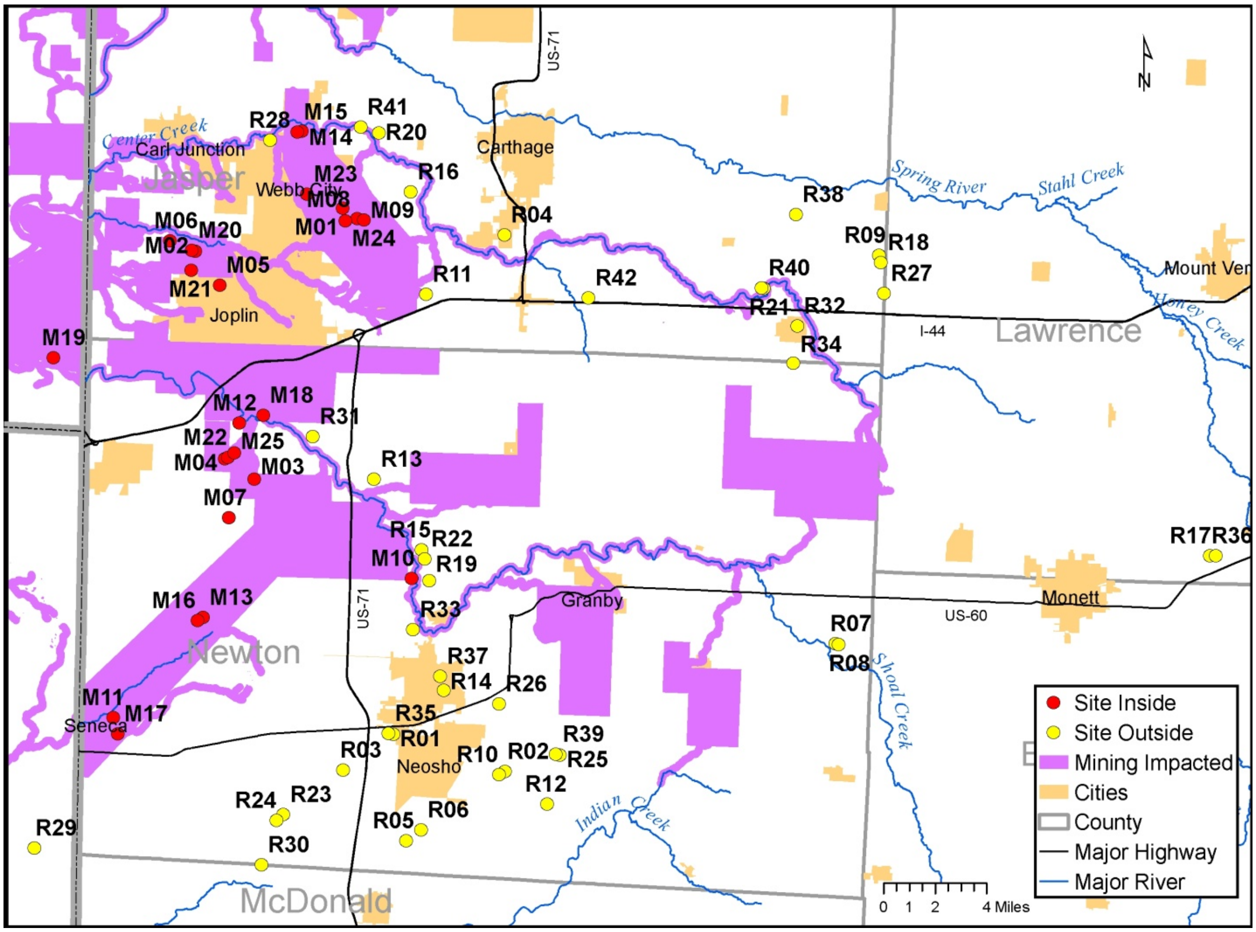


Figure 2. Location of sample sites categorized as inside (M#) or outside (R#) of designated mining-impacted areas.

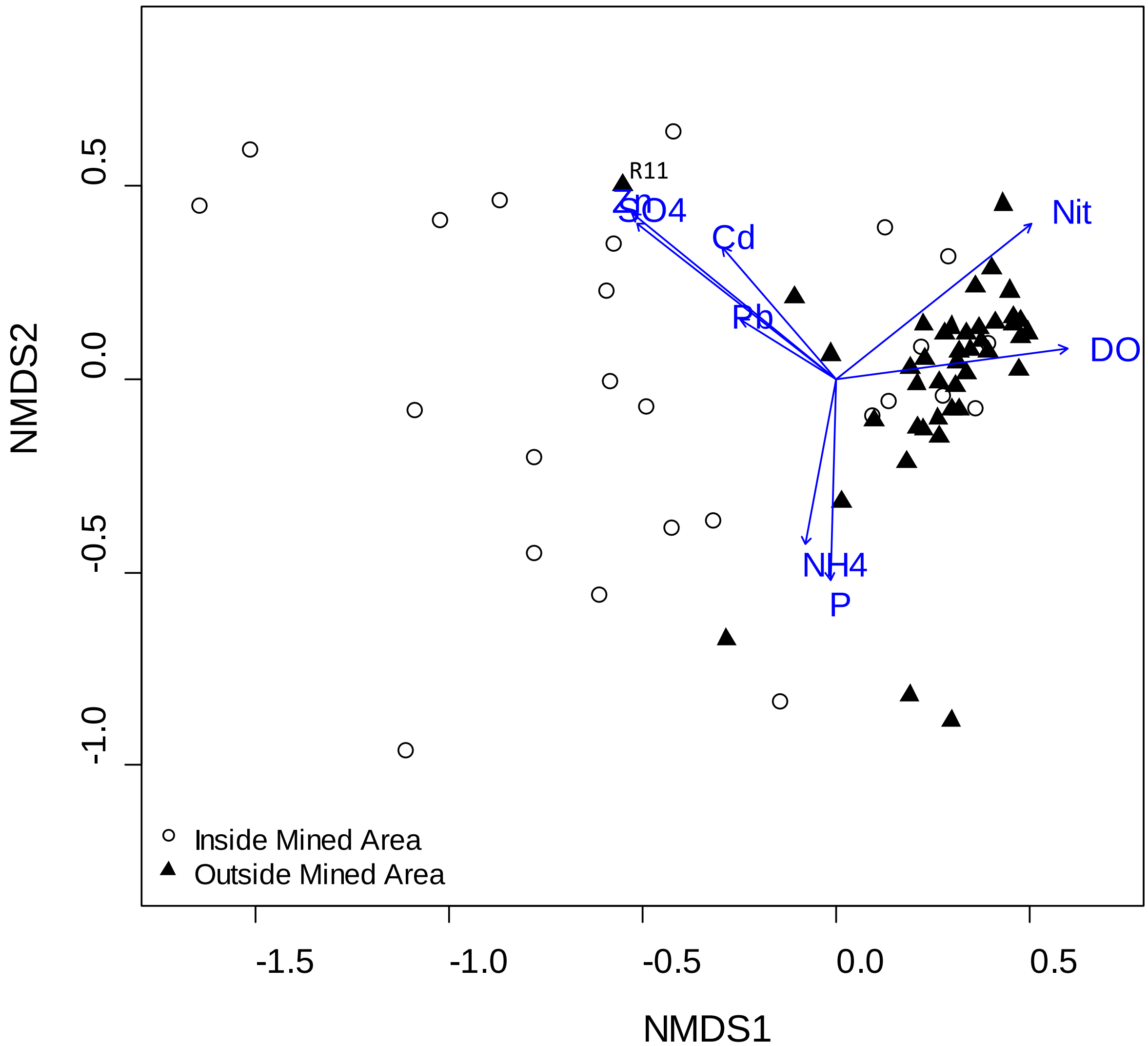


Figure 3. NMDS ordination of sample sites based on water quality characteristics and symbolized by location category (proximity to designated mining-impacted areas). Vectors indicate the relative strength and direction of increasing gradients in water quality variables including ammonia as N (NH4), cadmium (Cd), dissolved oxygen (DO), lead (Pb), nitrate+nitrite as N (Nit), total phosphorous (P), sulfate (SO4), and zinc (Zn).

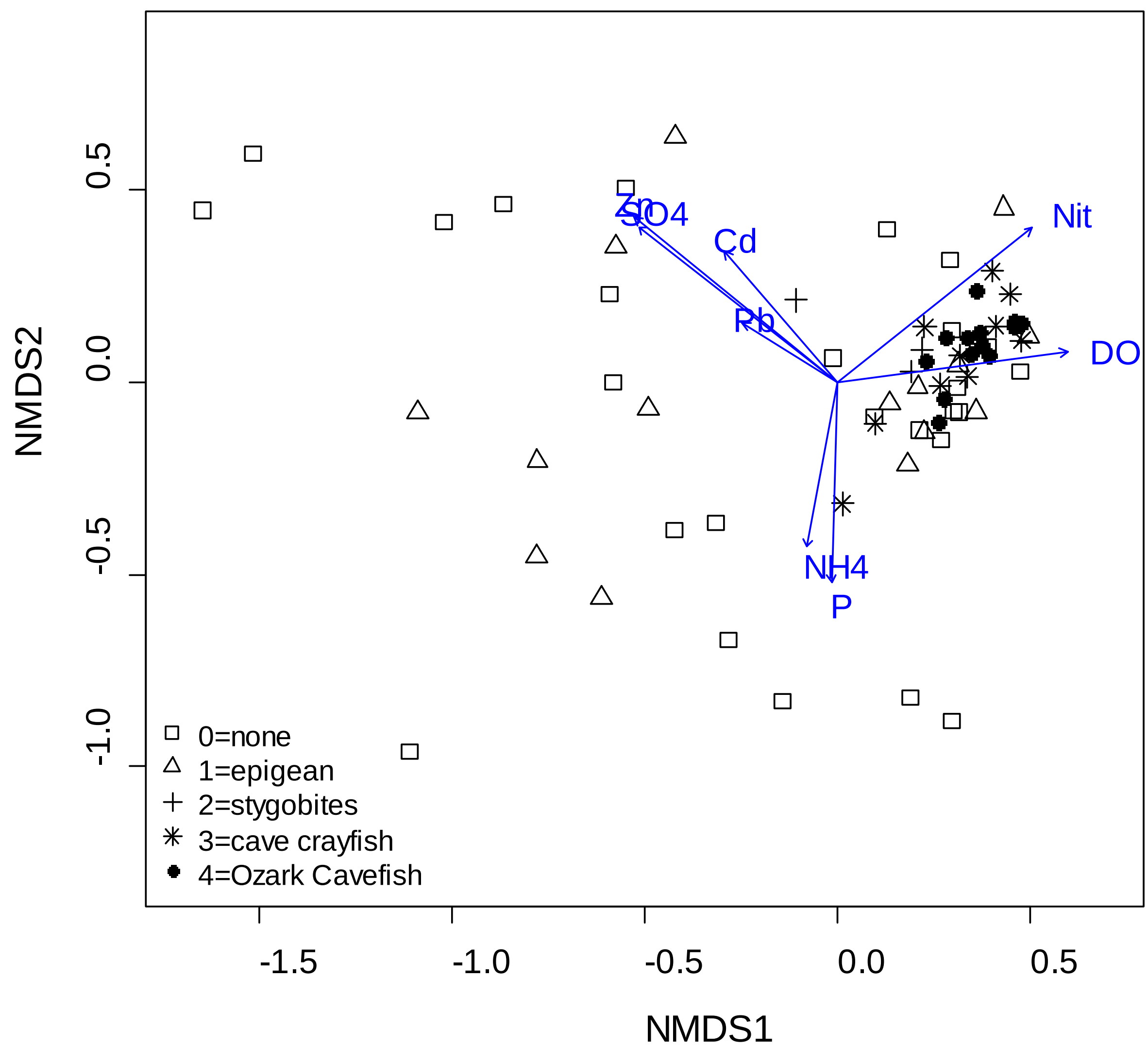


Figure 4. NMDS ordination of sample sites based on water quality characteristics and symbolized by type of aquatic life observed during surveys. Description of vectors fit to water quality variables follows Figure 3.

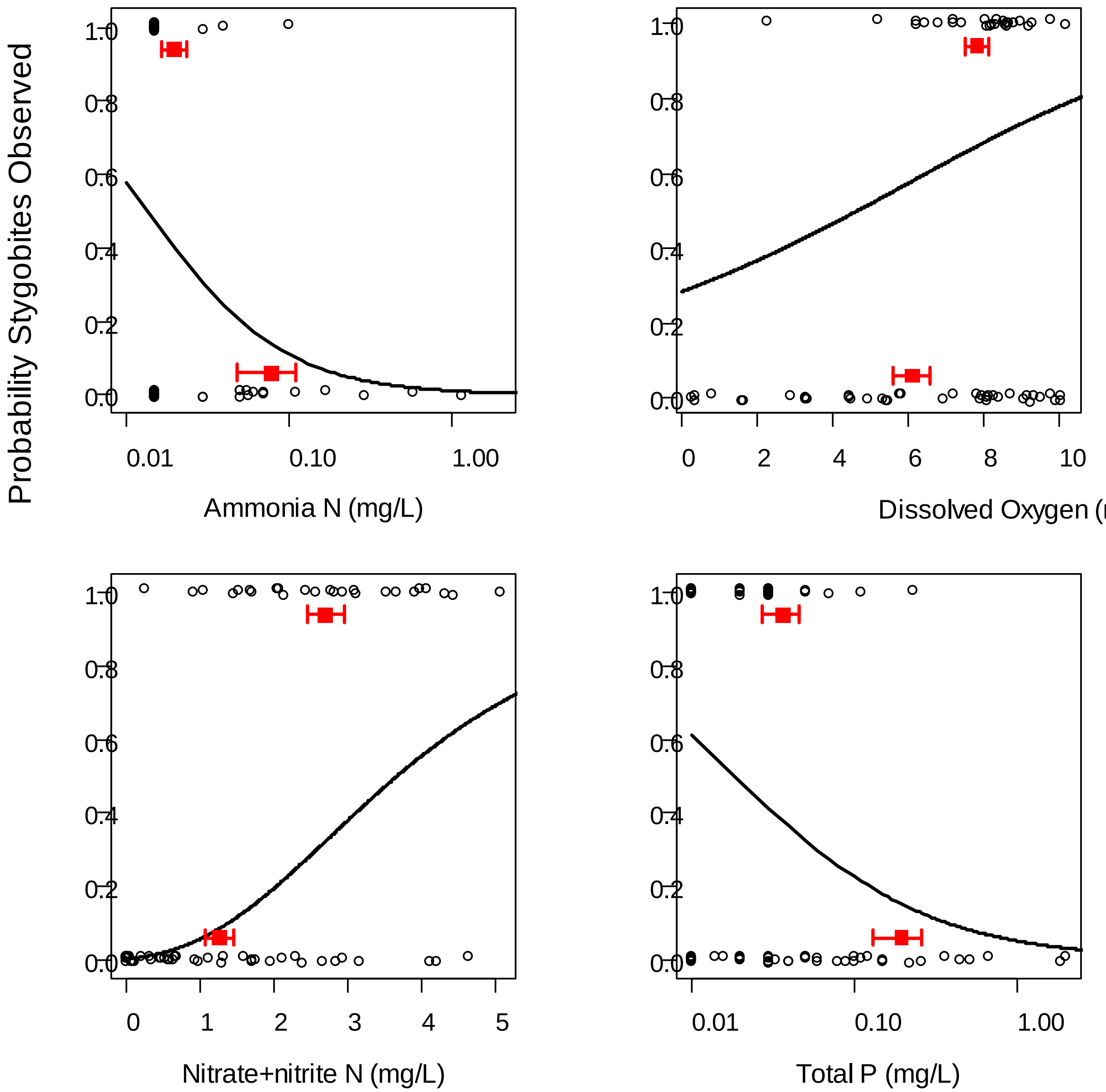


Figure 5. Logistic regression models (trend lines) for presence/absence of stygobites in sample sites (data points at probability = 1 and 0) fit to environmental water quality parameters. A random offset of 0.01 has been added to the probabilities to help distinguish overlapping data points. Also shown (in red) is the mean ± 1SE for each variable for sites with stygobites present and absent. Ammonia N and total P are plotted on a log-scale.

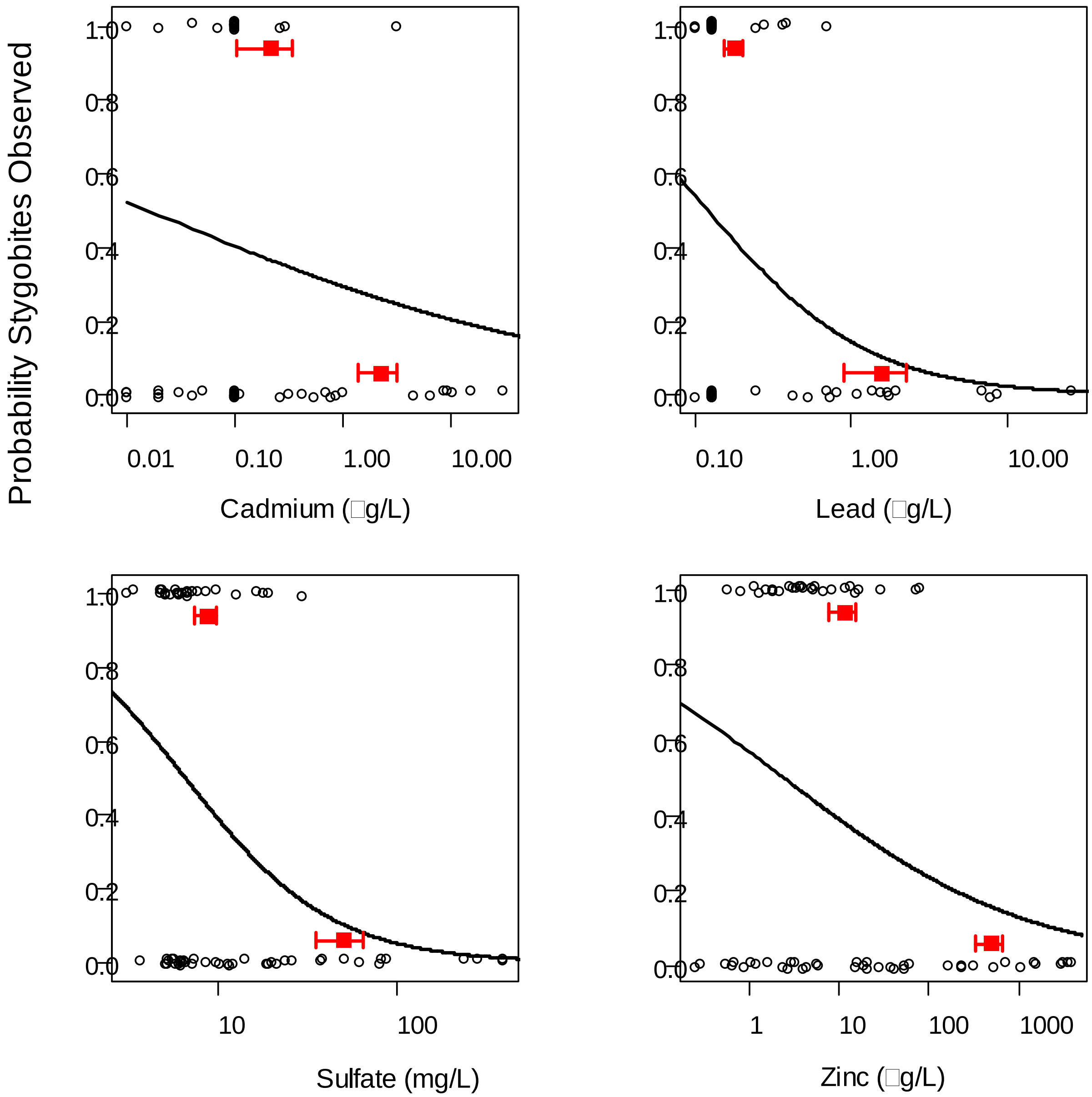


Figure 6. Logistic regression models (trend lines) for presence/absence of stygobites in sample sites (data points at probability = 1 and 0) fit to mining-associated water quality parameters. A random offset of 0.01 has been added to the probabilities to help distinguish overlapping data points. Also shown (in red) is the mean ± 1SE for each variable for sites with stygobites present and absent. The x-axes of each graph is plotted on a log-scale.

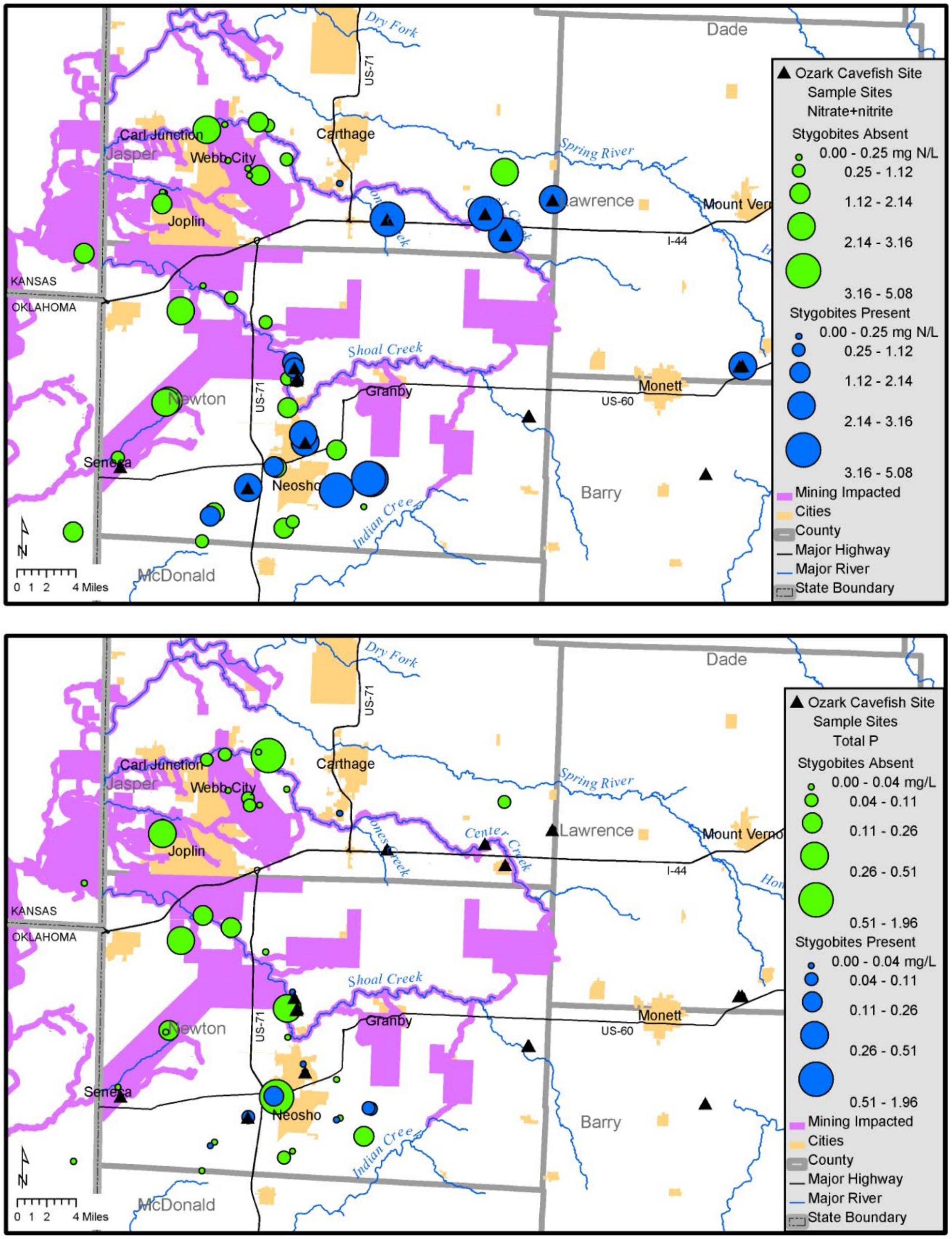
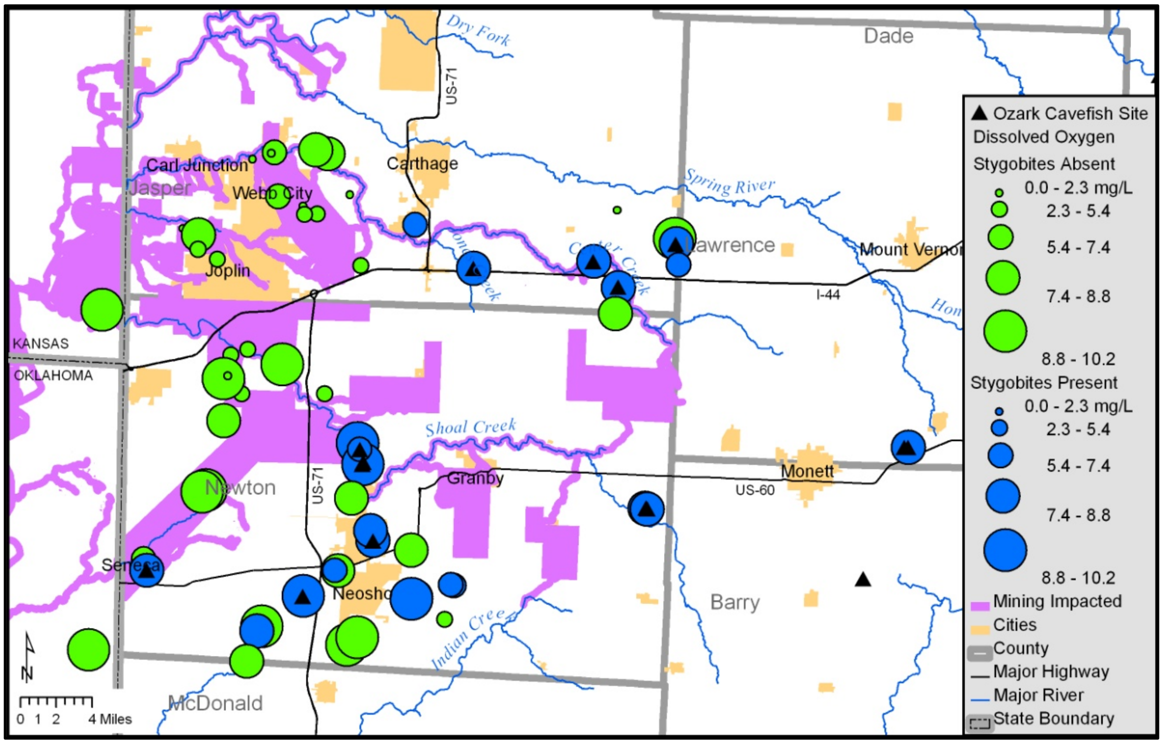


Figure 7. Concentrations of nitrate+nitrite (top panel) and total P (bottom panel) in water samples collected from 67 sites in relation to mining activities in Jasper and Newton counties. The size of symbols reflects the magnitude of concentrations with green vs. blue circles indicating sites where stygobites were absent or present.



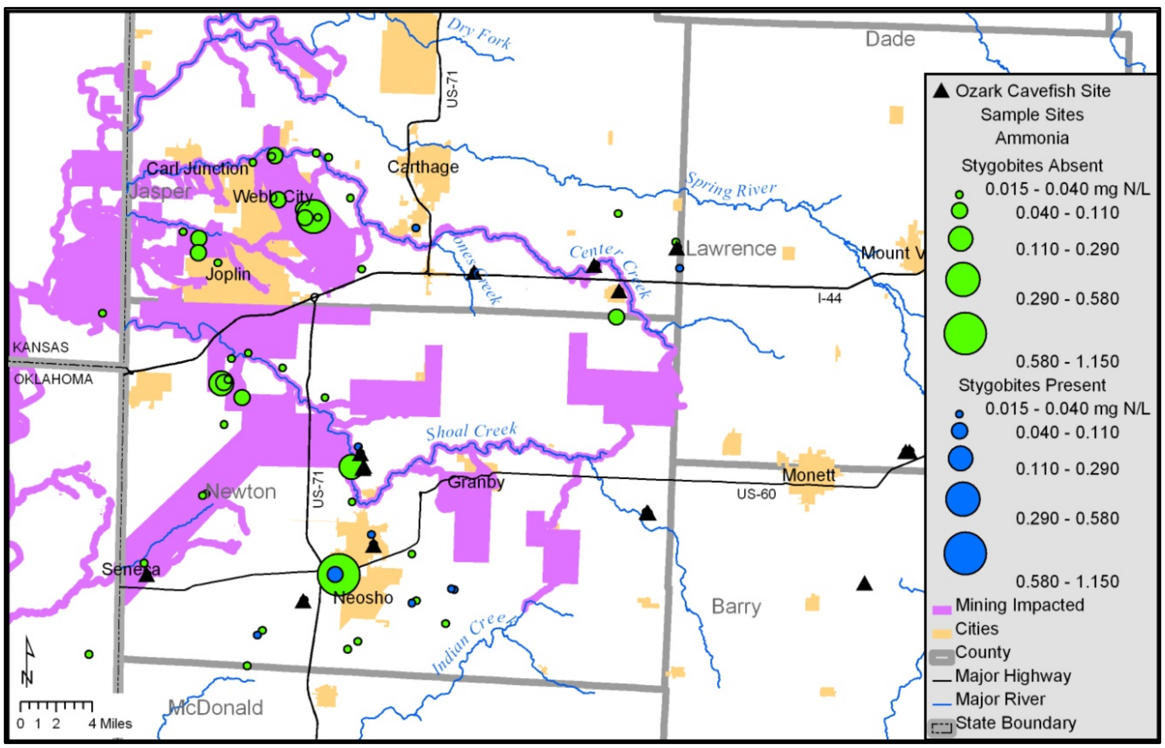


Figure 8. Concentrations of dissolved oxygen (top panel) and ammonia (bottom panel) in water samples collected from 67 sites in relation to mining activities in Jasper and Newton counties. The size of symbols reflects the magnitude of concentrations with green vs. blue circles indicating sites where stygobites were absent or present.

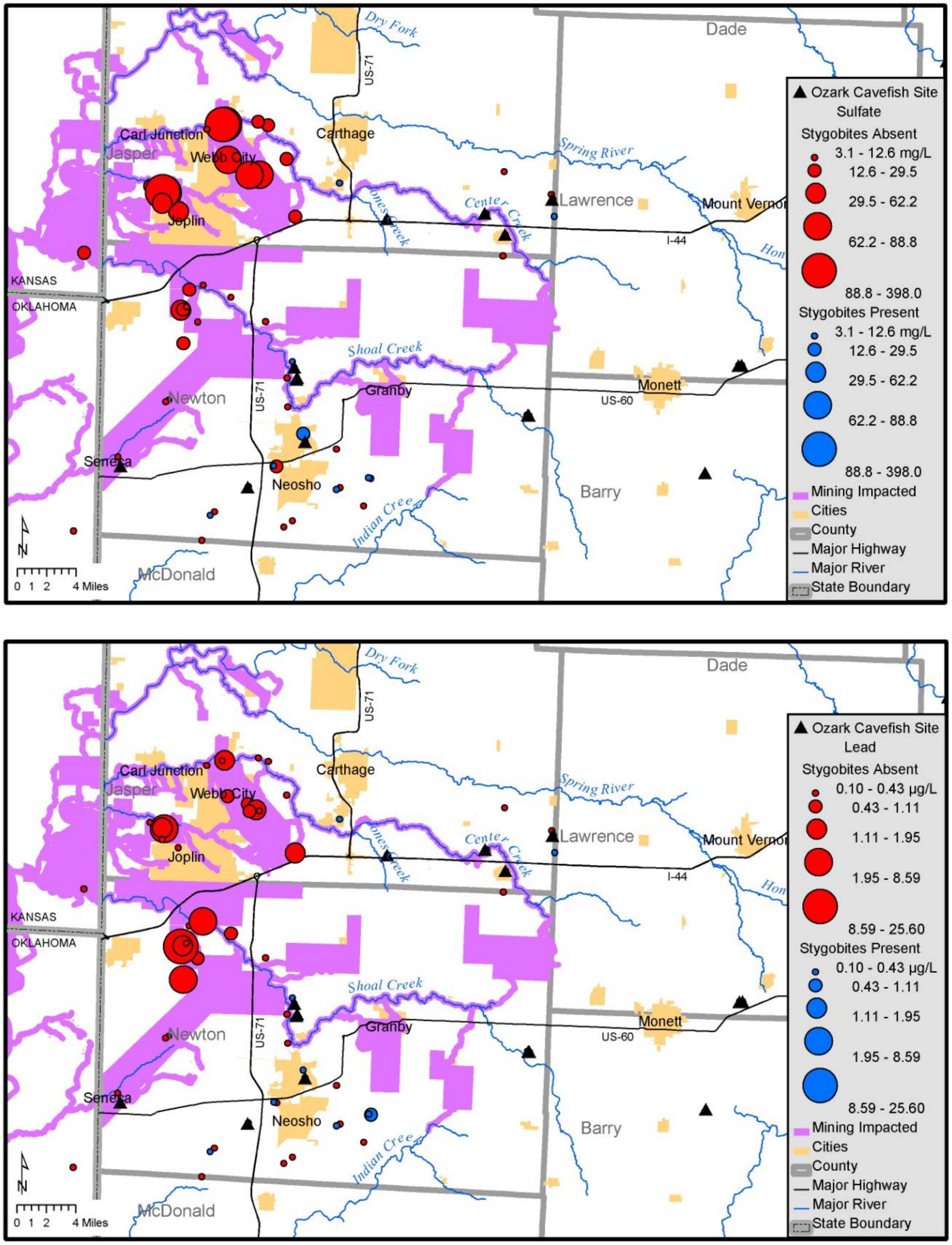


Figure 9. Concentrations of sulfate (top panel) and lead (bottom panel) in water samples collected from 67 sites in relation to mining activities in Jasper and Newton counties. The size of symbols reflects the magnitude of concentrations with red vs. blue circles indicating sites where stygobites were absent or present.

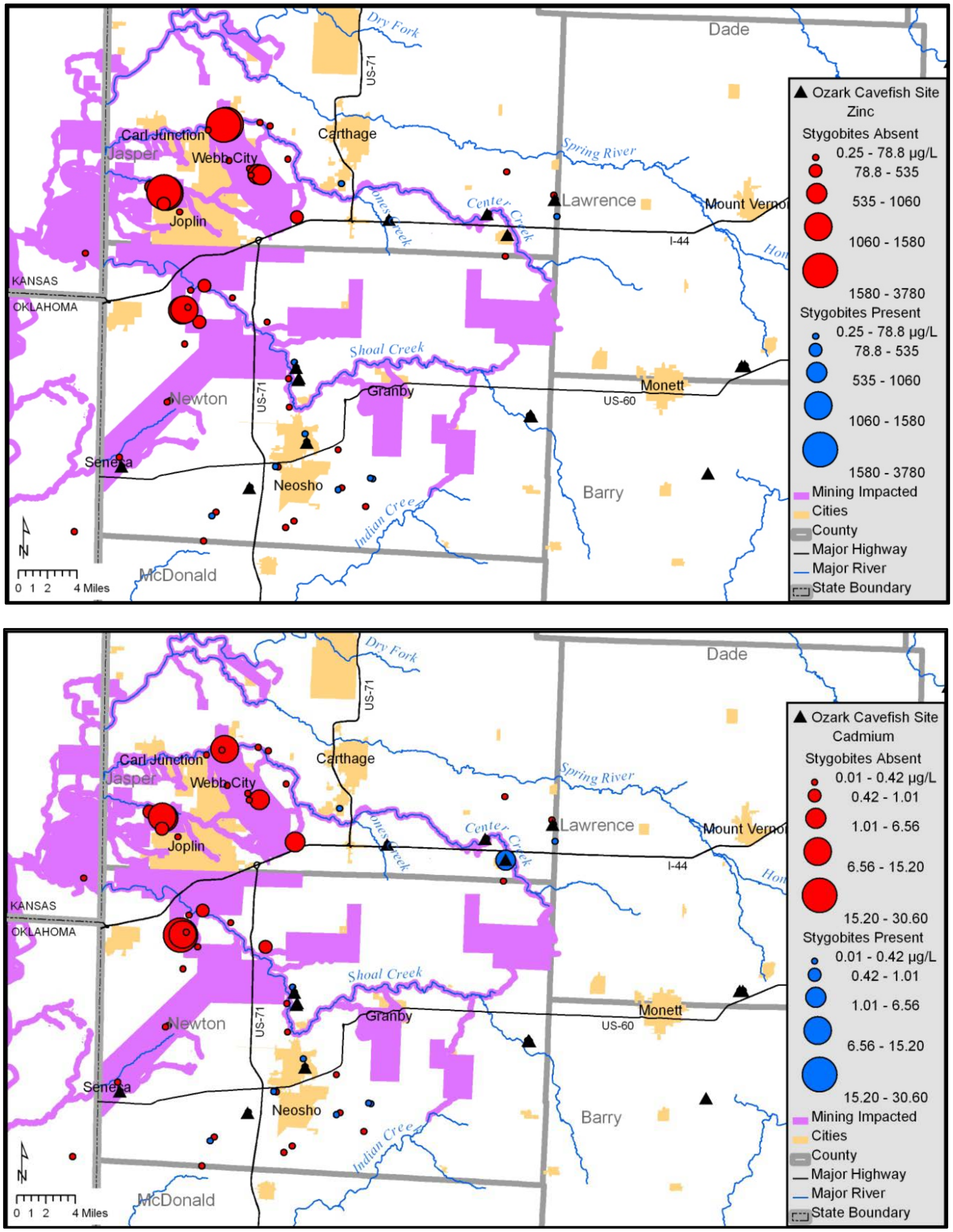


Figure 10. Concentrations of sulfate (top panel) and lead (bottom panel) in water samples collected from 67 sites in relation to mining activities in Jasper and Newton counties. The size of symbols reflects the magnitude of concentrations with red vs. blue circles indicating sites where stygobites were absent or present.

Appendix A. List of dissolved chemicals that water samples were tested for by MDNR.

Parameter	Test	Units	Method
Alachlor	507/508	µg/L	507/508
Aldrin	507/508	µg/L	507/508
Arochlor 1016 (PCB-1016)	507/508	µg/L	507/508
Arochlor 1221 (PCB-1221)	507/508	µg/L	507/508
Arochlor 1232 (PCB-1232)	507/508	µg/L	507/508
Arochlor 1242 (PCB-1242)	507/508	µg/L	507/508
Arochlor 1248 (PCB-1248)	507/508	µg/L	507/508
Arochlor 1254 (PCB-1254)	507/508	µg/L	507/508
Arochlor 1260 (PCB-1260)	507/508	µg/L	507/508
Atrazine	507/508	µg/L	507/508
Butachlor	507/508	µg/L	507/508
Chlordane	507/508	µg/L	507/508
Cyanazine	507/508	µg/L	507/508
Dieldrin	507/508	µg/L	507/508
Endrin	507/508	µg/L	507/508
gamma-BHC (Lindane)	507/508	µg/L	507/508
Heptachlor	507/508	µg/L	507/508
Heptachlor Epoxide	507/508	µg/L	507/508
Hexachlorobenzene	507/508	µg/L	507/508
Hexachlorocyclopentadiene	507/508	µg/L	507/508
Methoxychlor	507/508	µg/L	507/508
Metolachlor	507/508	µg/L	507/508
Metribuzin	507/508	µg/L	507/508
Propachlor	507/508	µg/L	507/508
Simazine	507/508	µg/L	507/508
Toxaphene	507/508	µg/L	507/508
Trifluralin	507/508	µg/L	507/508
Cadmium	6020 Metals-Dslvd	µg/L	SW 846 6020
Copper	6021 Metals-Dslvd	µg/L	SW 846 6020
Lead	6022 Metals-Dslvd	µg/L	SW 846 6020
Manganese	6023 Metals-Dslvd	µg/L	SW 846 6020
Nickel	6024 Metals-Dslvd	µg/L	SW 846 6020
Zinc	6025 Metals-Dslvd	µg/L	SW 846 6020
Ammonia as N	Ammonia as N	mg/L	EPA 350.1
Alachlor	Atrazine Mon Cpds	µg/L	507/508
Atrazine	Atrazine Mon Cpds	µg/L	507/508
Butylate	Atrazine Mon Cpds	µg/L	507/508
Cyanazine	Atrazine Mon Cpds	µg/L	507/508
Metolachlor	Atrazine Mon Cpds	µg/L	507/508

Appendix A continued.

Parameter	Test	Units	Method
Metribuzin	Atrazine Mon Cpds	µg/L	507/508
Pendimethalin	Atrazine Mon Cpds	µg/L	507/508
Propachlor	Atrazine Mon Cpds	µg/L	507/508
Simazine	Atrazine Mon Cpds	µg/L	507/508
Trifluralin	Atrazine Mon Cpds	µg/L	507/508
Chloride	Chloride	mg/L	SM 4500-CI-E
Calcium	CMNK 6010B Dslvd	mg/L	SW 846 6010B
Iron	CMNK 6010B Dslvd	µg/L	SW 846 6010B
Magnesium	CMNK 6010B Dslvd	mg/L	SW 846 6010B
Hardness as CaCO3	Hardness as CaCO3	mg/L	SM 2340-B
Nitrate + Nitrite as N	Nitrate +Nitrite as N	mg/L	SM 4500-NO3-F
Qualitative Organic Analysis	QOA		8270C
Sulfate	SO4	mg/L	EPA 375.2
Total Alkalinity as CaCO3	Total Alk as CaCO3	mg/L	SM 2320 B
Total Nitrogen	Total N	mg/L	I-2650-03
Total Phosphorus	Total P	mg/L	L 10-115-01-1-C

Appendix B. Site characteristics, visual survey results, onsite water chemistry measurements, water sample analyses, and sediment sample analyses for all sample sites. Life categories include 0 = no aquatic life, 1 = epigean aquatic life, 2 = stygobites (unidentified invertebrate), 3 = Bristly Cave Crayfish, 4 = Ozark Cavefish. Measurement units are reported in Appendix A.

Site Features & Visual Survey Results						Onsite Measurements						Water Sample Analyses																		Sediment Analyses		
Site	Date	Type	Loc	Flow	Life	T	Con	pH	DO mgl	DO %	Turb	Cd	Cu	Pb	Mn	Ni	Zn	Amm	Cl	Cal	Fe	Mg	Hard	Nit	SO4	Alk	T_N	T_P	QOA	Cd	Pb	Zn
M01	8/20/2010	mine	In	slow	1	27.4	NA	7.3	1.7	20.7	73.7	0.10	0.54	0.54	611.0	1.3	54.5	0.05	2.0	59	71.1	2.1	155	0.01	7.2	150	0.48	0.09	1	31.0	820	2483
M02	4/4/2011	mine	In	slow	0	14.3	900	7.2	0.8	7.7	0.9	8.74	0.85	6.90	33.1	10.2	3140.0	0.02	3.1	186	3.7	8.6	500	0.04	285.0	223	0.32	0.02	2	4.9	446	3167
M03	9/24/2010	well	In	slow	0	21.2	240	6.8	3.3	38.0	27.4	0.31	3.69	1.11	7.4	1.8	316.0	0.07	7.0	45	96.2	2.6	122	0.94	8.6	104	1.97	0.15	NA	NA	NA	NA
M04	8/6/2010	mine	In	slow	0	29.2	340	6.9	5.8	75.0	32.3	30.60	1.06	25.60	3.4	3.2	1500.0	0.17	4.9	96	21.2	4.8	258	2.31	50.8	160	4.17	0.36	2	23.0	1421	6164
M05	3/10/2011	cave	In	slow	0	21.4	740	7.1	2.9	32.7	64.9	0.01	0.69	0.13	516.0	0.8	5.7	0.02	9.9	143	1290.0	7.8	389	0.01	37.6	349	0.06	0.01	5	NA	NA	NA
M06	12/3/2009	mine	In	slow	1	15.2	470	6.7	0.3	3.5	0.8	0.77	1.45	0.13	197.0	4.0	230.0	0.03	8.9	66	7.0	4.1	182	0.09	19.2	156	0.39	0.04	2	NA	NA	NA
M07	4/15/2010	well	In	none	0	24.4	230	7.1	8.1	95.2	12.2	0.02	15.50	8.59	1.6	1.0	53.8	0.02	20.1	14	129.0	3.0	48	2.93	26.2	37	3.11	0.05	0	NA	NA	NA
M08	8/5/2010	well	In	slow	0	16.9	190	7.4	0.4	3.6	5.0	1.01	3.26	1.70	2570.0	13.9	721.0	0.58	2.8	23	155.0	1.2	63	0.33	38.4	64	1.47	0.66	0	NA	NA	NA
M09	8/5/2010	well	In	slow	1	19.9	470	7.0	3.3	36.3	1.0	4.51	2.37	0.13	1.0	5.8	1060.0	0.02	8.0	77	2.5	6.0	216	1.96	81.2	140	2.01	0.01	0	4.9	588	3636
M10	5/26/2010	well	In	slow	0	17.1	250	7.4	5.3	56.1	112.0	0.10	1.77	0.13	559.0	1.4	15.5	0.29	3.9	60	21.3	2.1	159	0.57	9.7	136	1.15	0.45	5	4.9	108	891
M11	8/13/2010	well	In	slow	1	14.6	NA	7.2	6.9	67.8	2.4	0.10	0.38	0.13	1.2	1.1	5.8	0.02	5.6	78	2.7	2.1	204	0.65	6.6	186	0.67	0.01	0	4.9	54	274
M12	9/24/2010	well	In	slow	0	20.1	390	7.1	5.4	59.5	2.3	0.26	0.61	0.13	1.6	0.7	40.8	0.02	4.2	85	0.5	3.2	226	4.22	21.4	196	4.40	0.10	NA	NA	NA	NA
M13	1/7/2011	cave	In	slow	1	9.3	350	8.5	9.2	80.3	3.6	0.01	0.96	0.13	2.8	0.5	2.8	0.02	15.5	67	8.3	2.6	179	2.39	6.2	131	2.79	0.22	6	4.9	117	436
M14	8/20/2010	mine	In	slow	0	22.2	NA	7.4	5.8	65.7	1.3	9.43	0.67	1.95	29.3	20.0	3780.0	0.05	7.1	168	10.3	4.2	437	0.06	239.0	170	0.22	0.05	1	1587.0	626	190356
M15	12/3/2009	mine	In	slow	0	14.4	120	6.7	0.2	2.4	6.1	0.42	0.51	0.13	97.4	20.2	2940.0	0.02	4.9	203	6.4	4.4	525	0.01	398.0	139	0.09	0.11	2	NA	NA	NA
M16	5/27/2010	cave	In	med	0	14.7	200	7.3	9.1	89.0	4.4	0.10	0.54	0.13	1.2	0.9	4.4	0.02	7.3	45	4.5	1.9	121	3.16	5.8	102	3.16	0.04	0	4.9	47	200
M17	12/18/2009	well	In	slow	4	15.0	320	7.5	8.1	81.0	1.0	0.02	1.03	0.10	3.3	0.1	2.2	0.02	5.3	65	4.3	1.6	168	NA	5.1	147	NA	NA	2	4.9	41	465
M18	1/27/2011	well	In	slow	1	12.8	360	7.6	5.4	51.5	18.5	0.54	0.97	7.83	19.9	1.6	535.0	0.03	14.7	74	311.0	2.7	196	0.12	6.1	192	0.40	0.15	4	4.9	108	1331
M19	10/2/2010	cave	In	slow	2	15.6	470	8.3	9.2	92.2	NA	0.10	1.12	0.13	2.8	1.1	15.6	0.02	33.7	72	7.4	5.0	200	2.14	29.5	151	2.11	0.02	NA	4.9	34	227
M20	4/1/2011	mine	In	Slow	0	18.9	890	7.9	8.7	94.0	7.3	15.20	2.57	1.38	500.0	10.8	3530.0	0.06	3.0	200	4.8	7.5	530	0.22	398.0	154	0.33	0.01	2	30.0	219	5124
M21	4/1/2011	mine	In	slow	0	16.0	610	7.1	4.5	45.2	67.2	0.87	2.09	0.43	89.7	3.8	167.0	0.06	35.9	106	17.0	6.5	291	1.76	62.2	211	2.41	0.51	2	4.9	446	3167
M22	4/15/2010	mine	In	none	1	15.4	350	7.4	9.1	93.3	3.3	10.40	0.81	1.55	8.0	3.8	1580.0	0.07	5.5	66	3.1	2.5	175	0.46	24.1	159	0.93	0.06	0	NA	NA	NA
M23	7/2/2010	mine	In	med	1	29.0	300	8.3	7.2	93.5	1.3	0.10	1.10	0.70	2.1	2.4	16.3	0.05	2.4	66	4.4	2.4	174	0.01	82.7	93	0.26	0.01	1	4.9	310	2685
M24	7/2/2010	mine	In	slow	1	25.7	380	7.6	4.5	54.4	10.0	0.10	1.33	0.82	28.2	2.3	21.0	0.11	2.0	79	17.9	4.4	216	0.01	88.8	140	0.66	0.05	2	4.9	708	3222
M25	4/5/2011	well	In	slow	0	13.2	260	7.8	NA	NA	2.4	0.11	1.94	0.13	1.6	0.4	19.2	0.02	1.5	53	10.2	1.2	138	0.36	6.2	127	0.34	0.03	1	NA	NA	NA

Site Features & Visual Survey Results						Onsite Measurements						Water Sample Analyses																		Sediment Analyses		
Site	Date	Type	Loc	Flow	Life	T	Con	pH	DO mgl	DO %	Turb	Cd	Cu	Pb	Mn	Ni	Zn	Amm	Cl	Cal	Fe	Mg	Hard	Nit	SO4	Alk	T_N	T_P	QOA	Cd	Pb	Zn
R01	2/18/2010	well	Out	med	0	15.8	370	7.5	7.9	79.3	891.0	0.10	1.69	0.13	604.0	1.1	27.6	1.15	23.2	54	92.0	4.8	155	1.71	18.8	117	3.21	1.83	2	4.9	73	1097
R02	1/14/2011	spring	Out	slow	0	13.6	330	7.2	8.1	78.0	0.4	0.01	0.25	0.13	0.5	0.9	0.6	0.02	2.8	63	0.5	1.1	161	0.49	5.3	148	0.59	0.01	NA	4.9	36	83
R03	1/6/2010	cave	Out	fast	4	14.0	300	8.2	9.0	87.2	7.3	0.10	0.63	0.13	2.3	0.8	3.1	0.02	8.6	43	15.1	2.5	117	2.77	7.3	88	2.48	0.05	2	4.9	49	213
R04	4/11/2011	spring	Out	slow	3	13.0	400	7.2	7.2	68.7	0.5	0.04	0.60	0.13	0.5	0.9	3.6	0.02	8.2	82	4.2	2.5	214	0.25	9.9	180	0.76	0.01	6	4.9	101	185
R05	2/25/2011	cave	Out	fast	0	11.1	110	7.4	9.8	80.6	12.5	0.02	1.26	0.13	3.0	0.8	1.6	0.02	3.2	17	146.0	1.2	47	1.60	5.7	37	2.06	0.10	5	4.9	30	95
R06	2/25/2011	cave	Out	fast	0	12.7	110	8.0	9.3	87.5	14.0	0.01	0.72	0.13	2.7	0.1	1.0	0.02	1.5	16	115.0	0.8	43	0.68	5.2	75	0.80	0.03	2	4.9	21	66
R07	1/13/2010	well	Out	slow	4	13.6	360	7.5	8.6	83.5	1.0	0.10	0.35	0.13	0.7	0.1	1.5	0.02	4.6	71	1.6	3.1	191	2.58	3.1	166	2.65	0.01	5	NA	NA	NA
R08	1/13/2010	well	Out	slow	4	13.3	400	7.6	8.0	77.0	1.0	0.10	0.98	0.13	1.9	0.1	3.9	0.02	4.6	77	1.6	4.8	211	3.98	3.4	177	3.94	0.01	5	NA	NA	NA
R09	12/15/2009	cave	Out	slow	1	11.5	400	8.2	10.0	92.2	1.2	0.02	0.43	0.10	1.0	0.1	0.9	0.02	10.9	71	3.6	2.3	187	4.13	5.1	162	4.04	0.03	NA	NA	NA	NA
R10	1/14/2011	spring	Out	slow	3	14.7	320	7.9	10.2	88.1	1.2	0.01	0.53	0.13	0.6	0.9	0.6	0.02	7.2	56	0.5	1.9	146	3.66	6.8	119	4.05	0.01	6	4.9	26	125
R11	6/17/2010	well	Out	slow	0	19.4	270	7.3	4.9	53.4	4.7	6.56	5.67	1.78	1.0	5.1	233.0	0.02	3.3	52	9.6	1.8	137	0.60	20.1	113	0.80	0.01	0	NA	NA	NA
R12	11/12/2010	well	Out	slow	0	18.3	260	7.2	3.3	34.2	11.2	0.10	5.58	0.13	55.8	2.6	38.7	0.02	42.4	19	29.1	2.0	56	0.08	11.3	44	0.21	0.26	NA	4.9	115	487
R13	3/4/2010	well	Out	slow	0	14.9	360	7.4	4.4	43.7	0.1	0.69	0.27	0.13	0.6	0.1	61.0	0.02	3.0	78	0.5	2.5	205	1.12	6.2	183	1.16	0.02	4	30.0	255	1560
R14	1/13/2010	spring	Out	med	3	14.9	380	7.9	8.5	84.6	0.9	0.10	0.49	0.13	2.8	0.7	2.9	0.02	12.1	61	5.3	3.1	165	2.45	8.6	128	2.56	0.03	5	4.9	43	324
R15	3/26/2010	cave	Out	fast	3	12.2	170	7.4	9.8	91.1	7.2	0.10	0.82	0.13	1.2	0.9	13.5	0.02	4.1	35	42.0	1.6	94	2.06	4.8	79	2.36	0.03	0	4.9	46	633
R16	6/18/2010	spring	Out	slow	2	13.7	480	7.0	2.3	21.9	1.3	0.10	0.90	0.28	50.2	0.1	3.5	0.02	16.6	91	27.5	2.1	235	1.06	16.7	213	1.01	0.01	1	4.9	55	111
R17	3/15/2010	well	Out	slow	4	14.2	380	7.2	7.4	74.3	0.9	0.10	0.58	0.13	1.0	1.3	11.9	0.02	9.5	67	12.0	2.9	178	3.11	7.3	154	3.20	0.02	6	NA	NA	NA
R18	1/27/2011	cave	Out	slow	4	12.2	530	7.5	8.6	80.1	6.0	0.07	0.63	0.13	4.5	1.3	1.8	0.03	8.6	87	12.6	2.4	226	3.13	12.6	147	3.32	0.03	4	4.9	41	182
R19	1/15/2010	cave	Out	slow	4	13.1	330	8.2	9.3	88.2	1.6	0.10	0.39	0.13	1.1	0.5	1.8	0.02	2.9	64	5.6	1.5	166	0.91	5.1	143	1.00	0.02	6	4.9	23	79
R20	4/26/2011	well	Out	slow	0	11.8	350	7.4	7.8	72.5	8.2	0.05	2.18	0.25	1.3	1.6	3.3	0.02	3.4	54	47.7	3.3	147	0.66	14.2	146	0.98	1.96	5	4.9	65	146
R21	12/2/2009	well	Out	slow	3	13.3	460	7.2	5.2	50.0	1.3	0.10	0.57	0.13	5.0	1.2	5.5	0.02	4.4	75	3.5	2.0	195	2.08	5.8	179	2.23	0.03	1	NA	NA	NA
R22	3/26/2010	well	Out	slow	4	13.6	270	7.4	6.4	62.2	4.2	0.10	0.66	0.13	1.4	1.0	4.1	0.02	4.2	54	12.8	1.3	141	1.70	6.8	128	1.85	0.01	0	4.9	37	220
R23	2/25/2010	cave	Out	fast	0	17.2	190	8.0	9.5	99.2	2.8	0.10	0.37	0.13	0.8	0.1	0.7	0.02	5.4	32	7.5	1.3	85	1.71	6.3	71	1.77	0.02	5	4.9	24	55
R24	2/25/2010	well	Out	slow	3	8.7	230	7.6	8.2	70.8	2.2	0.10	0.40	0.13	1.0	0.1	0.8	0.02	6.5	44	3.7	1.4	115	1.46	6.1	95	1.46	0.01	5	4.9	44	157
R25	9/16/2010	well	Out	slow	3	17.3	210	6.5	6.8	71.0	2.0	0.29	16.10	0.70	3.1	1.0	72.4	0.02	8.8	45	2.9	1.7	118	5.08	6.3	90	5.07	0.05	NA	4.9	60	1907
R26	1/14/2011	spring	Out	med	1	14.1	270	7.4	8.3	79.7	1.0	0.01	0.25	0.13	0.5	0.7	1.2	0.02	8.3	56	0.5	1.7	147	2.13	6.5	126	2.39	0.03	6	4.9	44	232
R27	2/18/2011	well	Out	slow	3	13.2	350	7.3	6.2	59.2	7.0	0.10	1.24	0.25	6.9	0.9	6.8	0.02	26.5	59	33.2	2.5	157	4.33	5.5	121	4.93	0.07	6	4.9	NA	NA
R28	12/10/2010	cave	Out	med	0	NA	350	8.5	NA	NA	34.0	0.10	0.51	0.13	4.3	1.2	2.4	0.02	7.5	67	17.8	3.3	182	2.86	12.1	153	2.76	0.08	6	4.9	34	240

Site Features & Visual Survey Results						Onsite Measurements						Water Sample Analyses																		Sediment Analyses		
Site	Date	Type	Loc	Flow	Life	T	Con	pH	DO mgl	DO %	Turb	Cd	Cu	Pb	Mn	Ni	Zn	Amm	Cl	Cal	Fe	Mg	Hard	Nit	SO4	Alk	T_N	T_P	QOA	Cd	Pb	Zn
R29	4/1/2010	cave	Out	med	0	11.3	250	NA	10.0	91.5	1.2	0.10	0.99	0.13	0.6	0.8	0.7	0.02	6.0	47	8.0	1.2	122	1.32	5.6	107	1.42	0.02	0	4.9	23	120
R30	12/23/2010	cave	Out	med	1	9.6	250	8.7	8.4	73.1	1.7	0.10	0.27	0.13	0.6	0.6	0.3	0.02	2.5	44	3.6	1.4	116	0.53	3.7	113	0.54	0.02	2	4.9	13	50
R31	6/25/2010	cave	Out	slow	1	15.1	280	7.5	9.9	97.8	12.6	0.10	0.79	0.74	6.2	0.1	20.8	0.02	9.6	50	5.2	3.2	137	0.99	5.2	124	1.04	0.15	0	4.9	101	370
R32	12/15/2009	cave	Out	med	4	14.1	380	7.7	8.6	83.4	2.8	3.12	0.64	0.10	5.9	1.0	8.3	0.02	10.7	63	14.9	3.3	171	3.53	6.1	142	3.61	0.03	2	4.9	33	168
R33	5/26/2010	spring	Out	slow	1	13.9	330	7.2	8.1	78.2	0.8	0.10	0.50	0.13	1.7	0.7	4.0	0.02	4.8	74	6.4	1.9	191	1.30	11.6	169	1.35	0.03	0	4.9	90	864
R34	2/18/2011	well	Out	med	0	11.7	180	6.9	7.9	73.4	17.3	0.03	2.05	0.13	4.6	1.0	3.0	0.06	8.8	26	159.0	2.6	76	4.65	7.4	51	5.42	0.12	6	NA	NA	NA
R35	2/18/2010	well	Out	slow	3	15.6	350	6.9	6.2	60.1	26.2	0.10	4.42	0.39	108.0	0.9	78.8	0.10	36.4	46	1360.0	3.0	128	1.53	7.8	102	1.93	0.23	4	4.9	86	319
R36	3/1/2011	well	Out	slow	4	13.2	140	7.5	8.3	79.3	1.8	0.10	0.69	0.13	1.0	0.3	16.4	0.02	5.9	22	22.2	1.7	61	2.82	4.8	43	3.14	0.01	4	4.9	40	879
R37	2/26/2010	spring	Out	slow	4	14.7	380	7.5	8.6	85.5	1.8	0.10	0.91	0.13	0.8	0.8	5.3	0.04	25.1	70	1.7	3.7	191	2.93	18.1	136	2.99	0.03	5	12.0	287	702
R38	3/25/2011	well	Out	slow	1	11.1	370	7.7	1.6	14.3	0.6	0.04	1.86	0.13	0.1	1.1	0.3	0.02	13.3	57	18.6	8.7	179	2.67	10.2	172	2.93	0.06	2	4.9	28	604
R39	9/16/2010	well	Out	slow	3	20.2	190	6.7	7.2	79.3	77.9	0.10	2.10	0.13	8.1	0.9	29.2	0.02	6.7	37	8.9	1.6	99	3.92	5.9	80	4.03	0.11	NA	4.9	52	554
R40	12/2/2009	cave	Out	slow	4	13.9	450	6.9	8.3	80.8	0.6	0.10	0.40	0.13	0.9	1.2	1.2	0.02	5.1	73	2.6	1.8	190	4.08	4.9	168	4.09	0.02	1	4.9	30	186
R41	4/23/2010	cave	Out	med	2	12.5	380	7.5	8.8	82.4	2.2	0.10	0.56	0.37	3.9	1.7	5.1	0.02	10.6	88	9.4	2.7	230	1.72	19.2	199	1.85	0.03	0	4.9	94	386
R42	2/19/2010	cave	Out	med	4	13.4	400	7.4	8.2	78.1	3.3	0.26	0.52	0.13	33.3	0.1	1.3	0.02	12.7	64	12.4	5.5	183	4.43	6.8	138	4.83	0.03	2	4.9	32	207