Mine Effluent Effects on the Macroinvertebrates and Habitat in a Small Stream in the Tar Creek Watershed (Ottawa County, Oklahoma)

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The Tar Creek watershed is in Tri-State Mining District, an area with extensive metals contamination that has been an Environmental Protection Agency Super Fund since 1983. We surveyed aquatic macroinvertebrates in an unnamed tributary of Tar Creek seasonally between fall 2004 and summer 2007. Quantitative Hester-Dendy samples and qualitative hand collection methods were used at three sites upstream and three sites downstream of a mine effluent discharge emanating from old exploratory boreholes. We found 81 macroinvertebrate taxa - 72 macroinvertebrate taxa upstream and 42 taxa downstream (33 taxa were found in both areas). Upstream sites had greater abundance and taxonomic richness than downstream sites. The damselfly *Argia*, the hydrophilid beetle *Berosus*, the pond snail *Physa*, and chironomids characterized the upstream reach, whereas ceratopogonid (biting midge) larvae and tubificid worms characterized the downstream reach. Upstream sites had higher percent of pooled mayflies, stoneflies, and caddisflies (Insecta: Ephemeroptera, Plecoptera, and Trichoptera; EPT) and downstream sites had higher percent dipterans (fly larvae). Tolerance values of macroinvertebrates indicated poor water quality upstream - likely a result of city drainage and the stream's intermittent flow. Downstream, mine effluent produced a permanent stream with faster flow and a deeper, narrower channel with heavy iron precipitation. Water pH averaged 7.1 at upstream sites and 6.3 at downstream sites. Planned treatment of the effluent may support recovery of the macroinvertebrate assemblages although sediment contamination will persist. The greater flow and accompanying habitat alterations will likely maintain taxonomic differences between upstream and downstream assemblages.

INTRODUCTION

The Tar Creek watershed in Ottawa County, northeast Oklahoma, receives effluent from former metal mines located in the Oklahoma and Kansas portions of the Tri-State (Oklahoma, Missouri, and Kansas) Mining District. Mine effluents are derived from above-ground chat (rock waste) piles and from discharge from filled mine cavities, which are associated with the shallow Boone Aquifer. Area mines produced primarily zinc and lead, and were an important source of zinc and lead during World Wars I and II. Mining and dewatering of mine cavities were discontinued in the late 1960's and contaminated waters from the underground mine began to surface in 1979 (Janik et al. 1982). The region was designated as an EPA SuperFund site in 1983, based on the combination of terrestrial, especially lead, contamination associated with the chat (Schaider et al. 2007) and the groundwater and surface water pollution in the Boone Aquifer and the Tar Creek watershed.

Major pollutants in the mine drainage

are zinc, sulfates, iron, manganese, and lead (Christenson 1995; Carroll et al. 1998). Precipitating iron is also present (Janik 1982). Impacts of these contaminants in the watershed have been documented primarily in fish. Fish diversity and biomass are lower in Tar Creek and its contaminated tributaries than in nearby uncontaminated sites (Franssen et al. 2006), and many fish have elevated lead, zinc and/or cadmium levels (Yoo & Janz 2003; Brumbaugh et al. 2005; Schmitt et al. 2005), which have physiological and reproductive effects (Franssen 2009). Local zinc toxicity in waterfowl and song birds has also been recorded (Sileo et al. 2003, Beyer et al. 2005).

Aquatic macroinvertebrate assemblages in Tar Creek are reported as being severely impacted (USFWS 2000), and two surveys have been conducted in the stream (Janik et al. 1982; Iverson 2003). Both surveys were limited to impacted areas, with no reference sites, making it difficult to distinguish between toxic effects of the mine effluent and compounding factors that contributed to poor water and habitat quality (Janik et al. 1982).

This study had two objectives: (1) to characterize the effects of a point-source mine contamination on stream macroinvertebrates assemblages in a portion of the Tar Creek watershed and (2) to provide pre-implementation data for a planned mitigation project on the study stream (described in Nairn et al. 2005). The study site was an unnamed tributary of Tar Creek that received effluent discharging from a cluster of exploratory boreholes.

Site description

The study site was an unnamed tributary creek draining an area of approximately 1.48 km^2 that includes parts of the communities of North Miami and Commerce (all within T28N R23E S7; Fig. 1). The borehole discharge area supports a cattail marsh south of the tributary and produces a channel that enters the tributary about halfway along its length. Although the tributary is depicted as a first-order temporary stream on maps, the discharge from the boreholes makes the lower portion of the tributary a permanent stream.

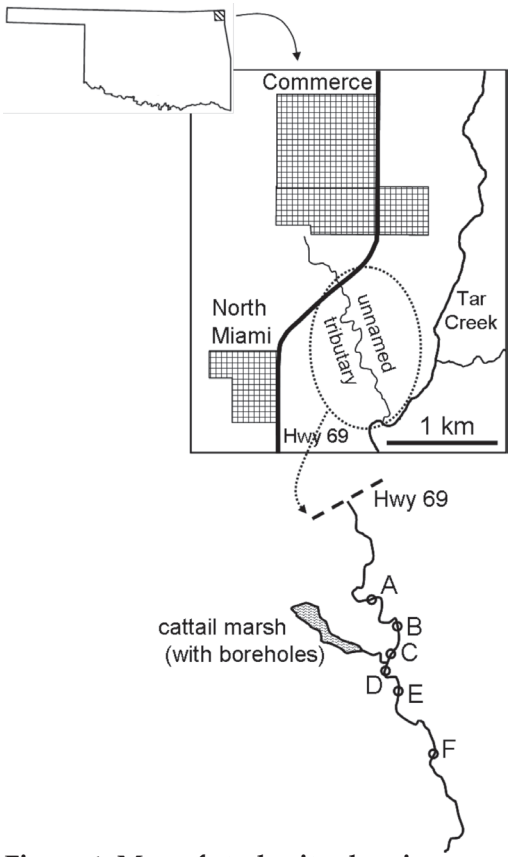

Figure 1. Map of study site showing sampling locations on the unnamed tributary to Tar Creek in Ottawa County, Oklahoma.

Riparian canopy is limited and adjacent land use includes urban areas in the upper watershed, hay pastures and fill areas along the upstream sites and a lateral marshy field, improved cattle pastures and hayfields along the downstream banks. A portion of the streambed and watershed just upstream of the study area was converted to baseball fields in year 3 of this project.

METHODS

Six invertebrate collection sites were established on the creek: three upstream of the discharge and three downstream of the discharge (Fig. 1). Sites were centered

on pools because of the necessity of pool depth for artificial substrate (Hester-Dendy sampler) placement. Samples were collected in fall, spring, and summer for three years, beginning in November 2004 (Table 1). Macroinvertebrate sampling included both qualitative (hand collection) and quantitative (Hester-Dendy sampler) methods. Qualitative samples allowed sampling from multiple microhabitats (e.g., pool sediments, emergent vegetation, backwaters, and runs), whereas quantitative artificial substrates provided the same habitat among sites and allowed comparison of macroinvertebrate abundance.

Hand collection involved sweeping with an aquatic net (mesh size = 500μm), visual searching (e.g., picking up and examining pieces of submerged wood; any invertebrates were removed with forceps), and kicking into a kick net or aquatic net. In addition to collecting visible invertebrates, small samples of substrate, such as leaf packs, were added to make a composite sample. These composite samples were later searched for invertebrates with a dissecting microscope. Field sampling continued until no new taxa were found.

Three, 15-plate, round Hester-Dendy samplers were placed in each of the six pools. Samplers were tied to a float, which was secured to the bottom with a bricks, suspending samplers in the water column, despite changes in water level. Samplers were deployed during qualitative sampling and allowed to colonize for approximately six weeks. Two samplers were randomly chosen for sampling (the placement of the third sampler provided a back-up in case of sampler loss or stranding). Samplers were retrieved with a fine-meshed aquarium net to prevent loss of invertebrates. Samplers were taken apart, the plates washed and the material removed was sieved through a fine mesh in the field. Processed samples were labeled and preserved with 90% ethyl alcohol. New samplers were used each sampling period to avoid possible pollutant contamination from previously used samplers.

In the laboratory, samples were sorted from debris using a dissecting microscope. Macroinvertebrates were identified using primarily Merritt & Cummins 1995 and Thorp & Covich 1991. Most taxa were identified to genus. Oligochaetes were identified to family, mites were morphotyped, and chironomids were identified to subfamily.

Site information was collected seasonally during qualitative sampling. Data included water temperature and conductivity (YSI meter, YSI Incorporated, Yellow Springs, Ohio), pH (ExStic PH100 meter, Extech Instruments, Waltham, Massachusetts), water depth, water velocity (Flo-Mate meter; HACH/Marsh-McBirney, Frederick, Maryland), notes on substrate and water conditions, and digital photographs.

Table 1. Sampling schedule for the six sampling sites along the unnamed Tar Creek tributary. H-D date is the date of Hester-Dendy sample collection and is followed by the number of sampler deployment days.

Sampling	Hand collection date	H-D date (deployed days)
Fall 2004	5 Nov 2004	(none)
Spring 2005	25 Mar 2005	6 May 2005 (42)
Summer 2005	7-8 Aug 2005	17 Sept 2005 (41)
Fall 2005	5 Nov 2005	19 Dec 2005 (44)
Spring 2006	24-25 Mar 2006	15 May 2006 (52)
Summer 2006	6 July 2006	17 Aug 2006 (42)
Fall 2006	23 Sept 2006	4 Nov 2006 (42)
Spring 2007	6 Apr 2007	17 May 2006 (41)
Summer 2007	15 Aug 2007	5 Oct 2007 (51)

Data analysis

Habitat data (stream width and depth, maximum water velocity, and discharge) were compared among sampling periods and between reaches (upstream versus downstream of the mine effluent discharge) using Multiple Analysis of Variance (MANOVA) with a 2-way Analysis of Variance (ANOVA) design, which maintained $\alpha = 0.05$. Tukey multiple comparison tests were used following significant ANOVA results in all ANOVA analyses. Physical data were log(x+1) transformed to increase normality; water chemistry (pH and conductivity) were not transformed because transformation did not increase normality. For clarity, all graphed data are untransformed.

Macroinvertebrate data were analyzed by richness, abundance, percent composition of specific taxonomic groups, and total community composition. Richness data combined Hester-Dendy and hand collection data for each sampling episode. Abundance data were summed from the two quantitative Hester-Dendy samples. In the two cases of missing samples (because of stranding by stream drying or beaver activity), the value for the remaining sample was doubled. Richness and abundance data were each analyzed by 2-way ANOVA (factors = stream reach and sampling period) following square root (x + 1) transformation of the data.

Macroinvertebrate percent composition metrics (% EPT = mayflies, stoneflies and caddisflies; % dipterans; % non-insects; and % hemipterans) were determined on percent richness, rather than percent abundance, which allowed use of combined Hester-Dendy and hand collection data. Data were based on total site richness (i.e., each of the 3 sites in each reach). Metrics were analyzed by MANOVA, using a 2-way AVOVA design (factors = stream reach and sampling period). These proportional data were arcsine square root transformed prior to analysis.

Macroinvertebrate community composition was analyzed using a variety of multivariate techniques, centered on Non-metric Multi-dimensional Scaling (NMDS). The presence-absence data from each Hester-Dendy and hand collection sample were used with the modification of deleting all samples with only a single taxon and all taxa with only a single occurrence. A Curtis-Bray similarity matrix among the 90 remaining samples was used for NMDS, which graphically illustrated the similarity of community composition among samples. ANOSIM (Analysis of Similarity) tested community similarity / difference among sets of samples (e.g., between reaches and among years), and Similarity of Percentages analysis (SIMPER) identified taxa that distinguished pairs of sample sets. These analyses used Primer 6 software (Primer-L, Ltd; Plymouth, U.K.).

Tolerance values were used to characterize the pollution tolerance of collected taxa. The North Carolina Biotic Index (Lenat 1993) was selected because it listed most of the taxa found in the Tar Creek tributary and was developed for both nutrient and toxic pollutants.

RESULTS

Habitat description

MANOVA results indicated significant differences in physical variables between the upstream reach, sites A-C, and the downstream reach, sites D-F, (Wilks' Lambda: $F = 45.5$, $p < 0.0001$) and among the 9 sampling periods ($F = 3.20$, $p < 0.0001$). The interaction between reach and sampling period was not significant ($F = 0.91$, $p = 0.60$), indicating that upstream-downstream pattern did not vary with sampling period.

Observation corroborated the statistical differences in the physical habitat of upstream sites and downstream reaches. Upstream sites were shallower and wider (Table 2, Fig. 2), and had a soft bottom, which was typically anoxic below the sediment surface. Aquatic and emergent plants were typically present, especially cattails at site A and a floating leafed *Potamogeton*

at site C. In contrast, downstream sites D-F were narrower, deeper (Table 2, Fig. 2) and had a noticeably harder bottom. The downstream sediment was red because of precipitated iron, and a reddish foamy film was often present at the surface at these downstream sites, especially at site D. On at least two occasions, we observed crickets

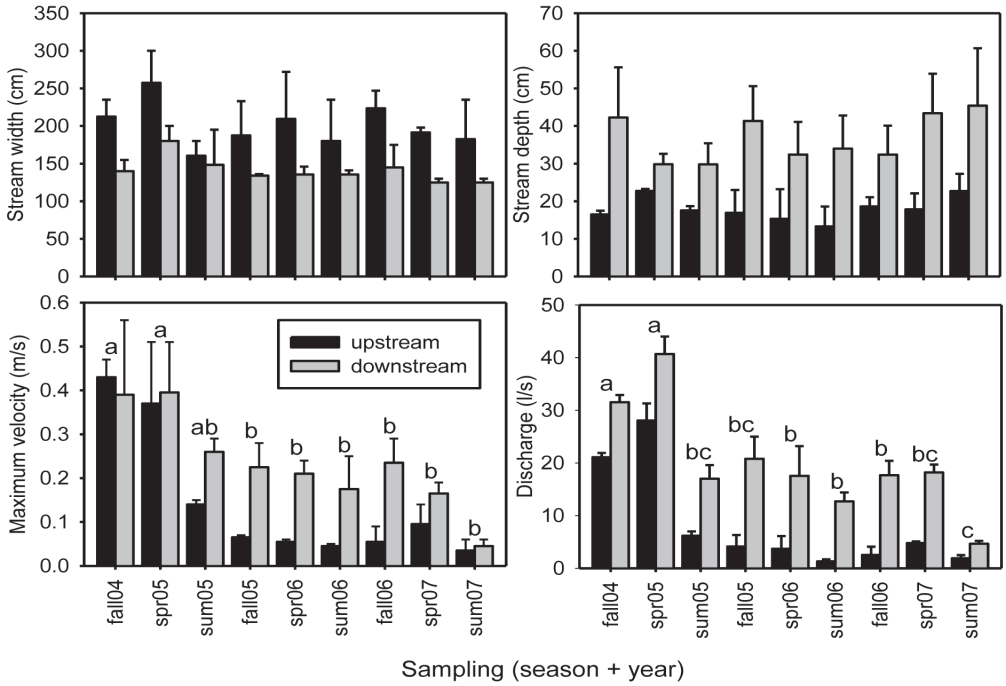

Figure 2. Mean values for habitat features (stream width, stream depth, maximum velocity, and discharge) in upstream (sites A & C) and downstream (sites D & F) reaches for each sampling period. Error bars are +1 SE and different letters above bars show significant differences among sampling periods.

Table 2. ANOVA summary for physical factors at the two upstream (A, B) and two downstream (D, F) sites over the 3-year survey of a Tar Creek tributary. 'Reach' compares upstream and downstream of the mine effluent input; 'Sampling' refers to the 9 sampling times (listed in Table 1).

Variable	Factor	df	F	p	Tukey test results
Width	Reach	1	15.52	0.0001*	upstream > downstream
	Sampling	8	0.81	0.060	
	Interaction	8	0.17	0.99	
Depth	Reach	1	20.66	0.0003*	downstream > upstream
	Sampling	8	0.41	0.90	
	Interaction	8	0.22	0.98	
Max. Velocity	Reach	1	10.64	0.004*	downstream > upstream
	Sampling	8	7.75	0.0002*	see Fig. 2
	Interaction	8	0.88	0.55	
Discharge	Reach	1	96.12	<0.0001*	downstream > upstream
	Sampling	8	16.15	<0.0001*	see Fig. 2
	Interaction	8	2.64	0.041*	

and wolf spiders crossing the stream on the thick foam. Aquatic plants were absent in the downstream reach, except for some *Potamogeton* at site D where the effluent plume had not yet mixed with the stream water along the far bank.

The downstream reach had greater discharge than the upstream reach because of the inflowing tributary of mine effluent between sites C and D (Table 2, Fig. 3). This pattern was apparent, despite temporal variation in upstream discharge. Discharge was greatest during the first two samplings (fall 2004 and spring 2005), intermediate through most of the study, and was lower during the final sampling. Upstream discharge was affected by drought (e.g., summer 2006) and upstream occlusion of the streambed because of the construction of baseball fields in the filled-in channel upstream of site A (beginning with the fall 2006 sampling).

The difference between discharge at sites C and D can be used to estimate the effluent discharge. This discharge averaged (SE) 13.0 (1.8) l/s, with a range of 3.9 to 19.4 l/s (Fig. 3). In comparison, the discharge at site C averaged less and had much higher temporal variability: 7.2 (3.3) l/s, with a range of 1.0 to 20.3 l/s. Discharge generally decreased between downstream sites D and F (Fig. 3). The tributary at site F ran through a wet meadow, which was not present at the other sites.

The pH was circum-neutral, being slightly alkaline at upstream sites and slightly acidic at downstream sites (2-way ANOVA: $F = 45.01$, $p < 0.0001$; mean (SE) = 7.12 (0.13) upstream and 6.31 (0.05) downstream). The pH was not significantly different among the nine sampling periods (2-way ANOVA: $F = 2.26$, $p = 0.07$).

Conductivity differed both between the two reaches (2-way ANOVA: $F = 37.82$, $p < 0.0001$) and among the sampling periods ($F = 17.52$, $p < 0.0001$). Conductivity was high throughout the tributary, but was lower in the upstream reach than in the downstream reach (mean (SE) = 1926 (264) μS/cm upstream and 2751 (161) μS/cm downstream).

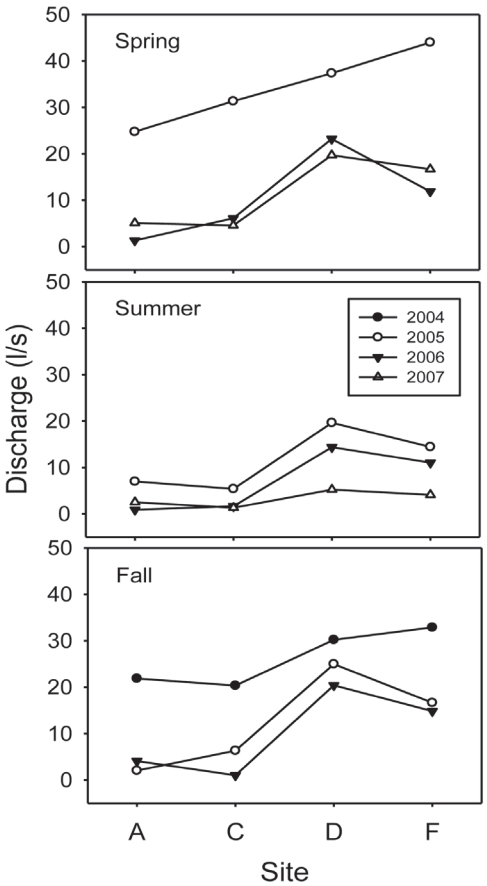

Figure 3. Downstream pattern of discharge along the study tributary among years and seasons. The discharge increase between sites C and D show mine effluent inflow.

Summer conductivities were higher than fall and spring conductivities (mean (SE) = 3334 (176) μS/cm during summer and 1627 (179) and 2054 (246) μS/cm during spring and fall, respectively).

Overview of taxonomic composition

Eighty-one taxa of macroinvertebrates were collected in the tributary over the 3-year study period (Appendix 1). Almost one-third, or 25 taxa, occurred in a single site on one collection period. The most common taxa were chironomid and ceratopogonid larvae (Diptera), pond snails (*Physa* sp.), *Berosus* (Coleoptera: Hydrophilidae) larvae and adults, and *Argia* (Odonata: Coenagrionidae) nymphs. *Hydra* were extremely

abundant during a single sampling period (spring 2005).

Biotic index tolerance values for tributary macroinvertebrates were mostly within the 7.5 to 10.0 range (Appendix 1), indicating poor water quality in both upstream and downstream reaches.

Hester-Dendy and hand collection methods were complementary. Hester-Dendy samples collected more taxa of dipterans, whereas hand collection was better at collecting non-dipteran insects, including odonates, hemipterans, lepidopterans, and beetles. When macroinvertebrates were rare, hand collection was more successful at finding organisms. Indeed, only three of the upstream Hester-Dendy samples (N = 42 because of 6 samples lost to stream drying or sampler vandalism) had no macroinvertebrates, whereas 24 of the downstream Hester-Dendy samples (N = 48) had no macroinvertebrates. Hand collection produced macroinvertebrates at all but one (site E in spring 2007) site and sampling period combination (N = 54).

Reach and date variation in macroinvertebrate metrics

Macroinvertebrate abundance, based on Hester-Dendy samples, was strongly dominated by the spring 2005 sample (Fig. 4), which contained high numbers of *Hydra* and chironomids (especially Orthocladinae and Chironominae) in the upstream sites.

Table 3. Statistical summary of macroinvertebrate metrics for 2-way ANOVA. 'Reach' refers to location upstream versus downstream of the mine effluent input; 'Sampling' refers to the 9 sampling times (listed in Table 1).

Variable	Factor	df	F	p	Tukey test results
Abundance[1]	Reach	1	112.66	<0.0001*	upstream > downstream
(all H-D samples)	Sampling	7	54.32	<0.0001*	
	Interaction	7	46.04	<0.0001*	
Abundance	Reach	1	27.33	<0.0001*	upstream > downstream
(minus Spr 2005)	Sampling	6	2.41	0.053	
	Interaction	6	3.24	0.015*	
Richness	Reach	1	133.36	<0.0001*	upstream > downstream
	Sampling	8	3.16	0.008*	
	Interaction	8	2.23	0.048*	
% EPT	Reach	1	12.31	0.0012*	upstream > downstream
	Sampling	8	4,98	0.0003*	
	Interaction	8	2.11	0.060*	
% dipteran	Reach	1	10.10	0.0030*	downstream > upstream
	Sampling	8	8.65	<0.0001*	
	Interaction	8	3.18	0.0079*	
% hemiptera	Reach	1	6.04	0.019*	upstream > downstream
	Sampling	8	3.09	0.0093*	
	Interaction	8	1.10	0.38	
% non-insect	Reach	1	0.312	0.58	
	Sampling	8	3.064	0.0098*	
	Interaction	8	1.611	0.16	

[1]Total number collected in Hester-Dendy samplers during a single sampling
[2]Abundance, with the exclusion the Spring 2005 samples, which had very high abundance (see Fig. 4)

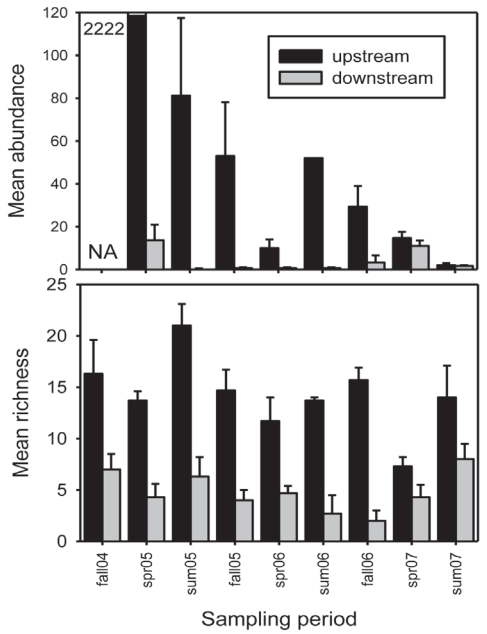

Figure 4. Abundance and taxonomic richness of macroinvertebrates at the three upstream and three downstream sampling sites. Abundances are the number on Hester-Dendy samplers (N = 2) at each of the sites; richness includes both Hester-Dendy and hand collection data. Error bars are +1 SE. Hester-Dendy samplers were not used in Fall 2004.

ANOVA results were significant ($p < 0.001$; Table 3) for sampling date, reach, and the sampling x reach interaction, with Tukey's test demonstrating that differences were centered on the high upstream spring 2005 samples; the corresponding downstream abundances were not elevated.

Exclusion of the spring 2005 abundance data demonstrated a significant reach effect (Table 3; Fig. 4), with upstream Hester-Dendy samplers averaging 32.8 (SE = 8.8) macroinvertebrates and downstream samplers averaging 4.3 (0.9) macroinvertebrates. Abundance did not differ significantly among the remaining sampling dates.

Total numbers of invertebrates collected (Hester-Dendy plus hand collections) were 7,966 for upstream sites and 307 for downstream sites.

Taxonomic richness, which encompassed both Hester-Dendy and hand collection data, was higher at upstream than downstream sites (Fig. 4, Table 3). Upstream sites averaged about 14 taxa per sample whereas downstream sites averaged about 5 taxa per sample. Richness was 72 taxa at upstream sites and 42 taxa at downstream sites. Thirty-three taxa were found in both upstream and downstream areas, giving a total richness of 81 taxa in the study area. Differences in richness among sampling periods were significant, but only because of the higher (upstream) richness in summer 2005 compared to the lower (upstream) richness in spring 2007.

MANOVA results for percent composition metrics were significant for reach, sampling date, and the reach x sampling date interaction (Wilks' Lambda: $F = 5.41$, $p = 0.0018$; $F = 4.41$, $p < 0.0001$; $F = 2.08$, $p = 0.0024$; respectively). EPT comprised 1 mayfly genus (*Callibaetis* sp.) and seven caddisfly taxa - no stoneflies were collected. EPT taxa occurred primarily at upstream sites; hence percent EPT was greater upstream (Table 3, Fig. 5). Percent EPT was significantly greater in one sampling (Fall 2004) than most other samplings. Dipterans included primarily chironomids and ceratopogonids. Percent dipteran taxa was higher downstream than upstream (Table 3, Fig. 5), although total dipteran richness was slightly higher upstream (11 taxa versus 8 taxa). High percent dipterans in fall 2006 (downstream) and a low percent in summer 2007 (upstream and downstream) produced much of the sample differences in percent dipterans. The percent of hemipteran taxa was higher upstream than downstream (Table 3, Fig. 5) – largely because hemipterans were always present upstream but were absent from downstream sites on four of the nine sampling dates. When hemipterans were present, the percent of hemipteran taxa was highly variable within and among sampling dates. Overall, the percent non-insect taxa was greater downstream than upstream, but the difference was not significant (Table 3, Fig. 5) and

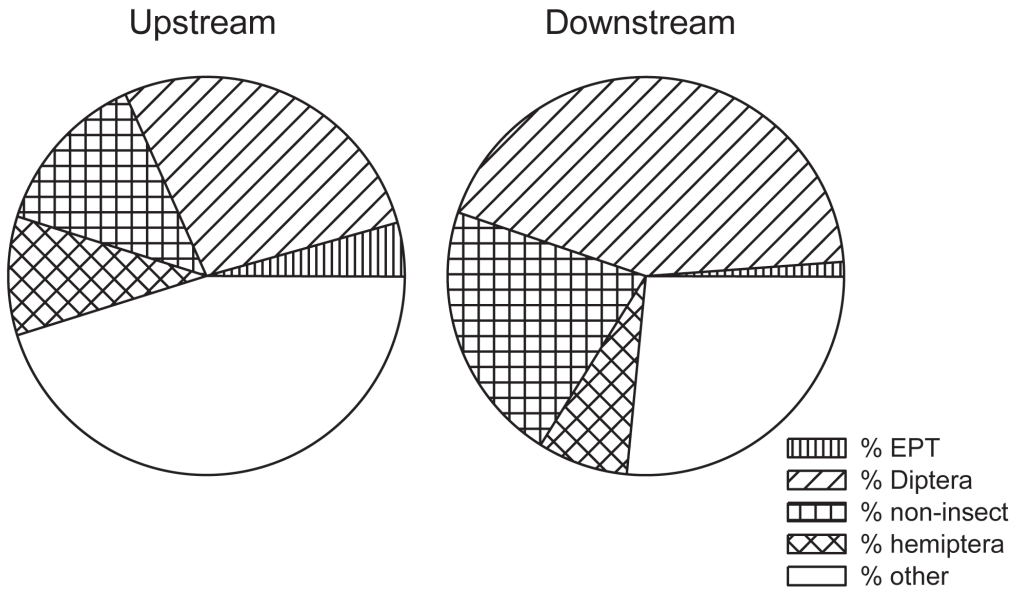

Figure 5. Summary of the percent composition of macroinvertebrates in upstream and downstream reaches, illustrating the relative contributions of EPT (Ephemeroptera, Plecoptera, and Trichoptera), dipterans, non-insects, and hemipterans.

non-insect richness was higher upstream (11 taxa versus 6 taxa). The downstream percent composition was highly variable among and within sampling dates.

Macroinvertebrate assemblage variation

NMDS produced a plot that depicted partial differentiation between the upstream and downstream reaches (Fig. 6A) and among sampling years (Fig. 6B), results consistent with the richness, abundance and metrics results. Sampling method (Hester-Dendy versus hand collection) also produced differentiation (Fig. 6C). The stress value of 0.14 for the 3-dimensional plot indicates a relatively good graphical depiction of similarities among samples (Clarke & Warwick 2001).

ANOSIM results (Table 4) showed not only differences between upstream and downstream reaches, but also similarity within the upstream (sites A-C) and downstream (sites D-F) reaches and dissimilarity between all combinations of upstream and downstream sites. The comparison of the nearby upstream site C and downstream site D had $p = 0.065$ and was by far the most similar upstream-downstream pair. ANOSIM also indicated differences among sampling years – with the exception of 2004 (which had only one sampling), all other years were distinct from each other. Among seasons, spring differed from summer and fall.

A set of eight taxa had the greatest contribution to the SIMPER dissimilarities among reaches and sites (summarized in Table 4). The upstream reach had more *Argia*, *Berosus*, chironomids (Chironominae, Orthocladinae, and Tanypodinae), and *Physa*; whereas the downstream reach had more ceratopogonids and tubificids. ANOSIM results indicated similarity between the lowest upstream site C and the highest upstream site D. SIMPER results indicated that site C had more *Argia* and Tanypodinae and fewer ceratopogonids than site D, and that there was a small decrease in abundance of several taxa between sites C and D. The aquatic caterpillar *Elophila*, which made a case of and was found on *Potamogeton*, was seasonally common at site C (SIMPER

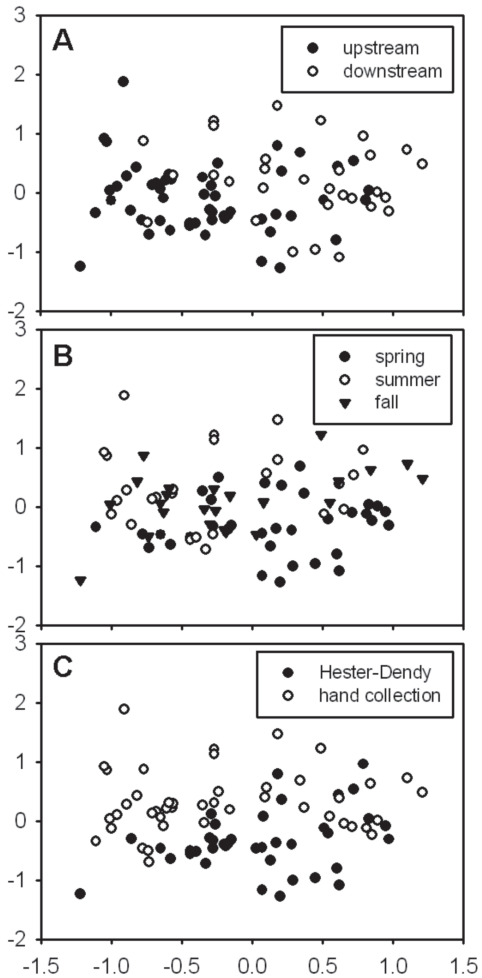

Figure 6. Plot of first two axes of a 3-dimensional non-metric multidimensional scaling analysis based on the presence-absence composition of individual samples (either Hester-Dendy or hand collection). The plots are coded to show (A) reach, (B) season, and (C) collection method.

abundance = 0.40), but was also occasionally found in hand collections at site D (abundance = 0.22), where *Potamogeton* persisted a short distance along the bank opposite the effluent inflow.

SIMPER results were used to identify the taxa associated with the temporal (season and year) differences found with ANOSIM. Seasonally, the spring samplings had more Chironominae and fewer *Berosus*, *Argia*, and *Physa* than the summer and fall samplings. Ceratopogonids were more abundant in fall samplings. Macroinvertebrate composition showed variable patterns among years (e.g., *Berosus* and Ceratopogonidae), a strong decreasing trend (e.g., *Argia*, Tanypodinae, and *Physa*), or weak increasing (e.g., tubificids) or decreasing (Chironominae) trends.

DISCUSSION

This study clearly shows that the inflow of mine effluent strongly affected local stream macroinvertebrates. Total numbers and richness were greatly reduced below the inflow. Only tubificids, collembolans, and a few uncommon taxa (e.g., *Hebris*, *Cyphon*) were moderately more abundant downstream than upstream.

The mine effluent has two obvious downstream influences: a large increase in discharge and mining-derived contamination. The increase in discharge likely made the downstream section a permanent stream, whereas the upper part of the upstream reach remained a temporary stream, partially drying in 2006. Another influence of the increased discharge was the loss of fine sediments in the streambed and the consequent incision of the channel. Channel incision, rather than channel widening, was apparently influenced by the tussock grasses along the banks. The resulting channel had faster flow and reduced pools.

These habitat changes likely affected macroinvertebrate assemblage composition but do not account for the drastic downstream reduction in richness and abundance.

In fall 1980, Janik et al. (1982) surveyed the macroinvertebrates at 5 sites along the Tar Creek mainstem, with their lowest site at Cardin, 12.5 km upstream of the Tar Creek-study tributary confluence. Using both hand collection and semi-quantitative kick net sampling, they found a depauperate fauna, consisting of a total of 19 taxa, with 2 to 11 species occurring at any one site. This richness is slightly higher than

Table 4. Summary of SIMPER results of comparisons of macroinvertebrate composition among reach (and the transitions between the reaches), season and year. Listed taxa are the greatest contributors toward dissimilarity and numbers are average abundance - or the proportion of samples with this taxon.

	Reach		Reach transition		Season			Year		
taxon	Upstream	Downstream	Site C	Site D	spring	summer	fall	2005	2006	2007
Argia	0.67	0.03	0.55	0.00	0.26	0.54	0.48	0.58	0.35	0.10
Berosus	0.71	0.37	0.65	0.56	0.38	0.77	0.64	0.53	0.70	0.43
Ceratopogonid	0.42	0.66	0.40	0.89	0.49	0.38	0.72	0.58	0.70	0.24
Chironominae	0.83	0.66	0.70	0.67				0.83	0.74	0.62
Orthocladinae	0.58	0.11								
Tanypodinae	0.54	0.11	0.50	0.00				0.63	0.17	0.05
Tubificidae	0.21	0.39	0.30	0.22				0.23	0.30	0.43
Physa	0.58	0.21	0.40	0.33	0.28	0.50	0.56	0.65	0.35	0.00

our finding of 13 and 7 taxa in the combined downstream sites during the summer and fall samplings of 2005, when the tributary was less impacted by drought or upstream construction than in 2006 or 2007.

A second macroinvertebrate survey was conducted in May and June 2002, with the upstream site near Cardin and extending about 2 .5 km downstream (Iverson 2003). In this survey, the upstream two sites had higher richness and was strongly dominated by dipterans (chironomids and, to a lesser extent, simuliids) and caddisflies (net-spinning hydropsychids) whereas the lower two sites had low richness and abundance and were dominated by chironomids and oligochaetes. The lower two sites, below the confluence with Lylte Creek had different water chemistry, with lower pH, higher iron, zinc, and conductivity (Carroll et al. 1998; Iverson 2003). This chemical transition was attributed to contamination from waste rock piles (Iverson 2003) and high iron sediment input from a collapsed mine shaft (Carroll 1998). Iron has been implicated in reducing macroinvertebrates in a similarly alkaline mine discharge in Pennsylvania (Letterman & Mitsch 1978).

The Janik et al. (1982) inventory of Tar Creek was one of 15 impacted-streams studies summarized by LaPoint et al. (1984). Tar Creek was highlighted for its very high metals concentrations and low taxonomic richness. Indeed, Janik et al (1982) concluded that the low number of taxa resulted from metals contamination and stated "in the view of the high metal concentrations it is remarkable that any form of aquatic life exists in Tar Creek." The limestone and chert of the Boone Formation result in a circum-neutral effluent – in contrast to the more common acid drainage associated with metals mines. Because the combination of low pH and metals is highly toxic to macroinvertebrates (e.g., Cherry et al. 2001), the toxicity of the metals in alkaline or higher pH mine effluents should be less toxic. However, local studies have demonstrated that chemical changes, especially conversion of metal sulfides to metal carbonates, produce bioavailable, highly toxic metals (Schmitt et al. 2005; Schaider et al. 2007).

The 1982 and 2002 surveys of Tar Creek did not include an upstream reference reach or a downstream recovery reach, unlike most of the other 14 studies in the larger study of impacted streams (LaPoint 1984). Although mine drainage is often a point-source or limited reach pollutant, Tar Creek is heavily impacted by contamination from the Tri-State Mining District from the stream's origin in Kansas (Janik et al. 1982) through it's junction with Neosho River and into Grand Lake (based on metals-contaminated sediments; McCormick & Burks 1987).

In contrast to the complexity of contamination along Tar Creek, this study on a small tributary has a single point of measureable discharge from a discrete set of boreholes and there is an upstream reach that was not contaminated from mining. A second advantage in our study is that the effects of municipal runoff is separable from the effects of mine effluent because they co-occur only at downstream sites (versus at all sites in the Tar Creek mainstem surveys).

Upstream of the mine effluent and its impacts, the macroinvertebrate fauna was characteristic of poor water quality, based on the nutrient and toxin-sensitive North Carolina Biotic Index values (Lenat 1993). For example, *Argia* and *Physa* were common in the upper tributary and are considered highly tolerant species (Lenat 1993). The dominance of tolerant species in the tributary is likely the result of stream impermanence and municipal pollutants. The tributary is marked as a temporary stream on the USGS 7.5 minute series topographic map (USGS, Denver, Colorado) and partially dried during 2006, which impacted sample collection in the upper two sites.

Although the taxa at the upper sites were pollution tolerant, they did not persist well under the added pollution of mine effluent. The change in abundance and richness between the nearby upstream site C and the immediate downstream site D hints

at the high toxicity of the effluent. Macroinvertebrates drifting downstream from above the effluent discharge evidently did not persist in noticeable numbers. Many of the macroinvertebrates found at downstream sites occurred in microhabitats that may have favored macroinvertebrate survival. Such microhabitats included the bank opposite the effluent junction, where mixing had not yet occurred (e.g., a plant or two of *Potamogeton* remained and aquatic caterpillars were sometimes present) or along the edges in the downstream-most sites, along a wet meadow and may have received uncontaminated groundwater inputs. Because of these refugial microhabitats, hand collection was much more successful at finding macroinvertebrates at downstream sites than using Hester-Dendy samplers.

If the proposed mitigation (Nairn et al. 2005) removes the iron, zinc, cadmium and other metals – the treated effluent should not be detrimental to downstream invertebrates. If the mine effluent is sufficiently cleaned, the higher discharge, altered geomorphology and contaminated sediments may still affect macroinvertebrate richness and abundance. The higher discharge will increase stream permanence and may increase the number of characteristic lotic species. The deeper channel is likely to persist because the effluent will introduce little sediment, while the increased discharge will maintain a high erosion environment. Contaminated sediments should not hamper macroinvertebrate colonization. Downstream sediments will remain toxic (e.g., McCormick & Burks 1987); however, macroinvertebrates are more affected by water quality than sediment contamination (Battaglia et al. 2005). Return of viable macroinvertebrate assemblages will provide a food base for recovery of fishes.

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Appendix 1. Taxonomic list of collected taxa, with total numbers collected at each site (A-F). Holometabolous taxa, which have ecologically distinct aquatic life history stages, are larvae (in Megaloptera, Lepidoptera, Trichoptera, and Diptera) or adults (in Coleoptera), unless noted (i.e., 'a' = adult, 'l' = larva, 'p' = pupa). Taxonomic richness and numbers collected are totaled for each site. Tolerance values are listed from the North Carolina Biotic Index (NCBI; Lenat 1993) and range from 0.0-10.0. Values < 5 are associated with excellent water quality; values > 7.7 may be associated with poor water quality.

		Site						
		A	B	C	D	E	F	NCBI
Planaria								
	unidentified	0	0	1	0	0	0	
Cnidaria								
Hydridae	*Hydra* sp.	599	709	379	0	0	0	
Annelida								
Oligochaeta	Naididae	3	0	0	0	0	0	
	Tubificidae	15	17	12	5	12	42	
	Helobdella triserialis	12	0	0	0	0	0	8.9
Crustacea								
Decapoda	*Procambarus* sp.	1	0	0	0	0	0	9.5
Collembola								
	Isotomidae	10	7	11	0	10	0	
	Onychiuridae	0	0	0	0	1	0	
	Poduridae	3	7	0	0	7	12	
	Sminthuridae	0	3	0	3	6	0	
Ephemeroptera								
Baetidae	*Callibaetis* sp.	1	1	1	0	0	0	9.3
Coenagrionidae	*Argia* sp.	55	97	38	0	0	0	8.7
	Enallagma sp.	7	13	9	1	1	0	9.0
Aeschnidae	*Anax* sp.	1	1	1	0	0	0	
	Basiaeschna sp.	0	1	0	0	0	0	7.7
	unidentified	1	0	5	3	0	0	
Libellulidae	*Erythemis* sp.	1	1	2	1	0	0	7.7
	Libellula sp.	1	1	0	0	0	0	9.8
	Nanothemis sp.	9	5	4	0	0	0	
	Perithemis sp.	1	1	2	0	0	0	10.0
	Plathemis sp.	3	2	1	1	0	0	10.0
	Sympetrum sp.	2	3	2	2	0	0	7.3
	unidentified	0	1	0	0	0	0	
Hemiptera								
Belostomatidae	*Belostoma* sp.	0	3	5	0	0	0	9.8
	Ranatra sp.	1	1	2	0	0	0	
Corixidae	*Trichocorixia* sp.	10	3	9	3	0	1	9.0
Gerridae	*Gerris* sp.	0	0	1	1	1	1	
	Trepobates sp.	0	1	0	1	0	0	
Hebridae	*Hebris* sp.	0	0	0	0	2	0	
Hydrometridae	*Hydrometra* sp.	2	1	2	1	0	0	
Mesoveliidae	*Mesovelia* sp.	1	1	4	1	1	0	
Pleidae	*Neoplea* sp.	0	0	1	0	0	0	
Veliidae	*Microvelia* sp.	1	1	1	1	0	0	
Megaloptera								
Corydalidae	*Chauliodes* sp.	1	0	0	0	0	0	
Sialidae	*Sialis* sp.	1	1	0	0	0	0	7.5
Trichoptera								

Hydropsychidae	*Cheumatopsyche* sp.	1	1	0	0	0	0	6.6
Hydroptilidae	*Hydroptila* sp.	1	3	1	0	0	0	6.2
	Oxytheira sp.	1	1	0	0	0	0	
Lepidostomatidae	*Lepidostoma* sp.	0	4	3	0	0	0	1.0
Leptoceridae	*Nectopsyche* sp.	1	6	2	0	0	0	3.8-4.2
	Oecetis sp.	3	0	0	0	0	0	5.7
Limnephilidae	unidentified	1	1	1	0	1	0	
unidentified		0	0	0	0	0	1	
Lepidoptera								
Crambidae	*Elophila* sp.	0	2	12	4	3	1	
Coleoptera								
Dytiscidae	*Agabus* sp.	1	0	0	0	0	0	
	Celina	0	0	0	0	1	1	
	Copelatus	0	1	0	1	0	0	
	Coptotomus (a,l)	2	0	0	0	0	0	9.0
	Desmopachria (a,l)	0	0	8	0	0	0	
	Hydactus sp.	2	0	0	0	0	0	
	Hydroporus sp.	0	3	0	0	0	0	
	Laccophilus sp. A	2	0	0	0	0	0	
	Laccophilus sp. B	3	2	2	1	1	4	
	Pachydrus sp.	0	0	0	0	0	1	
	Thermonectes sp.	1	0	0	0	0	0	
	Uvarus sp.	0	5	0	0	0	0	
	unidentified larvae	0	0	3	0	0	0	
Elmidae	*Macronychus*	0	3	0	0	1	0	
Haliplidae	*Peltodytes* (a,l)	12	6	10	2	1	5	8.5
Heteroceridae	unidentified	0	1	0	0	0	0	
Hydroplilidae	*Berosus* (a,l)	29	66	37	6	1	3	8.6
	Berosus sp. A	0	0	0	0	1	0	
	Helophorus sp.	0	0	0	0	1	0	
	Paracymus sp.	2	0	1	1	1	0	
	Tropisternus sp.	2	0	2	0	1	0	9.8
	Tropisternus lateralis	7	15	0	1	0	0	9.8
Scirtidae	*Cyphon* sp.	0	0	0	0	1	0	
Diptera								
Ceratopogonidae	unidentified (l,p)	21	21	39	19	6	24	
	Dasyhelea sp.	1	0	0	0	1	0	
Chironomidae	Chironominae	299	659	963	12	19	24	
	Orthocladinae	542	1358	1113	0	3	4	
	Tanypodinae	77	100	42	8	1	0	
	chironomid pupae	68	41	64	0	7	0	
Culicidae	*Anopheles* sp.	0	0	4	0	0	0	9.1
	Culex sp.	0	0	0	1	0	0	10.0
Simuliidae	*Simulium* sp.	1	0	0	0	0	0	4.4-8.7
Tabanidae	*Crysops* sp.	1	1	0	0	0	1	7.3
Tipulidae	*Limonia* sp.	1	0	0	0	0	0	10.0
	unidentified	1	0	0	0	0	0	
Acari								
Hydracarina	unidentified	0	3	0	0	0	0	5.7
Gastropoda								
Physidae	*Physa* sp.	35	76	45	8	0	2	9.1
	richness	53	49	41	25	27	16	
	total number	1860	3261	2845	88	92	127	