An Assessment of the Insect Community above and below Gravel Mine Operations on Little Piney Creek, Phelps Co., Missouri

A Report to

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by
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Abstract – Three riffles above and three below a gravel mining operation on Little Piney Creek, Phelps Co., Missouri were sampled for benthic insects on 9 September 2006. Ten quantified samples were taken in each riffle. Each riffle was considered a separate measure of the community with respect to the influence of the gravel mine. All insect specimens were sorted and identified to the lowest taxon possible with the current state of taxonomic knowledge. A total of 36 species were collected, some of which are known to be sensitive or tolerant to siltation, the most prevalent environmental problem with gravel mines and substrate perturbations. Little evidence of differences in insect density or richness in the riffles was found with respect to the effect of the gravel mine using Analysis of Variance. The taxonomic composition of the insect communities also showed no distinction with respect to position above or below the mine using Jaccard's similarity index and Cluster Analysis. However, a very clear difference in the community was found on each side of the gravel mine operation when the analysis combined aspects of density and taxonomic composition using Discriminant Function Analysis.

Introduction

The insect community associated with gravel stream beds is well known to be the primary diet for many species of fish, including for introduced rainbow trout in Missouri Ozark streams. However, anthropogenic disturbances to the stream poses a threat to particular components of the insect community because constituent taxa exhibit different levels of sensitivity or tolerance to various environmental perturbations.

Studies have shown that gravel mining has a detrimental impact on the environment. More specifically, as a result of gravel mining operations in Arkansas Ozark streams, the number of riffles decreased while the number of pools increased downstream (Brown and Lyttle 1992). Further, biomass and abundance of game fish and silt-sensitive fish were lower at downstream sites in comparison with upstream reference sites. Differences in benthic invertebrate communities, including insects, revealed similar associations; and in another study, density and biomass of large invertebrates and density of small invertebrates were lower at frequently mined sites (Brown et al. 1998). Similar community effects have been reported in streams in Wisconsin (Kanehl and Lyons 1992).

Little Piney Creek in Phelps County, Missouri is a spring-fed stream and is listed as an Outstanding State Resource Water for 30 miles from its mouth to S21, T35N, R8W. In 2001, a Wild Trout Management Area was formed on Little Piney Creek (mdc.mo.gov/fish/watershed/gascon/contents). Although the trout are introduced, the creek harbors a diverse natural fauna, including the grotto salamander, which is on the state watch list, at Little Piney Spring, and a great blue heron rookery (www.nps.gov/rtca/nri/states/MO.html). Although Little Piney Creek is noted for limited development in its watershed and high quality stream, some gravel mining is found on the creek, which represents a threat to the distribution and abundance of introduced rainbow trout.

The objective of this study was to determine if the gravel mine operation at T36N, R9W, S13 on Little Piney Creek has an effect on the richness and abundance of the benthic insect community.

Fieldwork

To determine if the gravel mining operation on Little Piney Creek has an effect on the insect community, three riffles above and three below the mine (Fig. 1) were sampled on 9 September 2006. Ten quantified samples of one square meter of substrate were taken in each riffle. Samples were taken by kicking the gravel immediately upstream from a D-net for a distance necessary to equal one square meter of substrate. The contents of the net were emptied into a white pan, and all insects were picked by hand with the help of Trout Unlimited membership. Insects from each sample were placed into one or two labeled, plastic containers with 80% ethyl alcohol. One container was for most insects, while the other was intended for larger specimens, such as Megaloptera, that would potentially damage smaller fragile insects, such as mayflies.

Figure 1. Google earth image of region of Phelps County in which Little Piney Creek was sampled at three riffles each above and below a gravel mine operation. GPS coordinates for locations of the riffles sampled are given.

Lower A: N 37° 50.738', W 091° 52.061', elev 772
Lower B: N 37° 50.699', W 091° 51.953', elev 773
Lower C: N 37° 50.672', W 091° 51.893', elev 774
Upper A: N 37° 49.756', W 091° 51.728', elev 785
Upper B: N 37° 49.623', W 091° 51.520', elev 786
Upper C: N 37° 49.576', W 091° 51.475', elev 787

In the laboratory, all insect specimens were sorted and identified to the lowest taxon possible with the current state of taxonomic knowledge. Systematic specialists were consulted to be certain of tenuous identifications.

Each riffle was considered a separate measure (i.e., replicate) of the community with respect to the influence of the gravel mine. It should be noted that use of same-stream sites represents pseudoreplication if the objective is to assess the effects of gravel mines in Ozark streams. However, if the desired objective is to assess the effect of only this mine, the results can be considered meaningful.

Analyses

A database of the densities of individual species for each of the 60 samples was constructed. From this database, and from three database subsets, values of richness and density were calculated. The database subsets were (1) taxa with exposed gills (analyzed because they are known to be sensitive to siltation), (2) taxa with concealed gills, including fly larvae because their respiration is primarily cutaneous (analyzed because they are known to be tolerant of siltation), and (3) taxa that feed using a filtering mechanism. The treatment of stream position (i.e., above or below the gravel mine) was analyzed using Analysis of Variance for differences in richness and density separately, and for each of the four databases, separately.

Taxonomic composition was analyzed using Jaccard's Community Similarity Index and Discriminant Function Analysis (DFA). Jaccard's Similarity Index is calculated as:

$$Cj = j / (a + b - j)$$

where j is the number of shared species, a is the number of species in site A, and b the number of species in site B.

Discriminant Function Analysis (DFA) is a powerful classification procedure in which differences among *a priori* groups are maximized, while simultaneously minimizing intragroup variation, by adjusting the linear combination of independent variables on each of a number of orthogonal axes. In this analysis, multidimensional site (dependent variable) distribution was predicted by the densities of all taxa (independent variables) for each site. Pair-wise F-tests associated with DFA determined differences between sites. The subsequent classification phase of DFA then assigned each sample to a site based on the linear combination of species densities from each discriminant function axis. Percent of correct assignments was used as a separate measure of community distinction among sites.

All hypothesis-testing was conducted with $\alpha = 0.05$. All analyses were performed using SPSS, version 4.0 (SPSS Inc., Chicago, IL).

Results

Descriptive Data. A total of 5,621 specimens representing 8 orders, 25 families, 33 genera, and 36 species were collected (Table 1). Of these, *Rhagovelia knighti* D&H (Heteroptera: Veliidae) was discounted from the analyses because it is a marginal surface dweller and not associated with riffles, thus its collection in our samples was considered adventitious.

Table 1. All taxa collected in Little Piney Creek, 9 September 2006.

Order	Family	Species
Ephemeroptera	Baetidae	*Acentrella turbida* (McDunnough)
	Baetidae	*Baetis* prob *tricaudatus* Dodds
	Isonychiidae	*Isonychia bicolor* (Walker)
	Leptohyphidae	*Tricorythodes* prob *cobbi* (A-T & Flannagan)
	Heptageniidae	*Stenonema modesta* (Banks)
	Heptageniidae	*Stenonema mediopunctata* (McDunnough)
	Heptageniidae	*Stenacron interpunctatum* (Walker)
	Heptageniidae	*Rhithrogena pellucida* Daggy
	Ephemerellidae	*Serratella deficiens* (Morgan)
	Caenidae	*Caenis latipennis* Banks
Plecoptera	Pteronarcyidae	*Pteronarcys pictetii* Hagen
	Leuctridae	*Leuctra tenuis* (Pictet)
Trichoptera	Hydropsychidae	*Cheumatopsyche* sp A
	Hydropsychidae	*Cheumatopsyche* sp B
	Hydropsychidae	*Cheumatopsyche* sp C
	Hydropsychidae	*Hydropsyche* sp.
	Hydropsychidae	*Ceratopsyche bronta/morosa*
	Helicopsychidae	*Helicopsyche borealis* (Hagen)
	Philopotamidae	*Chimarra* prob *obscura* (Walker)
	Polycentropodidae	*Polycentropus* sp.
Coleoptera	Elmidae	*Optioservus sandersoni* Collier
	Elmidae	*Stenelmis* sp.
	Elmidae	*Dubiraphia* prob *vittata* (Melsheimer)
	Dryopidae	*Helichus suturalis* LeConte
	Psephenidae	*Psephenus herricki* Dekay
Odonata	Gomphidae	*Stylogomphus sigmastylus* Cook & Laudermilk
	Coenagrionidae	*Argia moesta* (Hagen)
Heteroptera	Veliidae	*Rhagovelia knighti* Drake and Harris
Megaloptera	Corydalidae	*Corydalus cornutus* (Linnaeus)
	Corydalidae	*Nigronia serricornis* (Say)
Diptera	Athericidae	*Atherix lanta* Webb
	Tanyderidae	*Protoplasa fitchii* Osten-Sacken
	Simuliidae	*Simulium* sp.
	Chironomidae	*Cricotopus* nr *fasciatus*
	Tabanidae	*Chrysops* sp.
	Tipulidae	*Tipula* sp.

Of the entire list of taxa, all were present in both upstream and downstream sites with the following exceptions: *Rhithrogena pellucida* and *Tipula* were not present in the upstream riffles. *Polycentropus, Stenelmis, Dubiraphia,* and *Protoplasa fitchii* were not present in the downstream riffles.

Density and Richness using ANOVA. The complete database (Table 1) and subsets of taxa (Table 2) were examined separately for differences in density and richness using Analysis of Variance. No significant differences in density or richness above and below the gravel mine were found in the analyses including all insects (Table 3). Further, no significant differences in richness or density for the database subsets (Tables 4-6) were found with the exception of density for insects with concealed gills ($p = 0.013$). In this case, riffles below the gravel mine harbored more than twice the density of those above the mine (Table 7). All other comparisons of density and richness for each database resulted in non-significance ($\alpha = 0.05$).

Table 2. Taxa associated with each of three categories of insects important in considerations of impact of the gravel mine.

Filterers

Isonychia bicolor (Walker)
Cheumatopsyche sp A
Cheumatopsyche sp B
Cheumatopsyche sp C
Hydropsyche sp.
Ceratopsyche bronta/morosa

Exposed Gills

Acentrella turbida (McDunnough)
Baetis prob *tricaudatus* Dodds
Stenonema modestum (Banks)
Stenonema mediopunctata (McDunnough)
Stenacron interpunctatum (Walker)
Rhithrogena pellucida Daggy
Serratella deficiens (Morgan)
Pteronarcys pictetii Hagen
Cheumatopsyche sp A
Cheumatopsyche sp B
Cheumatopsyche sp C
Hydropsyche sp.
Ceratopsyche bronta/morosa
Helicopsyche borealis (Hagen)
Chimarra prob *obscura* (Walker)
Polycentropus sp.
Corydalus cornutus (Linnaeus)
Nigronia serricornis (Say)

Concealed Gills

Tricorythodes prob *cobbi* (A-T & Flannagan)
Caenis latipennis Banks
Leuctra tenuis (Pictet)
Optioservus sandersoni Collier
Stenelmis sp.
Dubiraphia prob *vittata* (Melsheimer)
Helichus suturalis LeConte
Psephenus herricki Dekay
Stylogomphus sigmastylus Cook & Laudermilk
Atherix lanta Webb
Protoplasa fitchii Osten-Sacken
Cricotopus nr *fasciatus*
Chrysops sp.
Simulium sp.
Tipula sp.

Table 3. ANOVA table for richness and density differences above and below gravel mine operation for **all riffle-dwelling** insects.

ANOVA - overall

		Sum of Squares	df	Mean Square	F	Sig.
Mean Density	Between Groups	4845.0	1	4845.0	1.165	.341
	Within Groups	16637.8	4	4159.5		
	Total	21482.9	5			
Mean Richness	Between Groups	2.667	1	2.667	1.065	.360
	Within Groups	10.013	4	2.503		
	Total	12.680	5			

Table 4. ANOVA table for richness and density differences above and below gravel mine operation for **filter-feeding** insects only.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Mean Density	Between Groups	2760.62	1	2760.615	.944	.386
	Within Groups	11700.9	4	2925.218		
	Total	14461.5	5			
Mean Richness	Between Groups	1.602	1	1.602	1.701	.262
	Within Groups	3.767	4	.942		
	Total	5.368	5			

Table 5. ANOVA table for richness and density differences above and below gravel mine operation for insects with **exposed gills** only.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Mean Density	Between Groups	2616.68	1	2616.682	.692	.452
	Within Groups	15117.3	4	3779.327		
	Total	17734.0	5			
Mean Richness	Between Groups	1.707	1	1.707	1.450	.295
	Within Groups	4.707	4	1.177		
	Total	6.413	5			

Table 6. ANOVA table for richness and density differences above and below gravel mine operation for insects with **concealed gills** only.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Mean Density	Between Groups	340.507	1	340.507	18.241	.013
	Within Groups	74.667	4	18.667		
	Total	415.173	5			
Mean Richness	Between Groups	.167	1	.167	.704	.449
	Within Groups	.947	4	.237		
	Total	1.113	5			

Table 7. Mean values for density and richness for insects of each database above and below the gravel mine.

Density	all insects	filterers	concealed gills*	exposed gills
Above	63.20	23.17	10.03	54.03
Below	123.27	66.07	25.10	98.20
Richness	all insects	filterers	concealed gills	exposed gills
Above	9.23	1.97	3.17	6.03
Below	11.17	3.00	3.50	7.50

* indicates significant difference (ANOVA, $p<0.05$)

Taxonomic Composition using Jaccard's Similarity Index and Cluster Analysis. Cluster analysis based on Jaccard's similarity scores resulted in close, paired associations between particular riffles above (1, 2, 3) and below (4, 5, 6) the gravel mine (Fig. 2). Cluster dendrograms on the database subsets revealed identical associations of riffle pairs as that for the entire dataset, and are not depicted here.

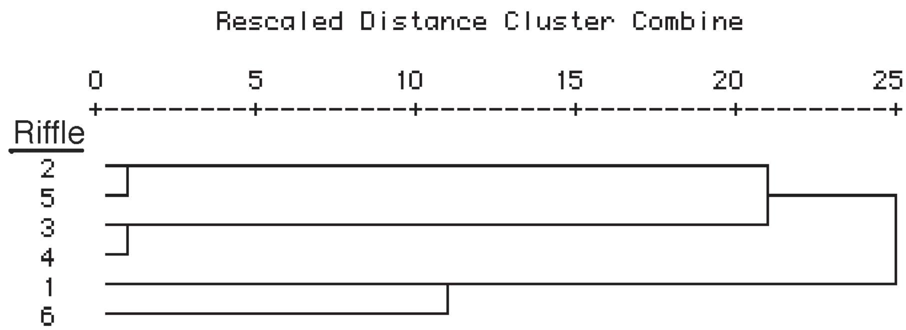

Figure 2. Cluster dendrogram based on taxonomic composition depicting degrees of community similarity among riffles above (1-3) and below (4-6) the gravel mine.

Community Distinction using DFA. Using the overall database, the six riffles were distinguished by five orthogonal discriminant functions, accounting for 100% of the variation among riffles (Fig. 3). The most important taxa accounting for inter-riffle variation in the first Function (axis) were the mayfly *Rhithrogena*, the caddisfly *Ceratopsyche*, and the fly larvae *Simulium* and *Tipula*. Those accounting for the greatest variation in Function 2 were the mayflies *Serratella deficiens* and *Baetis tricaudatus* (Table 7). Of the 15 pairwise contrasts between riffles, 12 resulted in significant differences. Those that were not significantly different were riffles 1-4, 1-6, and 4-6. The classification phase of DFA resulted in 90.0% accuracy in placement of samples to the correct riffle.

Table 7. Discriminant function coefficients for taxa accounting for the greatest amount of variation in the first two functions of analysis to distinguish among six *a priori* riffle groups.

Taxon	Function 1	Function 2
CERATOPSYCHE A	0.16980*	0.01258
SIMULIUM	0.09579*	0.05004
RHITHROGENA	0.07300*	-0.01097
TIPULA	0.07300*	-0.01097
SERRATELLA	0.18036	0.42895*
BAETIS	-0.07198	0.17701*

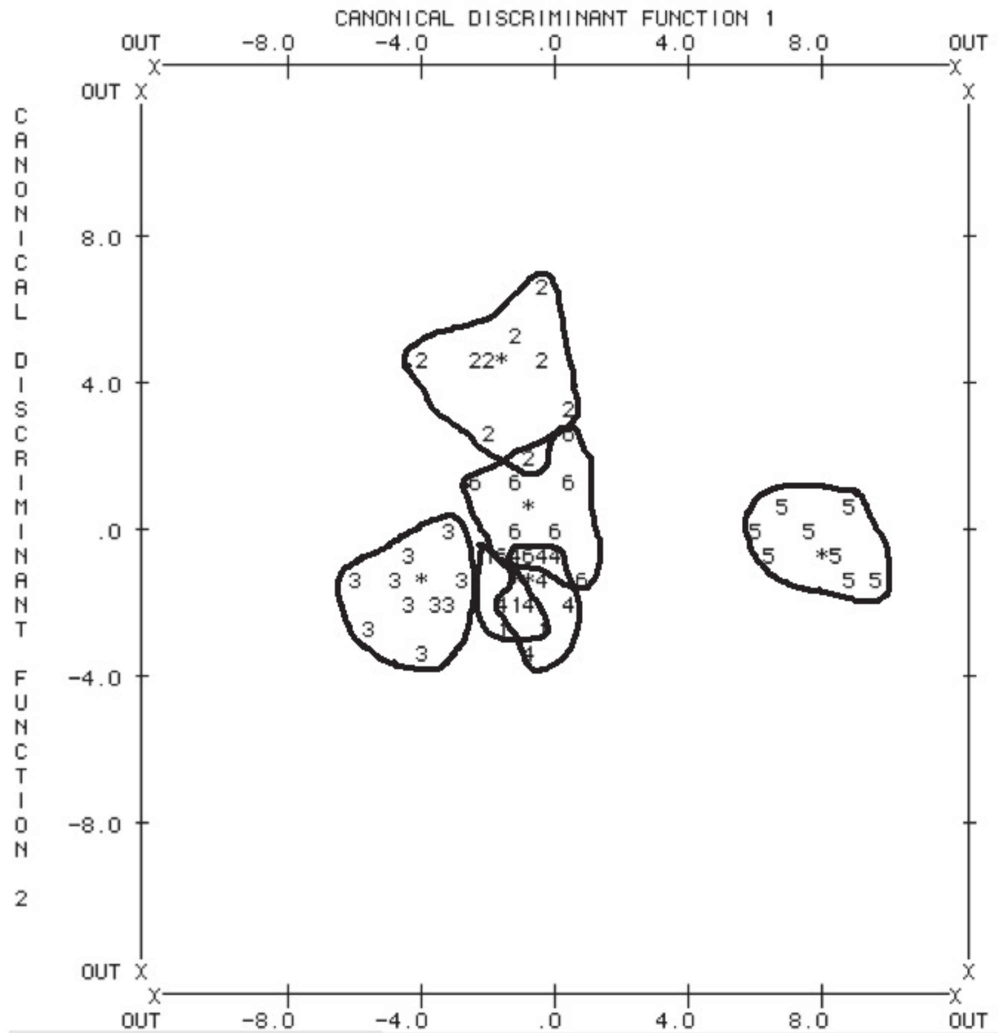

Figure 3. Scatterplot territory map for six riffles based on all taxa. Riffles 1-3 were above the gravel mine, 4-6 were below the mine.

When discounting riffle affiliation and considering only the two stream positions above and below the mine, DFA was able to account for all variation with only a single function (Fig. 4). Taxa considered the most important with respect to discriminating between sites above and below the gravel mine are given in Table 8. The pairwise F-test between positions was very highly significant (F=5.9504, p<0.0001). In the classification phase of DFA, 91.67% of the samples were accurately placed back to the correct stream position. More specifically, 28 of 30 riffles above the mine were correctly classified, and 27 of 30 riffles below the mine were correctly classified.

Table 8. Discriminant function coefficients for taxa accounting for the greatest amount of variation in a single function of analysis to distinguish among two *a priori* stream position groups.

Taxon	Coefficient
STENONEMA FEMORATA	-0.83100
OPTIOSERVUS	0.64841
PSEPHENUS	0.55353
CHEUMATOPSYCHE A	0.51516
CRICOTOPUS	-0.46802
DUBIRAPHIA	-0.44793
ACENTRELLA	-0.42927
HELICOPSYCHE	0.38886
SIMULIUM	0.36379
CORYDALUS	0.27928
ARGIA	0.26612
NIGRONIA	-0.24050
PTERONARCYS	0.20579

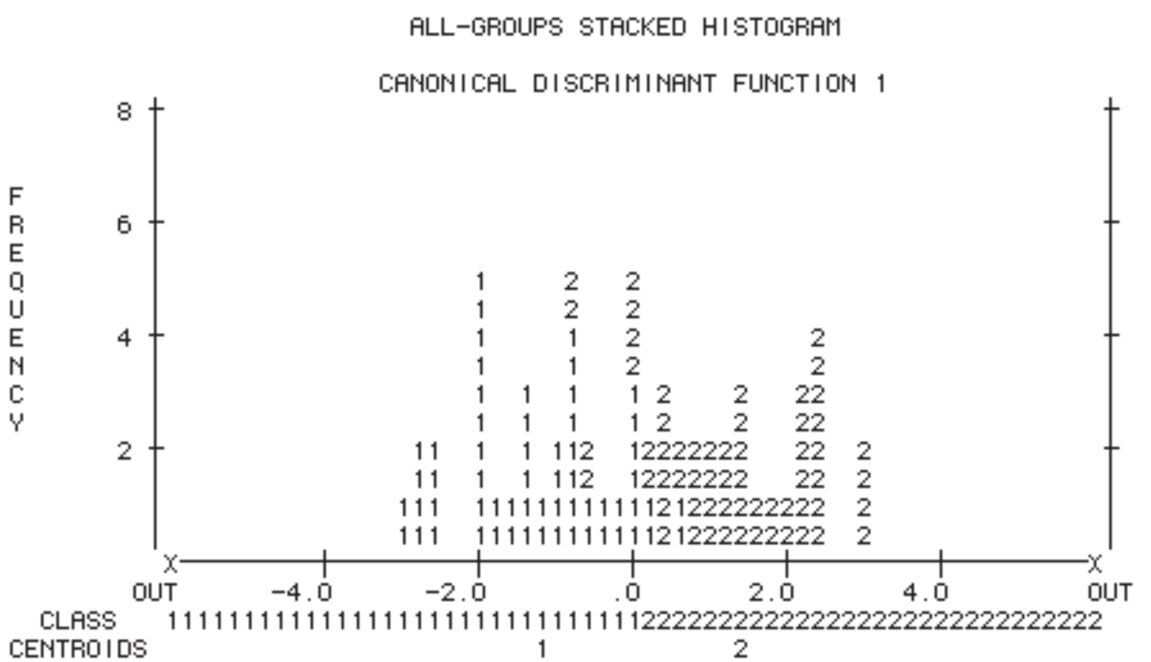

Figure 4. Histogram depicting distinction between sites above (1) and below (2) the gravel mine based on discriminant analysis of individual densities and species composition.

Discussion

Little Piney Creek had been surveyed previously by the Missouri Department of Conservation on October 17 1983 upstream at 35N, 8W, 32SW, elev 815 m near Yancy Mills; and 36N, 8W, 4SE, elev 842 m near Piney Spring. Their samples were taken from all habitats, including riffles, leafpacks, marginal vegetation, pools, etc. Those samples included 26 species recorded at Yancy Mills and 33 species at Piney Spring (Table 8). Our samples were restricted to riffles only, in which we recorded 36 species.

Table 8. Insect taxa collected in 1983 by Missouri Department of Conservation at two locations on Little Piney Creek.

Yancy Mills	Piney Spring
Hydropsyche simulans/incommoda	Antocha sp. (Tipulidae)
Antocha sp.	Stenelmis sp.
Agapetus sp.	Optioservus sandersoni Collier
Limnephilidae	Ectopria nervosa (Melsheimer)
Optioservus sandersoni Collier	Psephenus herricki (DeKay)
Tipula sp.	Ochrotrichia sp.
Hydroptilidae	Hexatoma sp.
Simuliidae	Elimia potosiensis plebeius (Gould)
Chironomidae	Helicopsyche borealis (Hagen)
Protoplasa fitchii Osten-Sacken	Bezzia/Probezzia...
Atherix lanta Webb	Chironomidae
Ceratopsyche slossonae Banks	Simuliidae
Isonychia sp.	Atherix lanta Webb
Empididae	Empididae
Stenonema pulchella (Walsh)	Muscidae
Acentrella sp.	Oxyethira sp.
Baetis tricaudatus Dodds	Ephydridae
Stenonema mediopunctata (McDunnough)	Stratiomyidae
Ceratopsyche (morosa grp.)	Agapetus sp.
Stenonema femorata (Say)	Acentrella sp.
Eurylophella temporalis (McDunnough)	Baetis tricaudatus Dodds
Tricorythodes sp.	Isonychia sp.
Taeniopteryx metequi Ricker & Ross	Heptagenia sp.
Acroneuria sp.	Cheumatopsyche sp.
Psychomyia flavida Hagen	Stenonema pulchellum (Walsh)
Cheumatopsyche sp.	Ceratopsyche slossonae Banks
	Ceratopsyche (morosa grp.)
	Chimarra obscura (Walker)
	Veliidae
	Leuctra tenuis (Pictet)
	Argia moesta (Hagen)
	Caenis sp.
	Tricorythodes sp.

In the Trichoptera, the family Hydropsychidae is particularly diverse in Ozark streams and is useful in studies of environmental perturbations. However, the distribution and density of members of the family in this study are confounding. Specifically, the genus *Ceratopsyche* tends to be sensitive and prefers cool, undisturbed stream and spring conditions. Surprisingly, in our samples, *Ceratopsyche bronta/morosa* was collected in only one sample above the mine, but in 9 samples below the mine. The genus *Hydropsyche* is more tolerant. So, typically in disturbed areas, only *Hydropsyche* will persist whereas *Ceratopsyche* will be absent. We collected *Hydropsyche* in 4 samples above the mine and only 2 below the mine. *Cheumatopsyche* is the most disturbance tolerant and does well in silty conditions. Three species of *Cheumatopsyche* were present in our samples, and they predominated in downstream samples.

Among the mayflies, *Baetis tricaudatus, Stenonema modestum, Rhithrogena pellucida,* and *Serratella deficiens* are especially adapted for cool waters. Thus, in the Ozarks, these species tend to be limited to reaches of streams that would be inhabitable by rainbow trout. Similarly, the stonefly *Leuctra tenuis* is adapted for cold to cool waters with perennial flow, including spring branches in the Ozark region.

Density or Richness Alone.
The ANOVAs resulted in only one difference among 8 separate contrasts of density and richness, that being the insects with concealed gills are in greater density downstream from the gravel mine. Although this was the only contrast that resulted in significance, all groups were numerically higher downstream. High levels of variation among samples and riffles resulted in the inability to detect other differences with statistical significance.

Taxonomic Composition Alone.
Testing for richness, an important community variable, using ANOVA is straight forward, but the absence of significant differences can be misleading. Richness is a taxonomically indiscriminant variable, and testing for differences in stream position with respect to the gravel mine does not take into account which species are which. Thus, although the number of species in all contrasts are not significantly different, the possibility remains that the species identities might be different. Therefore, similarity analyses were performed to examine the communities for affinities based on taxonomic composition.

Each riffle was characterized and compared with each other riffle based on Jaccard's Similarity Index. These similarity scores were then used in an unweighted group means cluster analysis. The resulting cluster dendrogram revealed paired communities on each side of the gravel mine that exhibited the greatest degree of similarity. More specifically, each pair of riffles 2 and 5, and riffles 3 and 4 were nearly identical to each other based on taxonomic composition (i.e., presence or absence of particular species). Riffles 1 and 6 were less similar, but still more similar to each other than they were to either of the other two riffle pairs (Fig. 2). Thus, there clearly is no taxonomic difference in the biota associated with presence of the gravel mine. In fact, the composition of the community on each side of the gravel mine could not be demonstrated to be more similar than it is here.

Density with Taxonomic Composition.
DFA revealed a that the benthic insect community downstream is highly significantly different from that upstream based on the combination of density and composition. This is a powerful multivariate technique that creates models for each of a number of axes to mathematically find differences that might otherwise not be detectable when analyzing individual community attributes. Here, using the individual densities of all taxa, DFA was able to distinguish among each of the 6 riffles with 90.0% accuracy. The accuracy improved slightly when discounting the riffles and focusing solely on sample position above or below the mine. Because the F-test for sample position was significant at $p<0.0001$, there is clearly a different community below the mine as compared to that above the mine.

Thus, based on richness alone and density alone without taxonomic distinctions (ANOVA), there are no clear differences. Based on taxonomic distinction alone, there are no clear community distinctions with respect to the mine. However, based on densities combined with taxonomic identities (DFA), very highly significant differences were discovered.

Perspectives
Why should we expect differences below the gravel mine? Gravel operations can affect the water column in several ways. Probably the greatest impact is by increased siltation resulting from disturbance of settled gravel. In our project, subtle effects of siltation were found, including a higher density of insects with concealed gills below the mine. Insects with concealed gills, such as the mayfly genus *Caenis*, are thought to have evolved to take advantage of habitats in which siltation is prevalent. In this case, *Caenis* has the first pair of gills enlarged, acting as an operculum to cover the subsequent pairs. Other than this lone significant contrast, little biological impact of siltation was evident.

Disturbance to gravel bars can also result in detritus and other food particles stirred up into water column. In areas in which this is a regular occurrence, typically there is a prevalence of insects that employ filter-feeding mechanisms downstream to take advantage of the suspended food resource. However, singling out filter-feeding insect taxa for a specialized ANOVA did not reveal a significantly higher density or increased richness of these taxa downstream. The mayfly *Isonychia bicolor* is the most prevalent of the filter feeding insects in Little Piney Creek. Although not statistically significant, much higher numbers of *Isonychia* were collected in downstream riffles, although their densities were highly variable among samples. Moreover, five of the downstream samples contained more than 100 *Isonychia* individuals per square meter, and one had 312 specimens.

Current research being conducted by scientists at the University of Kentucky have revealed an interesting phenomenon that might explain our higher numbers of individuals of particular taxa downstream of the gravel mine. Their unpublished data suggest that during times of the year when water levels are high and precipitation washes nutrients into the stream from the watershed, the nutrients are absorbed into porous gravel bars. Very slow horizontal movement of water through the gravel bar results in an extended time-release of these nutrients downstream from the gravel, including during the summer when water levels are low. In the case of the gravel mine, disruption of the gravel bar by the mine might result in more massive release of nutrients than previously considered, thereby providing an elevated level of food resource for insects that are able to take advantage of it.

Finally, the objective of this project was to determine if there is an effect on the benthic insect community by the gravel mine that might have a secondary effect on the trout population. There is no basis from which to infer a negative effect of mining on the insect community. The diversity of the insect community is clearly different above and below the mine based on species-specific densities combined with taxonomic identities. How this affects the trout population would depend on trout preferences for particular insect species as food items. Statistical significance was difficult to obtain in our project because of high variability among riffles. Thus, future studies should include more riffles to increase statistical power, and repeat this sampling in multiple streams of similar hydraulic characteristics to obtain true replicates. In this way, inference could be made about the effect of gravel mines on aquatic insects in Ozark streams, rather than only the effect of this gravel mine on the aquatic insects in Little Piney Creek.

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Figure 5. Trout Unlimited members collecting specimens from samples.