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# Use of Algal and Macroinvertebrate Indicators to Assess the Impact of Agricultural Practices on Surface Water Quality in the Mammoth Cave National Park Region, Kentucky

Final Research Report to
Kentucky Water Resources Research Institute
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## Summary of Funded Research

The karst aquifer underlying the Mammoth Cave area supports a unique and diverse cave aquatic ecosystem as well as providing the principal source of drinking water for many local residents of this region. This unique and fragile ecosystem is surrounded by one of the most intensively used agricultural areas in the Commonwealth with more than 80 % of the surrounding region used in beef, dairy, burley tobacco and alfalfa production. Agricultural practices resulting in run-off of pollutants into the aquifer via sinkholes and sinking streams have a pronounced impact on an important drinking water source and on the unique cave ecosystem.

Our hypothesis was that the biological communities in unprotected (no BMPs) stream sites adjacent to agricultural operations will be significantly different from those found in the un-impacted reference streams (when such streams are available). Specifically, we expected taxa richness to be lower, and the ratio of EPT (Ephemoptera, Plecoptera and Trichoptera) to Chironomidae to be lower in the impacted streams compared to the reference streams. We also expected there to be significant loss of benthic taxa richness in the areas. We expected that community structure and composition in those streams which are protected by Best Management Practices (BMPs) would not be significantly different from reference streams. Since bioindicators may reveal chronic or synergistic effects of pollutants that may be missed by physio-chemical sampling, the biological data can provide a unique and sensitive way of assessing stream ecosystem health.

## Introduction

In order to determine ecological integrity of freshwater aquatic systems, government and private monitoring organizations employ water chemistry analysis, as well as habitat and biotic surveys. The latter method, particularly benthic macroinvertebrate bioassessment protocols, enables the detection of site specific environmental stress. Macroinvertebrate communities in impacted watersheds are often subjected to stressors such as temperature extremes to mine tailings effluents, inorganic and organic contaminants, or agricultural waste runoff. Certain measures of community structure, such as overabundance of tolerant macroinvertebrates or the absence of sensitive species, may indicate diminishing ecological integrity due to anthropogenic impacts. Intensive livestock rearing facilities produce large quantities of waste and often must adopt best-management practices (BMPs) to reduce impacts on local biotic communities. The efficiency and effectiveness of BMPs in reducing non-point source run-off into streams may be evaluated using benthic macroinvertebrate indicators.

Although a variety of bioassessment protocols have been established for benthic macroinvertebrate sampling within the last decade (Plafkin *et al.* 1989, Klemm *et al.* 1990, Kentucky Division of Water 1993, Lenat and Barbour 1994), stream disturbances have been evaluated by determining the integrity of macroinvertebrate communities since the late 1960s (Berkman et al. 1986). Wohl *et al.* (1995) found distinct patterns between macroinvertebrate species distribution in Appalachian streams and habitat attributes, thereby demonstrating a relationship between environmental parameters and invertebrate community structure. Pollutants are environmental parameters largely influenced by or originating from anthropogenic practices and can affect invertebrates either directly by physiological responses or indirectly by alterations in habitat (Lemly 1982). For example, Lemly (1982) established that variation of benthic insect communities was correlated with sedimentation combined with nutrient enrichment. Berkman *et al.* (1986) tested and established the validity of utilizing benthic macroinvertebrates as bioindicators of environmental stress from agricultural practices. This research supported by the Knetucky Water Resources Research Institute examined the impact of two different BMP systems (waste lagoon/stack pad and constructed wetlands) on water quality in streams in agricultural landscapes. The research evaluated the impact of pollution (e.g. nutrient run-off) from dairy operations on one stream in the Glasgow, KY and from a swine operation on one stream in Franklin, KY using algae and macroinvertebrates as indicators of ecological integrity.

**Research Sites**

Glasgow dairy operation

This study site is located in Barren County on an un-named tributary (hereafter called "Judd Creek") of Beaver Creek. The operation includes a feedlot for dairy cattle located on a hill overlooking the tributary. Runoff from the feedlot drains directly into Judd Creek, which in turn drains into Beaver Creek.

In the spring of 1996, we conducted a series of pre-BMP samples on Judd Creek to assess stream condition. The stream was in poor condition, with large amounts of manure in the channel and very low oxygen readings (< 2 mg $L^{-1}$). There was very little substrate available for colonization by invertebrates or algae and the benthic community was dominated by chironomids, a group of insect larvae that are very tolerant of poor water quality. In August and September of 1996, the National Resource Conservation Service (NRCS) installed a holding lagoon as a Best Management Practice on the farm. This BMP was designed to intercept manure and run-off from the feed lot and prevent impacts on Judd Creek.

We sampled the farm stream in April 1997 and the data collected showed a sharp increase in the number of "intolerant" (clean water indicator) taxa, in total numbers of organisms and in species diversity in the stream, especially in the sites furthest from the BMP. For example, the EPT:Chironomid ratio went from 0:2 to 4:1, indicative of higher water quality in the stream.

The post-BMP periphyton data at the sites showed high species richness (16-30 species collected), with pollution tolerance scores in the 2.3-2.6 range, indicating a diatom community which was moderately intolerant of organic pollution. The diatom diversity index ranged from 2.68 to nearly 3.6 (maximum=4.00) indicative of a diverse periphyton community. These metric scores are consistent with much improved water quality in the stream.

The physical assessment of the stream revealed that habitat quality (as measured by KYDOW score sheet) greatly improved in the stream. We suggest this was primarily due to the interception of manure and the re-establishment of cobble/ gravel areas which provide good habitat for invertebrates and algae. In the upstream sites there is still some sedimentation, but this is likely the result of cows using an upstream crossing and disturbing the substrate. Overall, the data collected indicate that the BMP installed on the Judd Farm was effective in improving water quality on site. There were no reference streams in the area which were suitable for comparison.

**Franklin Swine operation**

A large swine operation in Franklin, Kentucky, has installed a large, 16 cell constructed wetland on their property. Waste water from the swine operation is passed though these cells to reduce nutrient levels and then the processed filtrate is spread as a fertilizer onto adjacent cattle pasturage twice a year (cattle do not have direct access to the creek). Even though nutrient levels in the filtrate are reduced, these applications still result in considerable nutrient loading to the fields (David Stiles, personal communciation). There was concern that there might be significant nutrient run-off from the fields into Buck Creek, the major stream draining the watershed. In

1996-1997, benthic macroinvertebrates were sampled in Buck Creek and a reference stream as part of a larger water quality assessment project to test whether the nutrient loadings to the downstream site of Buck Creek were severely impairing the stream.

Buck Creek is a third order, perennial stream as it passes though the swine operation. Sampling sites in Buck Creek were chosen based on run-off patterns and the positions of effluent ditches from the surrounding fields and constructed wetland; the upstream (no impact) sites were positioned several hundred yards upstream from the effluent ditches while the downstream sites were located just below the effluent ditches. While fish collections have been made on Buck Creek since 1993, no macroinvertebrate collections were made until the spring of 1996.

May's Branch was selected as a reference reach in the spring of 1995. Data from reference streams are often useful in bioassessment studies because they provide a measure of sample variability due to biological or temporal factors that are not directly connected to anthropogenic impact. Both Buck Creek and May's Branch were surveyed in May of 1995 to establish baseline physical parameters for the macroinvertebrate bioassessment. Two riffle and two pool areas (=sites) were chosen in each upstream and downstream portion of both streams. The physical environment in the sites was assessed using Kentucky Division of Water (KDOW) protocols (Kentucky Division of Water 1993). Briefly, this includes qualitative assessments of riparian vegetation composition and condition, phytoplankton and periphyton abundance, and stream substrate structure. Current velocities were taken at each site using a Gurley current meter.

Macroinvertebrate samples were taken in riffles and pools using the modified traveling kick net (TKN) method (Plafkin et al. 1989). A D-frame net ( 35x33 cm, 600 μm mesh) was dragged upstream for 1 meter over a 60 second time period while the operator vigorously kicked the substrate to dislodge resident macroinvertebrates. The macroinvertebrates were swept by the current into the net and were then washed from the net into a white enamel pan where the larger specimens were separated out from the detritus and other material using fine forceps. The remaining contents of the pan were then passed and washed through a #30 sieve (500 μm mesh) to remove those organisms which may have been missed in the initial processing. At least two and usually three TKN samples were taken in each riffle and pool site using this method (total of 2 pools/riffles* 2/3 transects=4-6 samples per upstream and downstream area). Samples were not pooled. KDOW methods also require selected sampling of depositional habitats (snags, leaf packs, etc.), but these special habitats were almost completely absent in these streams. Leaf packs have been observed in this stream in the fall, but samples were scheduled specifically to avoid periods of litter fall, since the litter would interfere with the fish collections also taking place in this system. Pools, which were sampled using the same TKN methods, were the only significant depositional habitats in these streams. All macroinvertebrates were placed in labeled Whirl-pak bags in 90% alcohol for transportation to the laboratory. Macroinvertebrates were identified to the lowest practicable taxon (always to family, usually to genus/species) using an Olympus stereomicroscope and standard taxonomic references (e.g. Merritt and Cummins 1996, Brigham et al. 1982) and the advice of local experts familiar with the fauna (primarily Dr. Rudy Prins, WKU).

Taxonomic data was analyzed using the Principal Components Analysis (PCA) module of the statistical package SYSTAT. The entire data set (both streams) was analyzed first, followed by within-stream analyses. The purpose of these analyses was to determine if the downstream riffle and pool areas in Buck Creek had a different community composition than the upstream sites in Buck and/or both sites in May's Branch. The data were then evaluated using a series of biological metrics recommended by KDOW: total number of individuals (TNI), taxa richness, Ephmeoptera/Plecoptera/Trichoptera (EPT) index, EPT:Chironomidae ratio and the Jaccard index. The first two metrics are self-explanatory. The EPT index measures the number of distinct taxa from each of these three insect orders that are present. Members of these orders are generally intolerant of poor water quality and are often used as indicator species. The EPT:Chironomidae ratio compares the number of intolerant EPT taxa with the generally more tolerant chironomid taxa found at the same station. Thus, higher ratios indicate better water quality. The Jaccard coefficient is an index of similarity based on the presence and absence of taxa, in which scores vary from 0 to 1, increasing as the similarity of a site to its reference station increases. This ratio is calculated as $c/(a+b+c)$, where a= the number of taxa found in sample A but not B, b= the number of taxa found in sample B but not A, and c= the number of taxa common to both samples.

The PCA and biological metrics scores together, along with the physical assessment, were used to determine if the downstream site in Buck Creek was significantly different from the upstream site or the reference stream sites.

**Results**

The physical assessment of the two streams indicated that they were very similar in terms of canopy cover and phytoplankton in riffle areas (Table 1). Substrate was similar between the two streams in riffle areas, although there was more sedimentation evident in the downstream areas of May's Branch. A significant percentage of the exposed stream bed in upstream May's Branch was exposed limestone, which provides little suitable habitat for many macroinvertebrates. Average current velocity and alga cover were different between the two sites; filamentous algae was very common in downstream Buck Creek during all sample periods and uncommon elsewhere in the streams. Average current velocities were much higher in May's Branch than in Buck Creek. This is primarily the result of a series of small waterfalls in both portions of May's Branch which increased water velocities. These differences in the physical structure among riffle sites may confound the analysis of the macroinvertebrate data.

The physical structure of pool sites was generally similar to adjacent riffles, with the exception that sedimentation was more prevalent (up to 35%) in these areas. This probably impacted the suitability of this habitat for macroinvertebrates (see below)

An additional complicating factor was the loss of some data for the May's Branch downstream pool sites in 1995. Inadequate preservation in the field resulted in the samples being destroyed by fungal, and presumably bacterial, action. As a result, the pool data comparisons are not as complete in terms of community representation as the riffle community samples.

The PCA analysis revealed that macroinvertebrate data points in the downstream riffles of Buck Creek (Fig 1) fell outside the data cloud formed by the other Buck Creek areas along the two PCA axes, indicating that macroinvertebrate community structure in this area was different than that in other areas. The year and season factors had less explanatory power than the position of the sites.

The biometrics were quite variable in their scores for the pool sites and somewhat less so for the riffle sites (Tables 2 & 3). Pool sites had generally lower numbers of individuals and lower taxa richness, EPT scores and Jaccard coefficients than riffle habitats in the same reach. Because of this, and the loss of some pool data mentioned above, we will focus our analysis on the riffle reaches of Buck Creek and May's Branch.

The downstream Buck riffles showed higher numbers of individuals and greater taxa richness compared to the upstream riffles; the opposite was true for riffles in May's Branch.

EPT taxa were found in all habitats and the EPT:Chironomidae ratios were high in all of the riffles sampled. Jaccard coefficients between reference and impacted riffles, however, were rather low (all$< 0.60$).

## Discussion

One of the most useful aspects of bioassessments and biometrics is the potential for biotic communities to reveal subtle impacts in the environment through changes in macroinvertebrate community structure. The purpose of this project was to determine if applying biofiltered effluent on fields was having a deleterious effect on Buck Creek. Based on these results, there does appear to be impact, but not impairment, on Buck Creek downstream riffles.

Several lines of evidence support this conclusion. First, the PCA analysis indicates that macroinvertebrate communities in the downstream riffle portions of Buck Creek are different than the upstream riffles. Taxa richness and total numbers of individuals are higher in the downstream riffles than they are in the upstream riffles. This increase in numbers may be the result of nutrient enrichment. Studies have shown that stream periphyton can be nutrient limited (Stockner and Shortreed 1978; Shortreed and Stockner 1983) wherein low levels of nutrient input may enhance periphyton growth and thus the resource base for macroinvertebrates. In addition, these downstream riffles are the only areas were filamentous algae are common. These algae are often indicative of nutrient enrichment. The impact of nutrient run-off did not, however, result in a shift in community composition of species more tolerant of poor water quality (e.g. Chironomidae). There was no consistent pattern of loss of EPT species downstream and EPT:Chironomidae ratios remained high at all Buck Creek sites. It is likely that run-off from the fields is affecting the stream, but not significantly impacting water quality.

Community patterns seen in May's Branch differed from those in Buck Creek. Taxa richness and total numbers of individuals were greater upstream than downstream in 1995, yet the opposite was true for 1996 samples. This variation in community composition coupled with the physical differences already mentioned limits the usefulness of May's Branch as a reference site. In addition, considerable sedimentation (Table 1) was observed in the downstream areas of May's Branch, which may also affect macroinvertebrate colonization, further confounding the comparison between Buck Creek and May's Branch.

Our data indicate that run-off flowing from the swine operation to downstream Buck Creek is affecting this ecosystem's macroinvertebrate community structure, but no severe impairment is indicated. Correlation with water chemistry data is necessary to confirm that applying biofiltered water to pasture lands of the swine operation is not having a negative impact on downstream Buck Creek. May's Branch is of limited usefulness as a reference stream and perhaps should not be sampled in future.

Since the conclusion of 1997 sampling, a low-water ford has been constructed across Buck Creek bisecting the upstream and downstream stations. This bridge has significantly altered the downstream habitat (shifted flow patterns; created a new, deeper pool just downstream of the bridge, etc.). As a result, additional work performed on this stream will have to take into account the hydrologically and biologically altered environments. We do recommend that the monitoring of Buck Creek continue, however. Macroinvertebrate bioassessment of Buck Creek has given the owners of the swine operation valuable insights into the effects of their operation on this watershed. Biological indicators have provided them a cost-effective and scientifically sound ecological impact assessment approach thereby allowing them to be both environmentally conscious corporate citizens and maintain a profitable operation in a competitive agribusiness market.

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Table 1. Physical assessment of Buck and May's Branch riffle sites using Kentucky Division of Water metrics. 1995 data only.

| Parameter | Method | Buck Riffle | Mays Riffle |
|---|---|---|---|
| **Current velocity** | Pygmy | 0.22 m $s^{-1}$ | 0.78 m $s^{-1}$ |
| **Substrate** | transect, mean | 60% exposed bedrock, 40% mixed pebble/gravel | 50 % exposed bedrock, 30% pebble gravel, 10% sediment |
| **Canopy (spring)** | Estimation | 70% cover | 50% cover |
| **Condition** | | perennial | perennial |
| **Alga cover** | quadrat | < 5% up riffle<br>25-30% down riffle (Cladophora) | < 5 % |
| **Phytoplankton** | KDOW Index | 1 | 1 |

Table 2. Result of biometrics for upstream and downstream pool sites in the impacted stream (Buck Creek) and the reference stream (May's Branch) for 1995 and 1996 sample periods at Dogwood Ridge Farm. Pig Improvement Corporation, Franklin, KY.

| Site | Date | Taxa Rich. | TNI | EPT | EPT:Chiron. | Jaccard |
|---|---|---|---|---|---|---|
| **Buck up** | May '95 | 1 | 3 | 1 | 1:0 | 0.08 |
| **Buck dwn** | May '95 | 13 | 53 | 6 | 6:1 | NA |
| **Mays up** | May '95 | 8 | 53 | 3 | 3:1 | * |
| **Mays dwn** | May'95 | NA | NA | Na | NA | * |
| **Buck up** | Sept. '95 | 2 | 2 | 1 | 1:0 | 0.09 |
| **Buck dwn** | Sept '95 | 17 | 3 | 3 | 3:0 | 0 |
| **Mays up** | Sept'95 | 11 | 36 | 5 | 5:1 | * |
| **Mays dwn** | Sept '95 | 2 | 2 | 0 | 0:1 | * |
| **Buck up** | May '96 | 7 | 19 | 3 | 3:0 | 0 |
| **Buck dwn** | May '96 | 10 | 26 | 4 | 4:1 | 0.38 |
| **Mays up** | May '96 | 6 | 20 | 1 | 1:0 | * |
| **Mays dwn** | May '96 | 8 | 15 | 5 | 5:0 | * |
| **Buck up** | Sept. '96 | 1 | 3 | 1 | 1:0 | 0.11 |
| **Buck dwn** | Sept '96 | 3 | 5 | 2 | 2:1 | 0.33 |
| **Mays up** | Sept '96 | 9 | 22 | 5 | 5:1 | * |
| **Mays dwn** | Sept '96 | 5 | 13 | 3 | 3:1 | * |

Table 3. Result of biometrics for upstream and downstream riffle sites in the impacted stream (Buck Creek) and the reference stream (May's Branch) for 1995 and 1996 sample periods.

| Site | Date | Taxa Rich. | TNI | EPT | EPT:Chiron. | Jaccard |
|---|---|---|---|---|---|---|
| **Buck up** | May '95 | 9 | 36 | 3 | 3:1 | 0.13 |
| **Buck dwn** | May '95 | 17 | 515 | 4 | 4:1 | 0.11 |
| **Mays up** | May '95 | 19 | 76 | 8 | 8:1 | * |
| **Mays dwn** | May'95 | 4 | 56 | 2 | 2:1 | * |
| **Buck up** | Sept. '95 | 8 | 47 | 6 | 6:1 | 0.19 |
| **Buck dwn** | Sept '95 | 16 | 135 | 3 | 3:1 | 0.19 |
| **Mays up** | Sept'95 | 14 | 105 | 6 | 6:1 | * |
| **Mays dwn** | Sept '95 | 8 | 71 | 4 | 4:1 | * |
| **Buck up** | May '96 | 11 | 27 | 6 | 6:1 | 0.32 |
| **Buck dwn** | May '96 | 17 | 208 | 6 | 6:1 | 0.47 |
| **Mays up** | May '96 | 14 | 76 | 3 | 3:0 | * |
| **Mays dwn** | May '96 | 18 | 56 | 8 | 8:0 | * |
| **Buck up** | Sept. '96 | 11 | 47 | 4 | 4:0 | 0.38 |
| **Buck dwn** | Sept '96 | 12 | 145 | 6 | 5:1 | 0.60 |
| **Mays up** | Sept '96 | 11 | 105 | 3 | 3:0 | * |
| **Mays dwn** | Sept '96 | 14 | 71 | 5 | 5:1 | * |

# Buck Creek 1997-Macroinvertebrates
Figure 1