

BENTHIC INVERTEBRATE POPULATION CHARACTERISTICS
AS AFFECTED BY WATER QUALITY IN COAL-BEARING
REGIONS OF TENNESSEE



U.S. GEOLOGICAL SURVEY

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BY WATER QUALITY IN COAL-BEARING REGIONS OF TENNESSEE

Arthur D. Bradfield

U.S. GEOLOGICAL SURVEY

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CONVERSION FACTORS

For use of readers who prefer to use metric units, conversion factors for terms used in this report are listed below:

| <u>Multiply</u> | <u>By</u> | <u>To obtain</u> |
|---|-----------|---|
| square foot (ft ²) | 0.09294 | square meter (m ²) |
| cubic foot per second (ft ³ /s) | 0.02832 | cubic meter per second (m ³ /s) |
| micromho per centimeter at 25° Celsius (µmhos/cm at 25°C) | 1.000 | microsiemen per centimeter at 25° Celsius (µS/cm at 25°C) |

BENTHIC INVERTEBRATE POPULATION CHARACTERISTICS AS AFFECTED BY WATER QUALITY IN COAL-BEARING REGIONS OF TENNESSEE

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ABSTRACT

Benthic invertebrate and water-quality data collected during previous U.S. Geological Survey studies to provide background hydrologic information on streams draining Tennessee coal reserves were evaluated to identify possible relations between stream biota and water quality. Linear regressions produced low correlation coefficients relating the number of taxa per sample, total number of organisms per sample, sample diversity, and percentage composition of selected orders of invertebrates with average water-quality parameter values available at sampling stations ($r < 0.62$ at $p = 0.05$). Analyses of these data by linear regressions explained little of the variability in benthic invertebrate samples primarily because the distributions of benthic organisms along environmental gradients are nonlinear. Variability in substrate characteristics in the study area and seasonal insect emergence patterns also complicated interpretation of these data. However, analysis of variance tests did indicate significant trends towards reduced number of taxa, number of organisms, and sample diversity at stations with relatively poor water-quality conditions. Decreasing percentage composition of Ephemeroptera was generally accompanied by an increase in percent Diptera at stations with higher water-quality constituent concentrations and acidic pH (<6.0 units). These trends indicate significant differences in benthic communities at sites with evidence of more severe land-use impacts. Additional data on benthic invertebrates, water quality, and physical habitat conditions, along with analyses of data using multivariate statistical methods are needed to define ecological relations between specific groups of invertebrates and environmental conditions.

INTRODUCTION

Coal production by strip and deep mining methods often results in serious environmental impacts on adjacent water resources. Recognizing the need for hydrologic information on coal mining areas, the United States Congress enacted the Surface Mining Control and Reclamation Act of 1977 (hereafter referred to as the Act), Public Law 95-87. This law established limits for some chemical and physical properties of mine effluents and established regulations governing mining and reclamation practices. The Act also required that specific studies of mining impacts be carried out. Data used in this report were collected by the U.S. Geological Survey in previous studies to provide background information on hydrologic environments of coal resource areas.

Studies conducted in eastern Tennessee have shown that increases in concentrations of dissolved and suspended constituents, sedimentation, and acid mine drainage can significantly alter the water quality, benthic invertebrate communities, and physical habitat conditions of receiving streams (Tolbert, 1978; Parker and Carey, 1980; Swartley and Gore,

1982). In order to assess these environmental impacts and to efficiently monitor the recovery of streams affected by mining operations, water-quality investigations should include examination and interpretation of the physical, chemical, and biological interactions of hydrologic systems. Surveys of benthic organisms, when combined with measurements of water-quality characteristics, can provide evidence of water-quality degradation or recovery that may not be apparent when studying only concentrations of chemical constituents.

Benthic invertebrates occur commonly in stream environments, form complex community relations, and are more easily identified to species than many microorganisms. Life cycles of invertebrates are short with most taxa producing one generation per year, although aquatic stages for some invertebrates may last as long as 3 years (Pennak, 1978). These attributes make invertebrate populations potential indicators of recent instream conditions, as well as indicators of long-term water-quality trends.

Although particular invertebrate taxa have long been associated with certain environmental conditions, little is known about the actual environmental requirements of most invertebrates. This is due in part to a lack of computer synthesis of available invertebrate and water-quality data and to the overlap and conflicts encountered when comparing taxonomic and environmental data from different sources (Hellenthal, 1981).

Without readily available computer processing of the large volumes of data collected in biological surveys, interpretations of invertebrate data have been based almost entirely on summary statistics such as diversity indices. Results of diversity index equations reduce the data to a single value that allows quick comparisons between stations or samples. Although diversity indices are adequate tools for discerning gross differences in biological communities, they are of little use in identifying specific water-quality conditions at the sampled site. Many diversity equations fail to respond to decreasing organism density when the number of species remains constant (Chadwick and others, 1984), making them inappropriate for evaluating "nonselective" impacts such as sedimentation that can depress the entire benthic community.

"Indicator organisms" are also of limited value in determining water quality (Cairns, 1974). Despite the fact that little is known about the response of most aquatic organisms to pollutants, the concept of using a single species as a measure of pollution still persists. Other environmental factors such as physical habitat conditions, natural geographical distribution, or sampling error may account for the absence of a particular species at a station.

The failure of any single measure of invertebrate communities to adequately assess land-use impacts on aquatic habitats and water quality indicates a need for more comprehensive analysis of invertebrate data. Benthic invertebrates will be more useful in water-quality investigations conditions once taxonomic or ecological groups of organisms can be associated with specific environmental conditions. This report describes results of initial investigations into relations between benthic invertebrate and water-quality data from streams draining Tennessee coal reserves using selected parametric statistical tests.

METHODS

Benthic invertebrate and water-quality data used in this report were collected by the U.S. Geological Survey during two separate projects designed to provide background

information of hydrologic environments in coal bearing regions. Invertebrate data and water-quality data were often collected as much as several months apart at the same station. Benthic invertebrate samples were collected once at 33 water-quality stations in 1980 and twice at 40 stations in 1981. Nine of the 33 stations sampled in 1980 had pH values less than 5.5 units. These stations were resampled as a part of the 1981 study, making a total of 64 stations sampled for benthic invertebrates (fig. 1). Sample data and locations of individual stations are published in Pennington (1980) and Gore and others (1982). Quantitative benthic invertebrate data were collected once in the 1980 study and twice in 1981 using a 1-square-foot (ft²) Surber sampler (three replicates). A qualitative kick sample using a hand held dip net was also collected at each site to increase representation of the more rare taxa. Identification of organisms was usually to the genus level.

During these biological studies, streamflow and water-quality data were collected by the Geological Survey at more than 100 sites in coal-bearing regions of Tennessee. Water-quality analyses include the determination of pH, specific conductance, alkalinity, dissolved sulfate, and total recoverable and dissolved iron and manganese. These data are summarized in Hufschmidt and others (1981), May and others (1981), Gaydos and others (1982), Leist and others (1982), and Hollyday and others (1983).

Approximately 6,000 observations of benthic invertebrate data were coded using a hierarchically arranged species list from the computer program ECOSCAN (Larry Evans, U.S. Army Corps of Engineers, Huntington District, West Virginia, written commun., 1983). Formatted fields were specified for station number, date of collection, number of organisms, organism code, and method of collection. Because water-quality data were not collected along with benthic invertebrate samples, water-quality data from Geological Survey coal-hydrology studies were used in the analyses presented in this report. Measurements of discharge, specific conductance, and pH, and determinations of dissolved concentrations of iron, manganese, and sulfate made during the 2 years of invertebrate sampling were averaged to characterize water-quality conditions at each station (table 1). Water-quality data were not available during 1980 and 1981 at two stations. Benthic invertebrate data were merged in computer files with mean values for water-quality parameters available at collection sites. Associated with each observation of invertebrate data were total number of organisms per sample, number of taxa per sample, sample percentage composition, sample diversity, and water-quality mean values.

Brillouin's measure of information per individual was selected as the index to express community diversity (Brillouin, 1962). Brillouin's index, though once cumbersome to compute because of factorials, is now widely accepted over other commonly used indices. (Pielou, 1966; Zand, 1976; Kaesler and others, 1978). Diversity was calculated for each sample using the formula

$$H = \frac{1}{N} \log_2 \frac{N!}{N_1! N_2! \dots N_S!}$$

where H = diversity index ;
 N = total number of individuals ;
 N₁, N₂ = number of individuals of species 1,2... ; and
 S = total number of species.

Table 1.--Average values for stream discharge and water-quality parameters available at sampling stations during 1980 and 1981

[N = number of samples used to derive mean values]

| Station No. | N | Discharge (ft ³ /s) | Sulfate (mg/L) | Manganese (µg/L) | Iron (µg/L) | Specific conductance (µS/cm) | pH (units) |
|-------------|----|--------------------------------|----------------|------------------|-------------|------------------------------|------------|
| 03403697 | 2 | 12 | 200 | 210 | 10 | 515 | 7.4 |
| 03403710 | 4 | 49 | 110 | 200 | 40 | 353 | 7.7 |
| 03403715 | 7 | 61 | 66 | 690 | 920 | 214 | 7.1 |
| 03404150 | 4 | 22 | 100 | 1,000 | 70 | 259 | 7.1 |
| 03407850 | 3 | 51 | 210 | 120 | 40 | 573 | 7.8 |
| 03407873 | 3 | 26 | 64 | 30 | 30 | 245 | 7.8 |
| 03407876 | 16 | 34 | 75 | 26 | 40 | 221 | 7.4 |
| 03407877 | 19 | 4.0 | 27 | 310 | 180 | 71 | 6.8 |
| 03407881 | 19 | 1.0 | 25 | 84 | 220 | 84 | 7.2 |
| 03407908 | 19 | 204 | 110 | 100 | 20 | 320 | 7.5 |
| 03407920 | 3 | 17 | 34 | 190 | 100 | 143 | 7.0 |
| 03407960 | 6 | 16 | 48 | 230 | 70 | 195 | 7.5 |
| 03408500 | 19 | 518 | 93 | 210 | 40 | 285 | 7.3 |
| 03408600 | 14 | 1.1 | 17 | 1,300 | 2,700 | 56 | 5.5 |
| 03408700 | 4 | 118 | 6.2 | 90 | 120 | 51 | 7.0 |
| 03408815 | 15 | 5.9 | 35 | 1,100 | 220 | 122 | 6.7 |
| 03409350 | 6 | 24 | 4.0 | 42 | 60 | 37 | 6.9 |
| 03409400 | 20 | 128 | 21 | 73 | 70 | 141 | 7.1 |
| 03409500 | 20 | 383 | 11 | 22 | 70 | 69 | 7.1 |
| 03414340 | 7 | 281 | 58 | 590 | 870 | 150 | 3.9 |
| 03414346 | 5 | 197 | 7.2 | 26 | 110 | 37 | 6.4 |
| 03414430 | 5 | 744 | 480 | 3,600 | 4,400 | 1,020 | 3.3 |
| 03414500 | 19 | 753 | 68 | 300 | 50 | 219 | 7.3 |
| 03414680 | 6 | 337 | 21 | 23 | 20 | 195 | 7.8 |
| 03415000 | 19 | 424 | 42 | 52 | 20 | 282 | 7.9 |
| 03415960 | 5 | 466 | 7.6 | 14 | 30 | 139 | 7.8 |
| 03416000 | 19 | 247 | 28 | 17 | 20 | 234 | 8.2 |
| 03418520 | 2 | 1.6 | 86 | 3,000 | 800 | 233 | 4.8 |
| 03418925 | 3 | 268 | 3.7 | 80 | 310 | 43 | 6.5 |
| 03418935 | 3 | 541 | 4.3 | 210 | 320 | 51 | 6.6 |
| 03418995 | 7 | 388 | 15 | 43 | 220 | 79 | 7.0 |
| 03419200 | 2 | 1224 | 9.5 | 35 | 60 | 54 | 7.2 |
| 03420116 | 4 | 843 | 33 | 82 | 20 | 176 | 7.5 |
| 03420230 | 7 | 379 | 11 | 17 | 20 | 265 | 7.9 |
| 03420260 | 4 | 266 | 38.0 | 275 | 25.0 | 182 | 7.8 |
| 03421000 | 18 | 3,030 | 12 | 24 | 40 | 181 | 7.9 |
| 03532100 | 3 | 25 | 4.7 | 23 | 20 | 320 | 8.0 |
| 03532480 | 6 | 13 | 45 | 35 | 40 | 225 | 7.6 |
| 03534000 | 7 | 69 | 81 | 170 | 40 | 346 | 7.8 |
| 03538225 | 20 | 248 | 41 | 220 | 30 | 231 | 7.4 |
| 03538398 | 4 | 188 | 7.5 | 120 | 150 | 102 | 7.1 |
| 03539600 | 7 | 2,675 | 5.6 | 39 | 60 | 65 | 7.2 |
| 03539719 | 2 | 32 | 3.0 | 75 | 80 | 47 | 6.6 |

Table 1.--Average values for stream discharge and water-quality parameters available at sampling stations during 1980 and 1981--Continued

| Station No. | N | Discharge (ft ³ /s) | Sulfate (mg/L) | Manganese (μg/L) | Iron (μg/L) | Specific conductance (μS/cm) | pH (units) |
|-------------|----|--------------------------------|----------------|------------------|-------------|------------------------------|------------|
| 03539750 | 8 | 158 | 6.9 | 25 | 50 | 50 | 7.0 |
| 03540100 | 4 | 237 | 82 | 2,200 | 140 | 210 | 5.4 |
| 03540500 | 24 | 1,610 | 20 | 50 | 40 | 97 | 7.1 |
| 03541487 | 4 | 209 | 6.0 | 60 | 30 | 67 | 6.9 |
| 03541496 | 4 | 147 | 9.0 | 12 | 20 | 105 | 7.0 |
| 03542495 | 4 | 674 | 7.8 | 14 | 40 | 48 | 6.8 |
| 03544500 | 18 | 97 | 11 | 9.4 | 20 | 75 | 7.4 |
| 03566292 | 6 | 209 | 13 | 120 | 20 | 74 | 6.7 |
| 03566400 | 4 | 245 | 26 | 360 | 20 | 84 | 5.3 |
| 03566530 | 2 | 750 | 7.5 | 50 | 30 | 28 | 5.4 |
| 03569245 | 4 | 125 | 34 | 470 | 50 | 102 | 5.2 |
| 03570602 | 4 | 236 | 5.2 | 55 | 40 | 222 | 8.2 |
| 03571000 | 18 | 610 | 7.9 | 26 | 30 | 185 | 7.9 |
| 03571500 | 4 | 549 | 27 | 18 | 20 | 143 | 7.9 |
| 03571800 | 4 | 341 | 10 | 18 | 30 | 196 | 7.9 |
| 03572092 | 4 | 357 | 11 | 28 | 20 | 206 | 8.2 |
| 03577966 | 8 | 118 | 5.0 | 10 | 30 | 100 | 7.6 |
| 03578000 | 18 | 100 | 16 | 52 | 30 | 195 | 7.6 |
| 03578190 | 4 | 67 | 9.8 | 30 | 40 | 189 | 7.8 |

The uniformity of the distribution of individuals among the taxa in a sample was also computed and is expressed as relative evenness (Zand, 1976). Relative evenness ranges from zero for the least even sample to one for the most even sample and is the ratio,

$$e = \frac{H - H_{\min}}{H_{\max} - H_{\min}}$$

where

$$H_{\max} = \frac{1}{N} \log_2 \frac{N!}{(m+1)^r (m!)^s}$$

and

$$H_{\min} = \frac{1}{N} \log_2 \frac{N!}{[N-(s-1)]!}$$

where

- m = N/s,
- S = number of species,
- N = total number of individuals, and
- r = remainder of m.

Relations between benthic invertebrate data from Surber samples and mean values of water-quality parameters were examined using regression analyses and analysis of variance tests. Because ecological data are rarely normally distributed, transformations of invertebrate and water-quality data were necessary to meet the assumptions of parametric statistics (Zar, 1974; Elliott, 1977; Sokal and Rohlf, 1981). Log transformations were adequate for number of taxa per sample, number of organisms per sample, and water-quality values. Percentage composition of taxa in a sample were normalized by taking the square root of each percentage and then transforming the square root to its arcsin as recommended in Zar (1974).

Homogeneity of variances between groups used in analysis of variance (ANOVA) was evaluated using Bartlett's test (Bartlett, 1937; Zar, 1974) and Hartley's Fmax-test (Hartley, 1950; Sokal and Rohlf, 1981). Homogeneity of variances of all groups used in this report satisfied the requirements of the Fmax test. Homogeneity of some groups could not be accepted at the $p < 0.05$ level using Bartlett's test. However, because analysis of variance is robust in regard to heterogeneity of variance and to the assumptions of normality, results of ANOVA tests, as qualified, were used to evaluate available invertebrate data.

DISCUSSION OF RESULTS

Streams draining the coal regions of Tennessee support a diverse population of benthic invertebrates. A total of 261 genera were represented in 370 kick and Surber samples from 64 streams. Despite a high diversity in the region, the number of taxa in an individual sample was relatively low (mean (\bar{x}) = 16, standard deviation (SD) = 9), with a maximum of 47 taxa in a single sample. The number of organisms per sample also was variable (\bar{x} = 324, SD = 414), ranging from 0 to 3,000 organisms in a single sample. Variability in sample composition is due in part to the clumped distribution of benthic organisms on the streambed, seasonal emergence patterns, and the wide range of physical and chemical factors influencing the distribution of individual taxa.

Analyses of available data using Pearson correlation coefficients (r) indicated poor linear relations between benthic invertebrate data from Surber samples and water-quality parameters (table 2). Correlation coefficients (r) relating the number of taxa and the total number of organisms per sample with average dissolved constituent concentrations, specific conductance, and pH were less than 0.62. Coefficients of determination (r^2) obtained from multiple regressions using sulfate, manganese, iron, specific conductance, and stream pH as independent variables explained only part of the variability in number of taxa per sample ($r^2 = 0.51$ at a level of probability (p) = 0.05) and number of organisms per sample ($r^2 = 0.32$ at $p = 0.05$).

Of the water-quality variables studied, dissolved manganese accounted for the majority of the coefficients of determination obtained in regression analyses of invertebrate data. Although not considered toxic to biota in concentrations found in the study area, manganese can be important in forming oxides in aquatic systems (Hem, 1970). Hydrous manganese oxides have extremely high adsorption capacities for trace metals. Conditions of pH and Eh favorable for dissolution of manganese could possibly result in increased dissolved concentrations of trace metals known to be toxic to biota. However, in addition to the scarcity of data on trace metal concentrations in mine impacted streams, little information is available on the uptake mechanisms and responses of most invertebrates to elevated concentrations of trace constituents. Additional data on trace metal concentrations in streams impacted by mining are needed to study actual cause and effect relations between invertebrates and potentially toxic constituents.

Table 2.--Pearson product moment correlation coefficients (r at p=0.05) relating benthic invertebrate sample data with average water-quality values at sampling stations (N=278)

| Benthic invertebrate sample parameters | Sulfate, dissolved | Manganese, dissolved | Iron, dissolved | Specific conductance | pH |
|--|--------------------|----------------------|-----------------|----------------------|-------|
| Number of taxa per sample. | -0.39 | -0.58 | -0.26 | -0.12 | +0.62 |
| Number of organisms per sample. | -.25 | -.43 | -.19 | -.01 | +.51 |
| Sample diversity (H) | -.37 | -.56 | -.23 | -.14 | +.42 |
| Relative evenness (e) | -.27 | -.36 | -.11 | -.20 | +.20 |
| Percentage composition of Ephemeroptera. | -.19 | -.54 | -.27 | 0 | +.48 |
| Percentage composition of Plecoptera. | -.09 | +.04 | +.22 | -.33 | -.16 |
| Percentage composition of Diptera. | +.14 | +.33 | +.05 | +.02 | -.17 |

A comparison between the number of taxa per sample and mean dissolved constituent concentrations at sampling stations indicates a trend towards fewer taxa per sample, particularly at sites with relatively high concentrations of dissolved manganese (U.S. Geological Survey, unpublished data). The number of organisms per sample also showed little linear correlation with water-quality parameters as indicated by r values in table 2.

In addition to the number of taxa and the number of individual organisms in a sample, diversity indices are also commonly used to describe differences in benthic invertebrate communities. Diversity equations consider not only the number of taxa but also the relative abundance of organisms in the different taxonomic groups.

Diversity and relative evenness values for individual Surber samples were used as dependent variables in regression analyses with water-quality parameters; however, values for the three Surber samples collected at each station on a particular date were averaged for presentation in table 3 of this report. Standard deviations are reported as an indication of the variability in Surber samples collected at the same station on the same date. Although kick samples were not used in statistical analyses because of differences in sampling effort and area sampled, diversity values of qualitative kick samples were usually higher than for Surber samples, reflecting the increase in number of taxa and individual organisms obtained from a larger sample area.

Table 3.--Diversity values (H) and relative evenness (e) values
for benthic invertebrate samples

[Diversity and evenness values for the three Surber samples collected on
each date were averaged for presentation in this table]

| Station No. | Date | Sample diversity (H) | Standard deviation (H) | Sample evenness (e) | Standard deviation (e) |
|----------------|---------|----------------------------|------------------------------|---------------------------|------------------------------|
| 03403697 | 6/17/81 | 1.73 | 0.37 | 0.28 | 0.14 |
| 03403697 | 9/22/81 | 1.58 | .16 | .15 | .02 |
| 03403710 | 6/17/81 | 2.26 | .31 | .32 | .11 |
| 03403710 | 9/22/81 | 2.58 | .12 | .16 | .01 |
| 03403715 | 5/14/80 | 1.90 | .20 | .32 | .05 |
| 03404150 | 5/14/80 | .95 | .01 | .06 | .00 |
| 03407850 | 5/18/81 | 1.13 | .05 | .11 | .03 |
| 03407850 | 9/21/81 | .92 | .32 | .06 | .02 |
| 03407873 | 5/13/80 | 1.87 | .39 | .10 | .01 |
| 03407876 | 5/13/80 | 2.51 | .17 | .28 | .05 |
| 03407877 | 6/30/81 | 2.67 | .46 | .34 | .09 |
| 03407881 | 7/ 1/81 | 2.96 | .44 | .35 | .13 |
| 03407908 | 5/13/80 | .94 | .43 | .10 | .12 |
| 03407920 | 7/ 1/81 | 2.13 | .09 | .22 | .01 |
| 03407920 | 9/21/81 | 2.32 | .07 | .24 | .10 |
| 03407960 | 5/18/81 | 1.42 | .28 | .09 | .03 |
| 03407960 | 9/21/81 | 1.63 | .33 | .14 | .06 |
| 03408500 | 5/13/80 | 1.49 | .46 | .11 | .07 |
| 03408600 | 5/ 9/80 | 1.75 | .24 | .19 | .07 |
| 03408700 | 6/17/81 | 1.36 | .67 | .10 | .08 |
| 03408700 | 8/28/81 | 1.27 | .42 | .07 | .04 |
| 03408815 | 5/ 9/80 | 2.20 | .93 | .28 | .24 |
| 03409350 | 4/15/81 | 1.90 | .57 | .38 | .18 |
| 03409350 | 9/18/81 | 1.96 | .21 | .19 | .04 |
| 03409400 | 5/ 9/80 | 1.41 | .51 | .08 | .04 |
| 03409400 | 4/15/81 | 1.60 | .10 | .20 | .03 |
| 03409400 | 9/18/81 | 2.78 | .56 | .39 | .14 |
| 03409500 | 5/ 8/80 | 2.05 | 1.69 | .11 | .10 |
| 03414340 | 4/15/81 | .92 | - | .07 | - |
| 03414340 | 8/26/81 | .79 | - | .06 | - |
| 03414346 | 4/14/81 | 2.33 | .13 | .41 | .01 |
| 03414346 | 8/26/81 | 3.07 | .35 | .18 | .01 |
| 03414430 | 9/16/81 | .06 | - | .00 | - |
| 03414500 | 5/ 7/80 | 1.15 | .40 | .03 | .06 |
| 03414680 | 5/ 1/80 | 2.92 | .05 | .44 | .02 |
| 03414680 | 3/29/81 | 1.87 | .69 | .25 | .35 |
| 03414680 | 8/26/81 | 1.39 | .48 | .15 | .08 |
| 03415000 | 5/ 1/80 | 1.47 | .26 | .14 | .05 |
| 03415000 | 5/ 1/81 | 2.17 | - | .38 | - |
| 03415960 | 4/15/81 | 2.89 | .26 | .31 | .06 |
| 03415960 | 9/18/81 | 2.83 | .11 | .08 | .02 |
| 03416000 | 5/ 7/80 | 3.00 | .32 | .25 | .07 |

Table 3.--Diversity values (H) and relative evenness (e) values
for benthic invertebrate samples--Continued

| Station No. | Date | Sample diversity (H) | Standard deviation (H) | Sample evenness (e) | Standard deviation (e) |
|-------------|----------|----------------------|------------------------|---------------------|------------------------|
| 03418520 | 4/25/81 | 0.67 | 0.25 | 0.03 | 0.03 |
| 03418520 | 10/ 6/81 | .26 | .11 | .01 | .01 |
| 03418925 | 5/28/80 | 1.99 | .20 | .23 | .12 |
| 03418935 | 5/21/81 | 2.26 | .55 | .45 | .14 |
| 03418935 | 8/25/81 | 2.06 | .17 | .15 | .10 |
| 03418995 | 5/ 1/81 | 1.47 | .21 | .06 | .02 |
| 03418995 | 10/ 6/81 | 3.16 | .27 | .45 | .11 |
| 03419200 | 5/20/81 | 1.93 | .28 | .29 | .08 |
| 03420116 | 5/20/81 | 2.75 | .21 | .39 | .12 |
| 03420116 | 8/25/81 | 3.10 | .14 | .16 | .05 |
| 03420230 | 5/20/81 | 2.85 | .20 | .18 | .08 |
| 03420230 | 8/25/81 | 3.13 | .16 | .31 | .07 |
| 03420260 | 6/26/80 | 2.62 | .07 | .27 | .06 |
| 03421000 | 6/26/80 | 2.99 | .24 | .25 | .11 |
| 03532100 | 5/14/80 | 3.00 | .26 | .15 | .00 |
| 03532480 | 5/14/80 | 2.13 | .17 | .22 | .03 |
| 03534000 | 5/15/80 | 0.45 | .13 | .01 | .01 |
| 03534000 | 5/18/81 | 1.27 | .71 | .06 | .04 |
| 03534000 | 9/21/81 | 1.84 | .29 | .12 | .03 |
| 03538225 | 5/15/80 | .51 | .20 | .02 | .01 |
| 03538398 | 5/22/81 | 1.60 | .90 | .18 | .12 |
| 03538398 | 8/27/81 | 1.48 | 1.08 | .20 | .25 |
| 03539600 | 7/ 2/80 | 2.93 | .76 | .33 | .11 |
| 03539750 | 4/15/81 | 1.91 | .82 | .26 | .11 |
| 03539750 | 8/28/81 | 2.18 | .05 | .16 | .01 |
| 03540100 | 4/16/81 | .47 | .10 | .02 | .04 |
| 03540100 | 9/21/81 | .88 | .43 | .04 | .07 |
| 03540500 | 5/15/80 | 2.18 | .47 | .11 | .06 |
| 03540500 | 4/16/81 | .30 | .14 | .01 | .01 |
| 03541487 | 4/16/81 | 1.87 | .76 | .20 | .11 |
| 03541487 | 8/27/81 | 2.50 | .61 | .43 | .22 |
| 03541496 | 4/16/81 | 2.43 | .09 | .43 | .08 |
| 03541496 | 8/27/81 | 3.41 | .05 | .53 | .09 |
| 03542495 | 6/30/80 | 3.12 | .07 | .28 | .14 |
| 03544500 | 6/30/80 | 2.86 | .13 | .23 | .03 |
| 03566292 | 6/12/81 | 1.11 | .54 | .09 | .05 |
| 03566400 | 6/27/80 | .51 | .02 | .00 | .00 |
| 03566400 | 6/21/81 | .40 | .37 | .06 | .11 |
| 03566530 | 6/12/81 | .74 | .93 | .11 | .19 |
| 03569245 | 6/12/81 | .88 | .43 | .07 | .03 |
| 03569245 | 9/17/81 | 1.90 | .25 | .31 | .12 |
| 03570602 | 8/25/81 | 2.21 | .32 | .27 | .07 |
| 03571000 | 6/27/80 | 2.74 | .15 | .18 | .05 |
| 03571500 | 6/27/80 | 2.70 | .37 | .15 | .06 |
| 03571500 | 6/11/81 | 2.14 | .28 | .23 | .09 |
| 03571500 | 9/17/81 | 3.10 | .16 | .44 | .10 |

Table 3.--Diversity values (H) and relative evenness (e) values for benthic invertebrate samples--Continued

| Station No. | Date | Sample diversity (H) | Standard deviation (H) | Sample evenness (e) | Standard deviation (e) |
|-------------|---------|----------------------|------------------------|---------------------|------------------------|
| 03571800 | 9/17/81 | 2.45 | 0.36 | 0.30 | 0.10 |
| 03572092 | 5/21/80 | 1.59 | .26 | .14 | .06 |
| 03572092 | 6/11/81 | 1.07 | .06 | .04 | .00 |
| 03572092 | 7/ 1/81 | 1.85 | - | .12 | - |
| 03572092 | 9/17/81 | 2.18 | .49 | .31 | .02 |
| 03577966 | 5/21/80 | 2.34 | .22 | .29 | .06 |
| 03578000 | 5/21/80 | 2.79 | .19 | .23 | .02 |
| 03578190 | 6/11/81 | 2.17 | .15 | .08 | .01 |
| 03578190 | 9/17/81 | 1.83 | .34 | .13 | .04 |

Diversity and evenness of Surber samples showed little correlation with mean water-quality parameters using Pearson correlation coefficients (table 2). Dissolved manganese was the most significant independent variable in multiple regressions relating sample diversity with sulfate, manganese, iron, specific conductance and pH ($r^2 = 0.35$ at $p = 0.05$). A comparison of diversity values with average dissolved manganese concentrations indicates a trend towards lower diversity values for samples collected at stations with relatively high dissolved manganese (U.S. Geological Survey, unpublished data). Water-quality parameters had less impact on relative evenness of samples as measured by multiple regression coefficients ($r^2 < 0.15$ at $p = 0.05$).

The number of species, number of organisms, and diversity values of invertebrate samples are useful for describing benthic community structure; however, they provide no information on specific environmental conditions from which the samples are collected. These measures of biological communities do not consider the types of organisms present or their ranges of tolerance to fluctuating environmental conditions. For example, a sample comprised of tolerant organisms from a polluted environment may contain the same number of taxa and have the same diversity as a sample of intolerant organisms from a pristine environment.

Although reductions in sample diversity or numbers of species can be used to document degrading conditions, such studies require data collection over a long period of time. Insight into the distribution of invertebrate taxa along environmental gradients such as increasing constituent concentrations would be more useful in assessments of environmental quality. Knowledge of the types of fauna typically inhabiting a stream environment would enable evaluations of environmental impacts relative to the range of conditions possible.

While attempts to establish a single species as an indication of environmental conditions are probably futile, marked changes or differences in the taxonomic structure of benthic communities known to occur in a particular type of stream habitat may be an indication of environmental stress. Sample percentage composition of three dominant orders of invertebrates found in the coal regions of Tennessee was compared with mean

water-quality parameters (table 2). Although the percentage composition of taxa in invertebrate samples was too variable for defining linear relations using regression equations, percentage composition of Ephemeroptera (mayflies) and Plecoptera (stoneflies) was generally inversely related with increasing mean concentrations of water-quality constituents, indicating a reduction of these taxa. Percentage composition of Dipterans, generally considered to be more tolerant to pollution as a group, increased with increasing average constituent concentrations as indicated by positive correlation coefficients in table 2.

This shift in dominance from Ephemeroptera and Plecoptera to Dipterans at the most polluted sites supports the findings of an earlier study by Winner and others (1980). In Winner's study, there appeared to be a direct relation between the fraction of the invertebrate community composed of chironomids (midges) and trace-constituent concentrations in water. Such changes in benthic community composition accompanying increases in constituent concentrations may provide documentation of changing environmental conditions as well as information useful in reclamation monitoring.

The poor correlations obtained using regression analyses of these available data are not surprising considering the complexity of hydrologic factors influencing benthic communities. Statistically significant linear regressions were not obtained primarily because:

(1) Frequency distributions of species, when aligned along environmental gradients such as increasing constituent concentrations, tend to form bell-shaped, binomial curves, with densities declining gradually to scarcity and absence on each side of a central peak (Whittaker, 1967). Therefore, sample parameters such as number of taxa and sample diversity affected by species abundance, are not linearly related to changing environmental conditions.

(2) Species distributions are affected by environmental factors other than water quality such as substrate characteristics, temperature, stream velocity, depth, and available food. Physical habitat conditions were not documented at invertebrate sampling sites, resulting in comparison of streams with different particle-size distributions of bed material. Substrate characteristics can have a profound effect on species occurrence and abundance (Lium, 1974; Orth and Maughan, 1983).

(3) In addition to the nonlinear response of most organisms to increasing constituent concentrations, the water-quality parameters used in this study to characterize conditions at invertebrate sampling sites are not considered toxic to aquatic biota. These parameters are more useful as indices to the degree of environmental impacts affecting benthic invertebrate communities.

Although these data were too variable for defining meaningful linear relationships between invertebrates and generalized water-quality conditions, trends in the data indicate that relations do exist. To further investigate these trends, stations were grouped based on similar mean water-quality values. Analysis of variance (ANOVA) tests were used to determine significant differences in invertebrate sample parameters when invertebrate samples were grouped based on similar water-quality conditions at sampling stations. These tests showed significant differences in most invertebrate sample parameters as measured by the probability of obtaining a greater F statistic ($P > F$) when stations were grouped based on mean values for manganese (table 4), sulfate (table 5), iron (table 6), specific conductance (table 7), and pH (table 8).

Table 4.--Mean values from benthic invertebrate samples when grouped based on mean concentrations of dissolved manganese at sampling stations

[N, number of invertebrate samples used to compute mean values; *, group deleted from ANOVA due to significantly different variance; **, homogeneity of variance accepted at $0.025 < p < 0.001$ using Bartlett's test]

| Benthic invertebrate sample parameters (mean values) | Mean concentrations of dissolved manganese at collection sites | | | | Level of significance | |
|--|--|-----------------------------------|------------------------------------|----------------------------------|-----------------------|-----|
| | 0-100 $\mu\text{g/L}$ N = 162 | 101-500 $\mu\text{g/L}$ N = 86 | 501-1,000 $\mu\text{g/L}$ N = 6 | >1,000 $\mu\text{g/L}$ N = 24 | Pr | > F |
| Number of taxa | 18 | 10 | 6 | * | 0.0001 | |
| Number of organisms | 410 | 243 | 44 | * | .0001 | |
| Sample diversity (H) | 2.29 | 1.54 | 1.48 | .95 | .0001 | |
| Relative evenness (e) | .24 | .16 | .22 | .08 | .0001 | |
| Percent Ephemeroptera | .31 | .16 | .14 | .07 | .0001 | |
| Percent Plecoptera | .03 | .03 | .04 | .06 | .8772 | |
| Percent Diptera | .41 | .64 | * | .71 | .0001 | ** |

Table 5.--Mean values from benthic invertebrate samples when grouped based on mean concentrations of dissolved sulfate at sampling stations

[N, number of invertebrate samples used to compute mean values; *, group deleted from ANOVA due to significantly different variance; **, homogeneity of variance accepted at $0.025 < p < 0.001$ using Bartlett's test]

| Benthic invertebrate sample parameters (mean values) | Mean concentrations of dissolved sulfate at collection sites | | | | Level of significance | |
|--|--|------------------------|------------------------|--------------------|-----------------------|-----|
| | 0-20 mg/L N = 129 | 21-100 mg/L N = 121 | 101-300 mg/L N = 23 | >300 mg/L N = 5 | Pr | > F |
| Number of taxa | 17 | 12 | 10 | 2 | 0.0001 | ** |
| Number of organisms | 415 | 264 | 193 | 35 | .0001 | ** |
| Sample diversity (H) | 2.18 | 1.79 | 1.54 | .06 | .0001 | |
| Relative evenness (e) | .23 | .18 | .16 | .00 | .0016 | |
| Percent Ephemeroptera | .25 | .23 | .22 | .20 | .0042 | ** |
| Percent Plecoptera | .04 | .03 | .02 | * | .8722 | ** |
| Percent Diptera | .46 | .52 | .63 | * | .0812 | |

Table 6.--Mean values from benthic invertebrate samples when grouped based on mean concentrations of dissolved iron at sampling stations

[N, number of invertebrate samples used to compute mean values; *, group deleted from ANOVA due to significantly different variance; **, homogeneity of variance accepted at $0.025 < p < 0.001$ using Bartlett's test]

| Benthic invertebrate Sample parameters (mean values) | Mean concentrations of dissolved iron at collection sites | | | | Level of significance Pr > F |
|--|--|------------------------|--------------------------|----------------------|------------------------------------|
| | 1-100 mg/L N = 206 | 101-500 mg/L N = 52 | 501-1,000 mg/L N = 12 | >1,000 mg/L N = 8 | |
| Number of taxa | 15 | 16 | 6 | 4 | 0.0001 |
| Number of organisms | 352 | 303 | 95 | 66 | .0002 |
| Sample diversity | 2.00 | 2.01 | .93 | 1.33 | .0003 |
| Relative evenness | .20 | .22 | .11 | .15 | .1080 |
| Percent Ephemeroptera | .26 | .19 | .08 | * | .0001 |
| Percent Plecoptera | .02 | .06 | .03 | * | .0001 ** |
| Percent Diptera | .48 | .53 | .64 | * | .5464 |

Table 7.--Mean values from benthic invertebrate samples when grouped based on mean values for specific conductance at sampling stations

[N, number of invertebrate samples used to compute mean values;
*, group deleted from ANOVA due to significantly different variance]

| Benthic invertebrate Sample parameters (mean values) | Mean specific conductance at collection sites | | | | Level of significance Pr > F |
|--|---|--------------------------------------|--|------------------------------------|------------------------------------|
| | 0-50 $\mu\text{S/cm}$ N = 20 | 51-100 $\mu\text{S/cm}$ N = 70 | 101-300 $\mu\text{S/cm}$ N = 151 | >300 $\mu\text{S/cm}$ N = 37 | |
| Number of taxa | 15 | 14 | 14 | 10 | 0.0068 |
| Number of organisms | 278 | 302 | 333 | 351 | .5619 |
| Sample diversity | 2.17 | 1.93 | 2.03 | 1.52 | .0150 |
| Relative evenness | .26 | .21 | .20 | .13 | .0157 |
| Percent Ephemeroptera | .28 | .19 | .26 | .22 | .0371 |
| Percent Plecoptera | * | * | * | * | * |
| Percent Diptera | .39 | .56 | .46 | .62 | .0112 |

Table 8.--Mean values from benthic invertebrate samples when grouped based on mean pH values at sampling stations

[N, number of invertebrate samples used to compute mean values;
**, homogeneity of variance accepted at $0.025 < p < 0.001$ using Bartlett's test]

| Benthic invertebrate Sample parameters (mean values) | Mean values of pH at collection sites | | | Level of significance | |
|--|---------------------------------------|--------------------|----------------|--------------------------|-----|
| | 6.0 N = 41 | 6.1-8.0 N = 223 | >8.0 N = 14 | Pr | > F |
| Number of taxa | 4 | 16 | 18 | 0.0001 | ** |
| Number of organisms | 59 | 369 | 369 | .0001 | ** |
| Sample diversity | .84 | 2.10 | 2.10 | .0001 | |
| Relative evenness | .09 | .22 | .21 | .0001 | |
| Percent Ephemeroptera | .04 | .27 | .25 | .0001 | ** |
| Percent Plecoptera | .06 | .03 | .01 | .2283 | |
| Percent Diptera | .71 | .46 | .50 | .0139 | |

The mean number of taxa of samples from stations with low mean concentrations of dissolved sulfate [less than 20 milligrams per liter (mg/L)], manganese, and iron [less than 100 micrograms per liter ($\mu\text{g/L}$)] decreased from approximately 17 taxa to less than 6 taxa at stations with relatively high values for these constituents (sulfate, greater than 300 $\mu\text{g/L}$; manganese and iron, greater than 500 $\mu\text{g/L}$). The mean number of organisms per samples in these groupings decreased by 75 percent from approximately 400 organisms per sample to less than 100. Samples from streams with pH values less than 6.0 had substantially fewer taxa and individuals than those streams with higher pH. Such reductions in number of taxa and individual organisms would greatly effect the ability of the benthic community to assimilate and degrade instream detritus as well as to provide a variable food source for higher trophic levels.

Sample diversity values also decreased from a mean of approximately 2.15 at stations with low mean water-quality parameter values to less than 1.33 at stations with relatively high parameter values. Average values for relative evenness also decreased for samples from stations with relatively high water-quality parameter values. Evenness values of samples from stations with pH values less than 6.0 were substantially reduced. Relative evenness dropped from approximately 0.24 at stations with low constituent concentrations and mean pH values greater than 6.0 units to less than 0.15 for samples from stations with a mean pH of less than 6.0 units and high constituent concentrations.

Differences in percentage composition of Ephemeroptera and Diptera were most obvious when samples were grouped on average dissolved manganese concentrations at collection sites. Samples from sites with mean dissolved manganese concentrations less

than 100 µg/L were composed, on the average, of 30 percent Ephemeroptera and 40 percent Dipterans. Samples from sites with mean dissolved manganese concentrations greater than 1,000 µg/L indicated substantial changes in community composition. Ephemeropterans averaged less than 10 percent of the organisms collected at these sites while the mean percentage composition of Dipterans increased to more than 70 percent. This trend was also apparent when stations were grouped on stream pH values, illustrating the effect of pH on dissolved constituent concentrations that may be affecting benthic community structure. Differences in sample percentage composition when samples were grouped on other water-quality parameters were more subtle or not significant at the $p < 0.05$ level. Differences in the mean percentage composition of Plecoptera were not significant.

Although results of regression analyses and analysis of variance tests did identify trends in general measures of benthic communities such as number of taxa and relative abundance of major orders, additional work is needed so that specific groups of invertebrates can be associated with particular stream environments. Multivariate statistical methods such as cluster analysis, detrended correspondence analysis, and other ordination techniques are an alternative method for reducing large volumes of data to a usable form without a significant loss of information (Sokal and Rohlf, 1962; Hill, 1973; Sneath and Sokal, 1973; Boesch, 1977; Green, 1980; Hill and Gaugh, 1980; Gaugh and others, 1981). Preliminary analyses of benthic invertebrate data using cluster analyses based on the presence or absence of individual taxa indicate that some assemblages of invertebrates occur with great regularity throughout the database. Reduction of biological data sets using multivariate analyses along with more intensive sampling of habitat conditions could identify invertebrate assemblages indicative of a limited range of water-quality conditions. Such associations between aquatic biota and their environments would greatly enhance the utility of invertebrate data in future water-quality investigations and monitoring programs.

SUMMARY

Data used in this report were collected by the Geological Survey to provide background information on hydrologic environments in coal-bearing regions of Tennessee. While these data indicated a wide range of water quality and a diverse population of benthic invertebrates in the region, analyses using linear correlation techniques were inappropriate for defining relations between invertebrates and water quality. Pearson correlation coefficients were less than 0.62 when comparing benthic invertebrate sample parameters with mean values of dissolved manganese, iron, sulfate, and with specific conductance and stream pH available at collection sites. Multiple regressions with average water-quality conditions explained only part of the variability in number of taxa and individuals per sample, sample diversity, or the percentage composition of major orders of invertebrates as indicated by coefficients of determination (r^2) consistently less than 0.50 at a probability level equal to 0.05.

Although linear regressions explained little of the variability in benthic invertebrate communities as related to mean water-quality values at collection sites, trends were indicated by analysis of variance tests. Differences between invertebrate samples, when grouped based on water-quality conditions at sampling sites, were generally significant at a probability level less than 0.05, often indicating substantial differences in benthic community structure at sites with poor water quality. These analyses usually indicated fewer number of taxa, fewer organisms, lower sample diversity, and a higher percentage of

Dipterans at stations with relatively high average constituent concentrations, specific conductance, and pH values less than 6.0. These differences suggest lower community complexity at stations with evidence of more severe land-use impacts, thus impairing the ability of benthic organisms to process instream organic detritus and to provide an adequate food supply for higher aquatic life forms.

Of the water-quality information available, dissolved manganese was the most significant independent variable used in comparisons with benthic invertebrate data. Although manganese is not toxic to biota in the concentrations found in the study area, conditions resulting in increases in dissolved manganese may also result in elevated concentration of potentially toxic trace constituents. Dissolved manganese concentrations, number of invertebrate taxa, and percentage composition of Ephemeroptera and Diptera may be useful indices to the severity of impacts on invertebrate communities resulting from mining operations.

Poor correlation coefficients obtained in attempts to define linear relations between invertebrates and environmental conditions serve to illustrate a fundamental ecological concept. Distributions of species of plants or animals, when aligned along environmental gradients, typically form bell-shaped, binomial curves with densities declining gradually to scarcity and absence on each side of a central peak. The fact that the distributions of invertebrate species change and intergrade continuously along with changing environmental conditions accounts for the lack of linear relations defined by these available data. In addition to more complete information on benthic invertebrates, water quality, and physical habitat conditions, analyses of data using multivariate statistical methods are needed to explain relations between benthic invertebrate communities and environmental conditions.

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