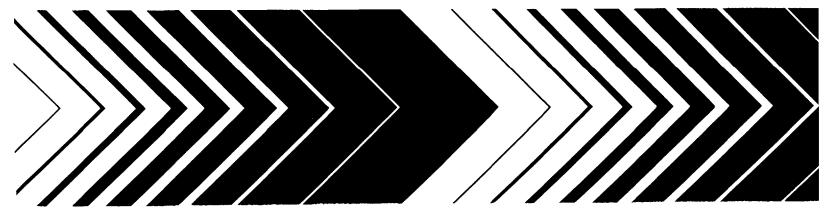
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Validity of Ambient Toxicity Tests for Predicting Biological Impact, Ohio River, Near Wheeling, West Virginia



## Validity of Ambient Toxicity Tests for Predicting Biological Impact, Ohio River, Near Wheeling, West Virginia

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### Disclaimer

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### Foreword

The Complex Effluent Toxicity Testing Program was initiated to support the developing trend toward water quality-based toxicity control in the National Pollutant Discharge Elimination System (NPDES) permit program. It is designed to investigate, under actual discharge situations, the appropriateness and utility of "whole effluent toxicity" testing in the identification, analysis, and control of adverse water quality impact caused by the discharge of toxic effluents.

The four objectives of the Complex Effluent Toxicity Testing Program are:

- 1. To investigate the validity of effluent toxicity tests to predict adverse impact on receiving waters caused by the discharge of toxic effluents.
- To determine appropriate testing procedures which will support regulatory agencies as they begin to establish water quality-based toxicity control programs.
- 3. To serve as a practical case example of how such testing procedures can be applied to effluent discharge to a receiving water.
- 4. To field test short-term chronic toxicity tests involving the test organisms, *Ceriodaphnia dubia* and *Pimephales promelas*.

Until recently, NPDES permitting has focused on achieving technology-based control levels for toxic and conventional pollutants in which regulatory authorities set permit limits on the basis of national guidelines. Control levels reflected the best treatment technology available considering technical and economic achievability. Such limits did not, nor were they designed to, protect water guality on a site-specific basis.

The NPDES permits program, in existence for over 10 years, has achieved the goal of implementing technology-based controls. With these controls largely in place, future controls for toxic pollutants will, of necessity, be based on site-specific water quality considerations.

Setting water quality-based controls for toxicity can be accomplished in two ways. The first is the pollutant-specific approach which involves setting limits for single chemicals based on laboratory-derived no-effect levels. The second in the "whole effluent" approach which involves setting limits using effluent toxicity as a control parameter. There are advantages and disadvantages to both approaches.

The "whole effluent" approach eliminates the need to specify a limit for each of thousands of substances that may be found in an effluent. It also includes all interactions between constituents as well as biological availability. Such limits determined on fresh effluent may not reflect toxicity of effluent after aging in the stream and fate processes change effluent composition. This problem is less important since permit limits are normally applied at the edge of the mixing zone where aging has not yet occurred.

The following study site was on the Ohio River near Wheeling, West Virginia, and was conducted in July and August 1984.

To date, eight sites have been investigated involving municipal and industrial discharges. They are, in order of investigation:

- 1. Scippo Creek, Circleville, Ohio
- 2. Ottawa River, Lima, Ohio
- 3. Five Mile Creek, Birmingham, Alabama
- 4. Skeleton Creek, Enid, Oklahoma
- 5. Naugatuck River, Waterbury, Connecticut
- 6. Back River, Baltimore Harbor, Maryland
- 7. Ohio River, Wheeling, West Virginia
- 8. Kanawha River, Charleston, West Virginia

This project is a research effort only and has not involved either NPDES permit issuance or enforcement activities.

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Project Officers Complex Effluent Toxicity Testing Program

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### **Executive Summary**

EPA recently issued a policy which provides for control of the discharge of toxic substances through the use of numerical criteria and effluent toxicity limits in NPDES permits. This is the first broad scale effort to use effluent toxicity limits in the NPDES permit program and a scientific basis for this approach is needed.

This study was the seventh in a series of eight and was conducted on the Ohio River near Wheeling, West Virginia, which receives discharges from many industrial facilities, including large steel mills. The study area comprises about 12 km of the Ohio River upstream from Wheeling, West Virginia, in the Pike Island pool. The Ohio River is a major inland waterway and is navigable throughout its length. Ambient toxicity tests were conducted on samples from eight river stations. Biological studies were conducted at these stations and included plankton, periphyton, and benthic macroinvertebrates.

This site study did not involve effluent testing as a requisite because it was impractical to do dye dilution studies. Without them, there was no way to use effluent toxicity data to predict instream impact. Effluent tests were planned however for use of the State agency. Due to both a problem in sample acquisition and a mistake in procedure, none were completed.

The impact in the river was not large but all indicators suggest some impact at Stations 2 and 3. The toxicity to *Ceriodaphnia* of samples from these two stations was lowest at these stations although not statistically significant. Fathead minnow toxicity was lowest at Stations 5 and 6 but the difference compared to the station with least toxicity was no larger than between duplicates.

The percent of correctly predicted stations ranged from 63 to 100 depending on the degrees of impairment compared. The *Ceriodaphnia* data gave exactly the same profile as the field macroinvertebrate data for species richness. Toxic impact is most difficult to predict in sites such as this one where the receiving water is large and the impact is not severe.

### **Quality Assurance**

Coordination of the various studies was completed by the principal investigator preceding and during the onsite work. A reconnaissance trip was made to the site before the study and necessary details regarding transfer of samples, specific sampling sites, dates of collections, and measurements to be made on each sample were delineated. The principal investigator was responsible for all Quality Assurance-related decisions. All instruments were calibrated by the methods specified by the manufacturers. For sampling and toxicity testing, the protocols described in the referenced published reports were followed. Where identical measurements were made in the field and laboratory, both instruments were cross-calibrated for consistency.

### 1. Introduction

The study site was the Ohio River near Wheeling. West Virginia. One large steel mill with multiple discharges for a total of approximately 7.3 m<sup>3</sup>/sec was located within the study area. The Ohio River is already large even this far upstream and the study area consisted of a tiny fraction of the river along one shore. Thinking of the study area as a mixing zone of a large discharge would give a representative picture. There are dozens of discharges upstream of the study area and the water quality entering the study area contained an unknown amount of effluents from these discharges. There was no plan to attribute any ambient toxicity measured to a source, rather the objective was to compare the ambient toxicity to community response in a large river system where there are many discharges. Previous studies completed in the study area had revealed reduced numbers of macroinvertebrates collected in artificial substrates downstream of a large steel mill complex. Effluent dilution tests of the steel mill were planned, but problems with sample acquisition and a randomization error required that these test results be disregarded. Since the intent was to compare ambient tests to community response, this problem did not affect the study objectives.

Several of the stations were located in the zone of effluent mixing as judged by color and temperature. The discharges and dilution volume were so large that dye studies were too expensive for the funds available. The Ohio River is very turbulent and, without elaborate dye studies, the effluent concentrations at various stations cannot even be approximated. Therefore, the effluent dilution test results could not have been used to predict impact since effluent concentrations at the sampling station were not known. The river flow variation was large when the substrates were in place, and there was no information as to how different flows affected the effluent concentrations at the sampling stations. Thus, the effluent exposure the substrates experienced before and after the toxicity test period may have been the same as, or quite different from the exosure concentrations during the test period.

Determining the impact of individual discharges to large rivers using stream surveys is very difficult unless the impact is dramatic. However, for rivers such as the Ohio River with many discharges, the combined effects could be quite large even though any single discharge would not have measureable effects on the aquatic community. A method is needed to assess such "undramatic" individual discharge effects. If it can be shown that ambient toxicity texts as used in this study are indicative of biological response, then there is some better justification for using effluent dilution tests to predict adverse effects even though those adverse effects from a single discharge cannot be measured by biological surveys.

This report is organized into sections corresponding to project tasks. Following an overview of the study design and a description of the site, the chapters are arranged into toxicity testing and ecological surveys. An integration of the laboratory and field studies is presented in Chapter 7. All methods and supporting data are included in the appendixes for reference.

### 2. Study Design and Site Description

The study area was on the upper Ohio River between Ohio and West Virginia and included about 12 km of the Ohio River upstream from Wheeling, West Virginia, in the Pike Island pool (Figure 2-1). The Ohio River is a major inland waterway and is navigable throughout. The Ohio River receives effluents from publically owned treatment works (POTWs), heavy industry, chemical plants, power generating stations, and steel mills. Within the study area, there was only a steel mill with multiple outfalls and a POTW. Upstream from this part of the river were many different types of dischargers including power plants, oil refineries, POTWs, and other steel mill installations.

Study components included 7-day Ceriodaphnia dubia toxicity tests and 7-day larval growth tests using fathead minnows (*Pimephales promelas*) on ambient samples from the river stations during 17-23 July. Water samples for the toxicity tests were collected near the locations of the artificial substrates. Quantitative assessment of the planktonic, periphytic, and benthic macroinvertebrate communities was conducted 5 July to 2 August 1984.

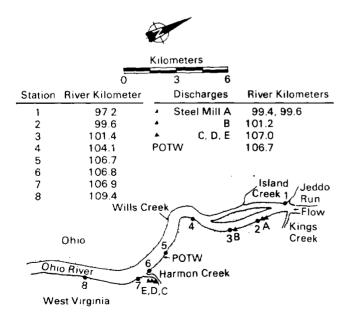


Figure 2-1. Study area on the Ohio River near Wheeling, West Virginia. Station locations are indicated.

Stations were also used to collect samples for zooplankton, periphyton, and benthic macroinvertebrates. The stations were located upstream, in and downstream of the effluent plumes which could be discerned in some areas by visible currents or color. At each station samples were obtained from two depths (0.6 and 1.5 m), since the steel mill discharge was warmer than ambient river temperatures and vertical mixing might be inhibited. Ambient water quality measurements in the field were not made. The stations descriptions are:

- Station 1 (RK 97.2)—Approximately 1.6 km downstream of a POTW, offshore approximately 26 m from the right bank, water depth 4.5 m. Artificial substrates were attached to the superstructure of a wrecked barge. The river bank was gravel and the river bottom was compacted sediment and rubble.
- Station 2 (RK 99.6)—Downstream of the first set of the large steel mill outfalls, offshore approximately 12 m from the left bank, water depth 3 m. Artificial substrates were attached to an icebreaker and mooring cable. The river bank was concrete and the bottom was uncompacted organic material.
- Station 3 (RK 101.4)—Downstream of the second set of steel mill outfalls, offshore approximately 7 m from the left bank, water depth 3 m. Artificial substrates were attached to mooring piers. The river bank was clay and the bottom was mud.
- Station 4 (RK 104.1)—At a marina, approximately 8 m offshore from the left bank, water depth 2 m. The artificial substrates were attached to the floating dock. The river bank and bottom were composed of mud.
- Station 5 (RK 106.7)—Approximately 1 km upstream of a POTW, 14 m offshore from the left bank, water depth 2 m. The artificial substrates were attached to a fallen tree. The river bank and bottom were composed of mud.
- Station 6 (RK 106.8)—Farther downstream of the POTW, approximately 14 m offshore of the left bank, water depth 2 m. The artificial substrates were attached to styrofoam floats. The river bank was composed of mud, whereas the river bottom was composed of sand and gravel.

- Station 7 (RK 106.9)—Immediately downstream of the confluence with Harmon Creek which receives the third set of steel mill discharges. The station was approximately 27 m offshore of the left bank, water depth 3 m. Artificial substrates were attached to styrofoam floats. The river bank was rock fill and the bottom was mud.
- Station 8 (RK 109.4)—Downstream of Harmon Creek by about 2.7 km, offshore approximately 14 m from the left bank, water depth 4 m. The artificial substrates were attached to styrofoam floats. The river bank was stone and the bottom was compacted sediment and rubble.

### 3. Ambient Toxicity Tests

Table 3-1.

The purpose of the toxicity tests was to measure the response of *Ceriodaphnia dubia* and fathead minnows (*Pimephales promelas*) exposed to ambient Ohio River water. The *Ceriodaphnia* toxicity tests measured reproductive potential (number of young per female) and survival. The fathead minnow tests measured the weight gain and survival of fathead minnows. Test results are to be compared with the macroinvertebrate populations on artificial substrates.

Samples of Ohio River water were collected daily for seven days from two depths at each of eight stations located upstream and downstream of a set of large steel mill discharges on the Ohio River near Wheeling, West Virginia. *Ceriodaphnia* and the fathead minnows were exposed to each sample for a 24-hour period and test water was renewed daily with new sample water. This procedure was used to approximate the continual exposures which would have been received had the test organisms been in the river and to approximate the exposure conditions where the artificial substrates were suspended. Descriptions of the sample collections, test methods, and statistical analyses are provided in Appendix A.

### 3.1 River Flow Measurements

The Ohio River flow data were used to estimate the relative effluent dilution and monitor the water flow over the study area. At stable river flows, a constant dilution of the effluents at each station would occur. River flows recorded daily by the National Weather Service are shown in Table 3-1. The flow data covers the entire period when the artificial substrates were in the Ohio River. Mean upstream river flow during toxicity testing (17-23 July) at East Liverpool (RK 69.2) was approximately 603 m<sup>3</sup>/sec and similarly downstream at Wheeling (RK 144.8) was approximately 625 m<sup>3</sup>/sec. The volume flow through the study area changed over time such that the mean river flows during the toxicity testing were midway (603 m<sup>3</sup>/sec) between the extreme flows. The pretest mean flows were 227 percent, and the post-test flows were 52 percent of the flows during the toxicity test. As a result of these changing river flows, the exposure of the artificial substrates to effluent concentrations differed from the exposure of Ceriodaphnia and fathead minnows. Effluent concentrations in the Ohio River would have been much reduced in early July during the period of high flow.

1984	East Liverpool (RK 69.2)	Wheeling (RK 146.4)
5 Jul	765	773
6 Jul	898	906
7 Jul	1,022'*'	1,051 <sup>(a)</sup>
8 Jul	841 <sup>(a)</sup>	886 <sup>14)</sup>
9 Jul	1,127	1,175
10 Jul	1,045	1,062
11 Jul	2,336	2,271
12 Jul	2,413	2,472
13 Jul	2,166	2,249
14 Jul	1,546 🖦	1,626
15 Jul	1,218 <sup>(a)</sup>	1,232**
16 Jul	1,014	1,053
Pre-Test Mean	1,366	1,396
17 Jul	844	872
18 Jul	807	838
19 Jul	719' <sup>a)</sup>	733'"
20 Jul	600	631
21 Jul	473'a'	491'"
22 Jul	416 <sup>(a)</sup>	430'*'
23 Jul	365	377
Ambient Toxicity Testing		
Period Mean	603	625
24 Jul	362	371
25 Jul	374	394
26 Jul	280	289
27 Jul	303	306
28 Jul	323%	328 <sup>(a)</sup>
29 Jul	320**	331 (*)
30 Jul	314	328
31 Jul	306	314
1 Aug	297	306
2 Aug	272	283
Post-Test Mean	315	325
Mean (5 Jul - 2 Aug)	819	841

Ohio River Flow (m<sup>3</sup>/sec)

<sup>(a)</sup>Projected flows.

Note: Flows recorded by National Weather Service.

The effluent concentrations to which the substrates were exposed increased as the flow decreased with concentrations probably highest after the toxicity test period, as the flow of the river decreased.

### 3.2 Chemical and Physical Test Conditions

Temperature for the *Ceriodaphnia* tests was maintained at 25  $\pm$  1°C. The fathead minnows were at temperatures determined by room temperature which ranged from 22-28°C. Most of this range was caused by the heat from lights during the daylight period. Vigorous air mixing assured uniform temperatures for all chambers at any one time and the water temperature changes were gradual when the lights came on and off in the morning and evening. Routine water chemistry measurements included pH, dissolved oxygen (DO), and conductivity for the Ceriodaphnia and fathead minnow tests (Table 3-2). Initial values of pH and DO for both test species were 6.6-7.4 and 7.9-8.2 mg/liter, respectively. Final values of pH were slightly higher than the initial values, ranging 7 0-7.5 for the fathead minnows and 7.1-7.7 for the Ceriodaphnia. Final values of DO were at least 6.6 mo liter for the fathead minnows and at least 7.4 ma liter for the Ceriodaphnia. The conductivities ranged from 210 to 292 umhos for the 0.6 m samples and from 263 to 286 umhos for the 1.5-m samples.

### 3.3 Ambient Toxicity Test Results

At each of eight stations, two water samples were used for the tests: samples collected at 0.6 m were identified T and 1.5-m depth samples were identified B. In addition, duplicate tests were conducted using the 0.6-m samples from Stations 1, 4, and 8 using the fathead minnows and are referred to as "A" samples. Duplicate tests using *Ceriodaphnia* were conducted only at Station 1 at both depths. For statistical comparison, a reference must be used. Stations T-1 and B-1 were selected for the fatheads because mean survival was near the highest and mean weight was the highest at T-1 and the weight of B-1 was within weighing error of the highest, B-8. Use of a T sample and a B sample from different stations did not seem reasonable in view of the small differences.

Mean survival of fathead minnows varied between 53 and 100 percent for the 0.6-m (T) samples (Table 3-3). The lowest survival at Station T-7 was significantly different when compared to Station T-5. Mean survival of fathead minnows for the 1.5-m (B) samples ranged from 75 to 95 percent and no significant differences were found when compared to Station B-1. The duplicate test results of the 0.6-m (T) samples were very similar for Stations T-1 and T-4, with the mean survivals varying by 7 and 5 percent, respectively (Table 3-3). The duplicate test results for Station T-8 (comparing T-8 and T-8A) varied by 16 percent.

The mean fathead minnow weights varied only from 0.259 to 0.406 mg (Table 3-4). The ranges for the 0.6- and 1.5-m depths were very similar. The 0.6-m stations were compared to the highest value T-1; and four stations (T-1A, T-4, T-5 and T-7) were significantly lower. However, T4 had a duplicate value that was not significantly different and the duplicate of T-1A (T-1) had the highest mean weight. Of the 1.5-m

Table 3-2.	Water Chemistry Data for Ambient Toxicity Tests with Fathead Minnows and Ceriodaphnia, Ohio River, Wheeling,
	West Virginia, July 1984

		Fathead Minnow	and Ceri	Minnow odaphnia al DO	Fathead Minnow		I Minnow			d <i>aphnia</i> al DO
Station	Conductivity (umhos)	and <i>Ceriodaphnia</i> Initial pH Range	Mean (mg∠L)	Range (mg∕L)	Final pH Range	Mean (mg∕L)	Range (mg∞L)	<i>Ceriodaphnia</i> Final pH Range	Mean (mg∠L)	Range (mg∠L)
T-1	268	7.0-7.4	8.2	78-8.7	7.1-7.5	6.8	6.1.7.1	7.4-7.5	7.4	7.2-76
T-1A				-	7.0-7.3	6.7	6.3-6.9	. –		
T 2	284	7.0-7.2	7.9	7.5-8.2	7.1-7.4	6.7	6.2-7.0		7.4	7.2.7.6
тз	284	6.8-7.1	7.9	7.8-8.1	71-7.5	6.8	6.8-8.1	7.1-7.4	7.4	70-7.8
T-4	265	6.8-7.2	8.1	7.9-8.5	7.0-7.4	6.7	61-7.2	7.2-74	7.5	70-7.7
Т 4А	210	7.0	78		7.1-7.3	6.7	6.2-6.9		-	
' 5	267	6772	80	7.6-8.4	7.0-7.4	6.6	6.0-7.0	7.3-7.4	76	7.4-7.8
T-6	266	68-7.2	7.9	7.5-8.5	7.0-7.4	6. <b>8</b>	6.1-7.2		7.6	7.2.7.9
7-7	292	67-7.4	7.9	7.5-8.3	7.1-7.4	6.6	6.1-7.0	7.4-7.5	7.7	7.4-79
- 8	272	6.8-7.2	7.9	76-8.3	7.1-7.4	6.6	6.4.70	7.4-7.5	76	7.2.79
T-8A					7.1-7 3	6.7	6.2-70		-	-
B-1	263	6.9-7.1	8.0	7.8-8.3	7.1-7.4	6.7	6.2-7.0		74	7 2-7.6
8-2	286	7.0-7.2	7.9	7.7-8.3	7.0-7.4	6.7	6270	74-75	7.6	73-7.9
B-3	285	6.7-7.1	8.0	7.8-8.2	7.0-7.4	6.8	6.2-7.1	7.4-7.5	74	7.0-7.8
B-4	268	6.9-7.1	80	7.6-8.5	7.0-7.4	6.7	6.4-7.3	74-7.5	76	7.3-7.8
Б-5	265	67.7.0	8.0	7.8-8 5	7.0-7.3	6.7	6.2-70	7 5-7.6	7.6	7.5-7.8
B-6	264	6.7-7.0	8.0	78-8.5	7.0-7.4	6.8	6.3-70	7.5-7.6	7.6	7.3-7.8
B 7	272	6.8-7.1	8.0	7.8-8.4	7.0-7.4	6.6	6.1-7.0	7.2-7.6	7.6	7.4 7.8
B 8	271	6.6-7.1	7.9	7.0-8.3	7.0-7 3	6.7	6.4 7 0	7.4-7.7	7.4	7.0.7.8

Note Stations T-TA, T-4A, and T-8A are duplicates.

T indicates samples were collected near surface at 0.6 m and B indicates samples were collected near bottom at 1.5 m

Replicate							
Station	A	В	- <u>c</u> -		Mean		
T-1	90	100	90	100	95		
T-1A	90	90	80	90	88		
T-2	80	100	100	100	95		
т.3	100	100	100	50	88		
T-4	100	90	80	80	88		
T-4A	100	80	100	90	93		
T-5	100	100	100	100	100		
T-6	100	100	90	80	93		
T 7	70	40	50	50	53'a'		
T-8	80	100	100	100	95		
T-8A	100	70	90	60	80		
B-1	100	90	100	80	93		
B-2	90	80	80	60	78		
B-3	100	90	100	<b>9</b> 0	95		
B-4	90	90	90	90	90		
B 5	90	90	90	80	88		
B-6	90	80	70	100	85		
B-7	100	100	80	80	90		
B-8	80	80	80	60	75		

Table 3-3. Mean Survival of Larval Fathead Minnows for Ambient Toxicity Tests, Ohio River, Wheeling, West Virginia, July 1984

<sup>(a)</sup>Significantly different using two-tailed Dunnett's test (P ≤ 0.05). The T ambient stations were compared against T-1, and B ambient stations were compared to B-1 in the statistical analysis. Note: Stations T-1A, T-4A, and T-8A are duplicates.

T indicates samples were collected near surface at 0.6 m. B indicates samples were collected near bottom at 1.5 m.

Table 3-4.	Mean Individual Weights (mg) of Larval Fathead
	Minnows for Ambient Toxicity Tests, Ohio River,
	Wheeling, West Virginia, July 1984

	ŀ	Replicate	Weight	s	Weighted	
Station	A	В	С	D	Mean	SE
T-1	0.486	0.380	0.344	0.400	0.402	0.024
T-1A	0176	0.254	0 308	0.303	0.259 <sup>(a)</sup>	0.023
T-2	0.459	0.367	0.323	0.330	0.365	0.024
Т-З	0.382	0.464	0.381	0.250	0.386	0.025
T-4	0.247	0.281	0.256	0.341	0.279 <sup>(a)</sup>	0.025
T-4A	0 3 9 0	0.365	0.325	0.346	0.356	0.023
T-5	0 302	0.294	0.287	0.288	0.293'*'	0.024
T-6	0.297	0.299	0.281	0.361	0.307	0.025
T-7	0.290	0 345	0.240	0.210	0.270'*'	0.033
T-8	0.404	0.315	0.305	0.302	0.328	0.024
T-8A	0.356	0.354	0.389	0.356	0.365	0.024
B-1	0.383	0.427	0.421	0.365	0.400	0.025
B-2	0.409	0.335	0.346	0.300	0.353	0.028
B-3	0 381	0.421	0.366	0.339	0.377	0.025
B-4	0.382	0.414	0.342	0.371	0.377	0.026
B-5	0.262	0.280	0.256	0.303	0.274'*'	0.026
B-6	0.244	0.215	0.293	0.344	0.277	0.026
B-7	0 3 2 7	0.269	0.290	0.469	0.344	0.026
B-8	0.335	0.349	0.469	0.492	0.406	0.028

<sup>ta</sup>'Significantly different using two-tailed Dunnett's test (P <> 0.05). The T ambient stations were compared against T-1, B ambient stations were compared to B-1 in the statistical analyses.

Note: Stations T-1A, T-4A, and T-8A are duplicates.

T indicates samples were collected near surface at 0.6 m B indicates samples were collected near bottom at 1.5 m. stations, B-5 and B-6 were different from B-1, but there were no duplicate values for comparison. Since half of the significantly different values were duplicates of values that were not different, the statistical differences found have questionable biological importance.

The mean survival of *Ceriodaphnia* ranged from 80 to 100 percent at the 0.6-m (T) samples (Table 3-5). For the 1.5-m (B) samples, *Ceriodaphnia* survival was greater than 80 percent, except at Station B-2. No significant differences in survival at either depth for any stations were found. *Ceriodaphnia* reproduction varied between 19.7 and 28.1 mean number of young per female for the 0.6-m (T) samples and between 21.7 and 28.8 mean number of young per female for the 1.5-m (B) samples (Table 3-5). Very similar young production occurred for the two depths. Using the highest value of young production at each depth for comparison, differences in the number of young produced were not significant.

#### 3.4 Discussion

The Ceriodaphnia ambient toxicity test results did not show any toxic effects for either survival or young production. There were some statistically significant differences between fathead minnow survival and weights which were confounded by the poor replicate data. For the 0.6-m sample at Station 7, fathead minnow survival was low, as was the mean weight which provides some evidence of toxicity at that location. However, there is no evidence that toxic effects, if any, are large.

			-
	Mean Number	95%	Mean
	of Young	Confidence	Percent
Station	per Female	Intervals	Survival
T-1	28.1	22.8-33.4	100
T-1A	25 7	20.6-30.8	100
T-2	19.7	14.6-24.8	100
T- <b>3</b>	20.5	15.9-25.0	80
T-4	27.5	23.1-31.9	100
⊺-5	24.9	21.9-27.7	80
T-6	24.3	19.5-29.5	90
T-7	23. <del>9</del>	19.2-28.6	100
⊺-8	24.6	21.0-28.2	100
B-1	22 4	18.8-26.1	80
B-1A	25.4	22.9-27.9	90
B-2	23.1	18.2-28.0	66
B-3	21.7	18.4-25.0	100
B-4	26.6	22.1-31.1	100
B-5	23.8	19.9-27.7	100
B-6	24.9	21.5-28.3	100
B-7	28.8	23.6-34.1	90
B-8	24.8	18.8-30.6	90

 
 Table 3-5.
 Mean Young Production and Percent Survival of Ceriodaphnia for Ambient Toxicity Tests, Ohio River, Wheeling, West Virginia, July 1984

Note. Stations T-1A and B-1A are duplicates. T indicates samples were collected near surface at 0.6 m and B indicates samples were collected at 1.5 m. There were no significant differences between stations or levels ( $P \le 0.05$ ).

### 4. Plankton Community Survey

The plankton community was investigated by measuring the occurrence and density of organisms in the Ohio River. Samples were collected at two depths: 0.6 m and at 1.5 m. The primary emphasis was to collect zooplankton, but algae were also collected and enumerated. Measures of the number of species and individuals are used to determine alterations in composition and/or density. The sampling and analytical methods are presented in Appendix B; additional data are included in Appendix C.

#### 4.1 Community Structure

Rotifers were the dominant taxonomic group and accounted for the highest zooplankton concentrations which occurred at Stations 6 and 8 (Table 4-1). *Brachionus* was the most common genus of rotifers and composed 50 percent or more of the rotifers at each station. Total densities of rotifers varied from lows of about 20 organisms/liter at Station 1 to over 100 organisms/liter at Station 4. Crustaceans were collected at all stations, but in low numbers; densities varied from 0.6 to 6.3 organisms/liter. Nauplii of cyclopoid copepods composed the majority of the crustaceans.

Algae represented a very small portion of the total plankton densities. Algal densities varied from less than 1 percent to near 10 percent of the total. With the use of an 80  $\mu$  net the proportion of algae retained would be small and so the density would be expected to be low due to sampling method.

### 4.2 Evaluation of the Zooplankton Community

The densities of crustaceans and rotifers were lowest for Station 1 for both depths (Table 4-1). Crustacean densities at Station 1 were 0.6 and 2.9 organisms/ liter for the 0.6- and 1.5-m samples, respectively. Rotifer densities were 20.5 organisms/liter at 0.6 m and 27.2 organisms/liter at 1.5 m for Station 1.

The results of a two-way Analysis of Variance (ANOVA) on the total zooplankton densities indicated significant ( $P \le 0.001$ ) differences between stations and nonsignificant differences between depths. The results of a two-way ANOVA on the total rotifer densities were similar and this is not surprising considering that rotifers were the overwhelming component of the zooplankton population. Tukey's Honestly Significant Difference Test on both zoo-

plankton and rotifer densities indicated that Station 1 was significantly different (P  $\leq$  0.05) from all other stations. Crustacean densities revealed significant differences (P  $\leq$  0.05) between stations and depths. Using a two-way ANOVA and Tukey's test (Sokal and Rohlf, 1981) results indicated that Stations 1 and 3 were significantly different (P  $\leq$  0.05).

The densities of crustaceans and rotifers were lowest at Station 1 (Figure 4-1). The abundance of rotifers increased dramatically between Stations 1 and 2 and this higher abundance level was consistent downstream. The steel mill outfalls are located above Stations 2, 3, and 7. Travel time from Station 1 to 8 is about 25 hours (Personal Communication, Wheeling Office, Region III). Any adverse effect due to the steel mill discharges is probably not measurable within the time that the organisms traverse the study area.

In contrast to the variability in the density of zooplankton, taxa were not significantly different either between stations or between depths.

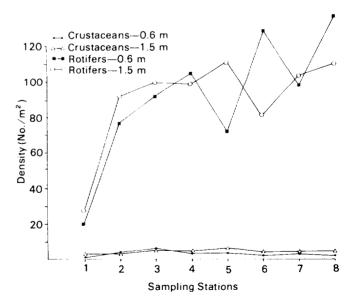


Figure 4-1. Densities of crustaceans and rotifers collected in the Ohio River near Wheeling, West Virginia 1984.

	Stati	on 1	Stati	ion 2	Stati	on 3	Stat	on 4
Taxa	0.6 m	1.5 m	0.6 m	1.5 m	0.6 m	1.5 m	0.6 m	1.5 m
Crustaceans								
Cyclopoid copepods	0.3	11	1.4	1.6	1.2	1.6	1.0	14
Calanoid copepods		0.3	0.2		0.7	0.4	0.1	0.4
Nauplii	0.1	1.2	1.9	2.0	3.0	2.5	1.7	1.1
Bosmina sp.	0.2	0.1	06	0.2	0.9	0.9	0.7	0.9
Daphnia sp		0.2			0.4	0.2		
Eubosmina sp					0.1			
Diaphanosoma sp								0.1
Total crustaceans	0.6 <sup>(b)</sup>	2.9	4.1	3.8	63	5.6	3.5	3.9
Rotifers								
Brachionus budapestinensis		0.3	2.2	1.2	0.9	2.7	0.7	1.4
B. calyciflorus	4.6	9.5	19.3	19.4	35.8	37.8	19.6	21.3
B. caudatus	0.6	3.6	16.1	16.2	9.8	13.3	16.9	12.8
B. angularis	6.3	10.2	17.2	20.4	22.9	21.2	27.2	31.6
B. urceolaris	0.1	0.5	3.0	0.9	2.6	2.9	2.1	2.9
B. quadridentatus	0.1	0.2		0.2	1.0	0.9	1.2	0.5
B. havanaensis			0.2		0.3		D.1	
B. bidentata		0.1		0.1			0.1	
B. variabilis					0.3	0.4		
Keratella sp.	8.7	2.4	16.8	31.2	16.5	18.8	36.1	275
Polyarthra sp.		0.1	1.1	0.6	0.7	0.3	0.6	0.1
Trichocerca sp.		0.1	0.3	0.6		1.0	0.2	0.3
Kellicottia sp	0.1			0.2			0.3	0.1
Platyias sp.					0.3		0.1	
Filinia sp.		0.2	0.1		0.4			
Monostyla sp.			0.2		0.1			
Euchlanis sp.								
Total rotifers	20.5 <sup>°°°</sup>	27.2 <sup>(c)</sup>	76.5	91.0	91.6	99.3	105.2	98.6
Algae								
Ceratium sp.	0.1	0.3	4.1	7.4	4.0	2.9	10.2	4.6
Closterium sp.			0.4			0.1		0.2
Total algae	0.1	0.3	4.5	7.4	4.0	3.0	10.2	4.8
Total density	21.2	30.4	85.1	102.2	101.9	107.9	118.9	107.3
Fotal number of zooplankton taxa' <sup>a</sup> '	9	15	14	13	18	14	16	15

Table 4-1.	Densities <sup>(a)</sup> (No./liter) of Plankton Collected from the Ohio River, Wheeling, West Virginia, July 1984
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	Stat	ion 5	Stati	Station 6 Station 7		Station 7 Station 8		
Taxa	0.6 m	1.5 m	0.6 m	1.5 m	0.6 m	15 m	0.6 m	1.5 m
Crustaceans								
Cyclopoid copepods	1.8	2.7	0.7	1.6	0.8	1.2	0.3	2.4
Calanoid copepods	0.3	0.3	0.2	0.2	02	0.1		03
Naupli	1.6	2.4	1.2	1.9	13	2.0	1.7	21
<i>Bosmina</i> sp.	0.5	0.7	0.2	0.2	0.6	0.6	0.2	0.1
Daphnia sp.	0.1							
Eubosmina sp.								
Diaphanosoma sp								
Total crustaceans	4.3	6.1	2.3	3.9	2.9	3.9	2.2	4.9
Rotifers								
Brachionus budapestinensis	2.3	11	2.5	1.1	2.3	1.7	1.8	0.2
B. calyciflorus	15.4	15.3	29.0	18.4	18.5	21.9	22.2	19.5
B. caudatus	14.8	10.6	23 1	13.1	21.8	16.6	32.8	24.3
B. angularis	19.1	25.1	36.8	23.8	29.3	32.0	35.8	24.5
B. urceolaris	0.4	1.5	2.1	2.8	2.7	1.9	1.4	2.7
B. quadridentatus	0.5	0.5	0.4	0.9	04	0.5	0.4	04
B. havanaensis	0.2	0.4	0.4	0.1	0.1	0.1	0.2	
B bidentata			0.1	0.1			0.1	
B. variabilis		0.9					0.1	
Keratella sp.	18.0	54.8	34.0	20.0	21.8	28.2	40.6	36.7
Polyarthra sp.	0.6	0.4	0.1	02	0.8	0.2	0.6	0.2

#### Table 4-1. (continued)

	Sta	tion 5	Stat	ion 6	Stat	ion 7	Stat	ion 8
Таха	0.6 m	1.5 m	0.6 m	1.5 m	0.6 m	1.5 m	0.6 m	15 m
Trichocerca sp	0.2	0.4	0.2		0.1		0.5	1.0
Kellicottia sp.	0.1	0.2					0.2	0.2
Platyias sp.		0.1	0.1	0.1	0.1			
Filinia sp.								
Monostyla sp	0.4				0.1	0.1		01
Euchlanis sp.		0.2						
Total rotifers	72.0	111.5	128.8	80.6	98.0	103.2	136.7	109.8
Algae								
Ceratium sp.	3.7	8.2	15.3	2.9	12.3	10.6	12.6	4.6
Closterium sp.		0.1			0.1			
Total algae	3.7	8.3	15.3	2.9	12.4	10.6	12.6	46
Total density	80.0	125.9	146.4	87.4	113.3	117.7	151.5	119.3
Total number of zooplankton taxa <sup>:d)</sup>	16	17	15	14	15	13	15	15

<sup>Tai</sup>Density estimates are based on one sample from each location. <sup>To</sup>'ANOVA and Tukey's test indicated Station 1 is significantly different from Station 3 (P = 0.05). <sup>To</sup>'Comparison by ANOVA and Tukey's test indicated Station 1 is significantly different from all other stations (P = 0.05). <sup>To</sup>'Comparison by ANOVA and Tukey's test indicated Station 1 is significantly different from all other stations (P = 0.05). <sup>To</sup>'Total number of taxa does not include crustacean nauplii or algae, and there were not significant differences between stations or depths

### 5. Periphyton Community Survey

This study investigated the periphytic community by measuring chlorophyll *a* and biomass. The relatively short reproduction time and rapid growth of periphytic algae results in quick response to changes in water quality. A change in the periphytic community may be either a reduction of an important habitat or food source for other organisms or the enhancement of nuisance species of algae that neither support higher trophic levels nor are aesthetically pleasing. Sampling and analytical methods are presented in Appendix B.

## 5.1 Chlorophyll *a* and Biomass Measurements

Samples for chlorophyll *a* and biomass determinations were collected from artificial substrates on 2 August 1984 at a depth of 1.5 m. None of the sample replicates at Stations 1 and 5 were recovered.

Chlorophyll *a* replicate values ranged from 1.9 to 151.6 mg/m<sup>2</sup> (Table 5-1). The variations within stations may be due to stream conditions, habitat availability, or sampling conditions. Mean chlorophyll *a* values ranged from 29.1 to 151.6 mg/m<sup>2</sup>. Three

upstream stations (Stations 2, 3, and 4) had similar values of 29.1-40.1 mg/m<sup>2</sup>, whereas the three downstream stations (Stations 6, 7, and 8) had higher values of 73.1-151.6 mg/m<sup>2</sup>. Results of one-way Analysis of Variance (ANOVA) indicated that there were significant differences (P = 0.008) in chlorophyll *a* between stations when all data were considered. When Station 8 was omitted, because there was just one replicate (with the highest value), a significant difference (P = 0.014) between stations was still found (Table C-11).

Periphyton biomass varied from 2.4 to 17.4 g/m<sup>2</sup> measured as ash-free dry weight (AFDW) (Table 5-1). Similar to the trend with chlorophyll *a* data, the biomass at Stations 2 through 4 (3.3-5.8 g/m<sup>2</sup>) was generally lower than at Stations 6 through 8 (5.8-11.1 g/m<sup>2</sup>).

Results of a one-way ANOVA, using natural logtransformed data, indicated that the differences in AFDWs between stations were significant (P = 0.04), with or without Station 8 data (Table C-11).

Colleges of faces Artificial Substantian in the Ohio Diver Near

lable 5-1.	Chlorophyll a and Blomass Measurements of Periphyton Collected from Artificial Substrates in the Onio River Near
	Wheeling, West Virginia, August 1984

				Sampling	Station <sup>'a</sup>			
Parameter	1	2	3	4	5	6	7	8
Chiorophyll a (mg/m²)								
Rep 1		53.8	47.2	10.5		147.2	91.6	151.6
Rep 2	· _	26.5	1.9	14.3		130.4	35.0	
Rep 3		~-	44.7	62.3		89.9	92.7	
Mean		40.1	31.2	29.1		122.5	73.1	151.6
Biomass (m≠m²) <sup>™</sup>								
Rep 1		4.1	3.9	2.4	~-	17.4	8.0	11.1
Rep 2		7.4	2.8	2.5		8.4	3.2	
Rep 3			4.1	5.1		7.1	6.2	
Mean		5.8	3.6	3.3		10.9	5.8	11.1
Autotrophic Index <sup>101</sup>								
Rep 1		76	82	228		118	87	73
Rep 2		276	1,469	172		65	91	
Rep 3	-		92	82		79	67	
Mean		176	547	161		87	82	73

<sup>tai</sup>Dash indicates that the substrate was missing.

<sup>(D)</sup>Measured as ash-free dry weight (AFDW).

"Weber 1973.

Values of an autotrophic index (AI) were calculated following that of Weber (1973), and were based on the ratio of AFDW to chlorophyll *a*. The Al values indicate that heterotrophic (nonalgal) taxa or nonliving organic matter dominated at Stations 2-4, whereas autotrophic (photosynthetic) taxa dominated at Stations 6-8 (Table 5-1).

# 5.2 Evaluation of the Periphytic Community

There is a difference in the chlorophyll a content and biomass for the periphytic community above and below Station 5. This transition area between Stations 4 and 6 covers almost 3 km and, unfortunately no data were available for Station 5. Chlorophyll a values increased downstream of Station 5. These increases suggest a source of enrichment between Stations 4 and 6, especially since the community downstream of Station 5 is dominated by photosynthetic taxa. Potential sources are a POTW located downstream of Station 5 and Harmon Creek, which receives some of the steel mill discharges. Station 7 is located downstream of the confluence of the Ohio River and Harmon Creek. However, the two other steel mill outfalls are located above Stations 2 and 3 where lower chlorophyll a values were obtained.

### 6. Macroinvertebrate Community Survey

This survey investigated the macroinvertebrate community along the Ohio River using artificial substrates. Substrate samples were collected at two depths (0.6 m and 1.5 m) for eight stations. The benthic community is considered to be a good indicator of changes in water quality due to restricted mobility. The degree of community stability can be ascertained by measuring species composition and dominance. An alteration in community structure, species composition, or biomass beyond normal variations would be regarded as an adverse effect.

A description of the sampling and analytical methods is presented in Appendix B. Additional data are included in Appendix C.

### 6.1 Community Composition

The macroinvertebrate community along the study area on the Ohio River was composed of 56 taxa. The number of taxa at each station, including the 0.6 m and 1.5 m substrates, ranged from 13 to 34 (Tables C-2 through C-5). Two taxonomic groups were extremely abundant: oligochaetes (unidentified Naididae) and amphipods (*Gammarus* sp.) (Table 6-1). These two macroinvertebrate taxa often composed over 50 percent of the population. Another seven taxa which contributed  $\geq$ 5 percent of the populations for at least one station were: chironomids (*Cricotopus cylindraceus* group, *Dicrotendipes* sp., *Polypedilum convictum* type, *Rheotanytarsus* sp., and unidentified chironomid pupa), hydropsychids (*Hydropsyche orris*), and polycentropodids (*Cyrnellus fraternus*) (Table 6-1).

The macroinvertebrate community from this area of the Ohio River is not diverse. Of the nine major taxa of the community, five are in the Chironomidae family (midges) and two are in the Trichoptera order (caddisflies). So, seven of the nine major taxa are insects, and the remaining taxa are the two most

		Sampling Stations						
Taxa	1	2	3	4	5	6	7	8
Oligochaeta								
Unidentified Naididae	6.0	43.5	38.8	37.8	5.9	17.3	68 5	21.9
Amphipoda								
Gammarus sp	48.0	40.8	42.6	18.4	25.4	32 2	10.3	22.0
Trichoptera								
Hydropsyche orris	0.6	0.0	0.0	1.8	6.7	2.9	1.9	1.8
Cyrnellus fraternus	0.9	0.1	1.7	0.7	74	3.2	01	1.1
Chironomidae								
Cricotopus cylindraceus								
group	1.8	1.5	0.7	1.1	10.0	2.3	19	5.3
Dicrotendipes sp.	3.3	1.5	9.0	9.1	7.9	4.7	1.3	7.0
Polypedilum convictum								
type	2.1	3.4	1.2	5.5	4.4	5.5	0.9	65
Rheotanytarsus sp.	17.1	0.0	0.0	0.4	36	8.1	0.1	2.0
Chironomidae pupae								
(unidentified)	6. <b>9</b>	0.9	0.7	5.7	6.4	5.7	2.6	6.7
Totai Chironomidae <sup>®</sup>	39.6	14.2	14.4	39.4	51.9	42.0	18.1	52.0
Total 0.6-m Taxa	23	16	14	26	26	26	20	25
Total 1.5-m Taxa	34	14	14	24	26	25	28	26

Table 6-1. Mean Percent Composition of Major Macroinvertebrate Taxa, " Ohio River, Wheeling, West Virginia

<sup>(a)</sup>Major taxa are those which composed five percent or greater of the total density for at least one station. The percents are for both substrate depths.

"Includes all chironomid taxa collected.

Source: Tables C-2 through C-9.

abundant: oligochaetes and amphipods. The number of oligochaete taxa is not known since further identification was not conducted.

#### 6.2 Station Comparisons

There are noticeable differences in the abundance of most of the major taxa between stations and depths. There are also differences in the abundance patterns between these taxa. Unidentified Naididae densities varied between depths at Station 7 (located downstream of Harmon Creek), and decreased by over an order of magnitude between Stations 1 and 2 and Stations 2 and 5 in the 0.6 m samples (Figure 6-1). Results of an Analysis of Variance (ANOVA) indicated that differences between depths and stations were significant (P=0.001) for the numbers of unidentified Naididae (Table C-12). Result of Tukey's Honestly Significant Difference (HSD) test indicated that the maximum abundance at Station 7 was different than Stations 1, 3, 4, 5, 6, and 8. Gammarus sp. densities were greatest at Station 1, then decreased to minimums at Stations 4 and 7 (Figure 6-2). The pattern of variation was similar for the two depths. The ANOVA results indicated that there were significant differences (P=0.001) in numbers of Gammarus between stations, but that the differences between depths were nonsignificant. The Tukey's HSD test results indicated that Station 1 was different than Stations 4, 5, 7, and 8 ( $P \le 0.05$ ).

The densities of each of the five major Chironomidae were less than  $350/m^2$  at each station, with the exception of *Rheotanytarsus* sp. at Station 1 (Figure 6-3). Results of an ANOVA for the abundance of all chironomid taxa indicated that there were significant differences (P=0.0007) between stations and Tukey's test results indicated that Stations 2 and 3 were different (P  $\leq$  0.05) from Station 8. The patterns of

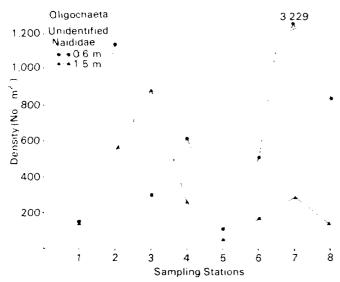


Figure 6-1. Mean density of Oligochaetes (aquatic earth worms) in the Ohio River.

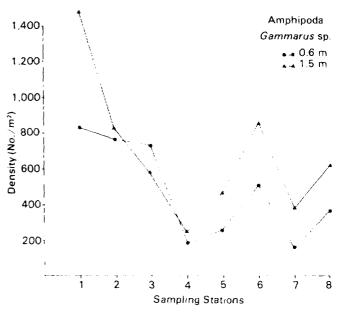


Figure 6-2. Mean density of *Gammarus* amphipods in the Ohio River.

abundance differed for the five major chironomid taxa. All had significant differences between stations and depths, except that only differences between stations were found for Dicrotendipes sp. (Table C-13). For *Dicrotendipes* sp. Station 2 was different ( $P \le P$ 0.05) from Stations 3, 4, and 5, and Station 7 was different ( $P \le 0.05$ ) from Stations 3 and 5. In contrast, for *Polypedilum* sp., Station 3 was different ( $P \le 0.05$ ) from Stations 8 and 6. Examination of differences between stations using Tukey's HSD test indicated that Station 1 was different (P  $\leq$  0.05) from all other stations and that Station 6 was different (P  $\leq$  0.05) from Stations 2, 3, 4, and 7 for Rheotanytarsus sp. Further, for Chironomidae pupae Stations 1 and 8 were different (P  $\leq$  0.05) than Stations 2 and 3 using Tukey's test. The abundance of Cricotopus cylindraceus at Station 5 was significantly different (P <> 0.05) from Stations 4 and 3.

Of the nine major taxa, the two Trichoptera had the lowest densities. Densities were 80/m<sup>2</sup> or less except at Stations 5 and 6 (Figure 6-4). In addition, there were two stations (Stations 2 and 3) where Hydropsyche orris was not collected and three stations (Stations 2, 3, and 7) where Cyrnellus fraternus were rare. ANOVA results indicated that the numbers of H. orris were significantly different (P  $\leq$  0.01) between stations, but were not nonsignificantly different between depths (Table C-14). The Tukey's HSD test results indicated that Station 5 was different from Stations 1, 2, 3, and 4. ANOVA results indicated that the numbers of C. fraternus were significantly different between stations and depth ( $P \leq 0.001$ ). Examination of the differences between stations using Tukey's test also indicated that Station 5 was significantly different ( $P \le 0.05$ ) from Stations 2, 4, and 7.

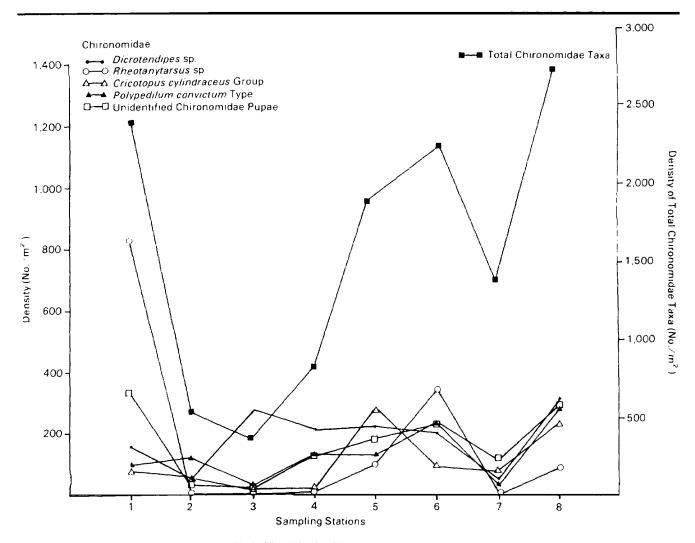


Figure 6-3. Mean density of Chironomids (midges) in the Ohio River. Densities for each depth are combined.

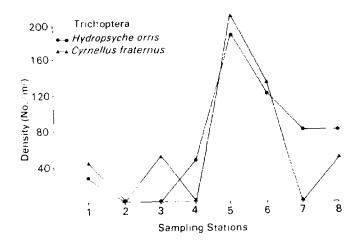


Figure 6-4. Mean density of Trichopterans (caddiflies) in the Ohio River. Densities for each depth are combined.

The macroinvertebrate community was dominated by the presence of unidentified Naididae and Gammarus sp. At Station 1, Gammarus sp. contributed 48 percent, while the next most abundant taxon was Rheotanytarsus sp. At Stations 2 and 3, the unidentified Naididae and Gammarus sp. each composed approximately 40 percent of the community. The contribution to the total abundance from all the chironomid taxa increased to nearly 50 percent at Stations 4 through 6. Almost 70 percent of the community at Station 7 was composed of unidentified Naididae. In contrast, the unidentified Naididae and Gammarus sp. were similarly represented at Station 8, each composing about 20 percent of the community while chironomid taxa again contributed approximately 50 percent.

## 6.3 Evaluation of the Macroinvertebrate Community

Examination of the abundance trends of the major taxonomic groups indicates that the pattern of

oligochaete and amphipod density by station appears to be inversely related. Densities of oligochaetes were high at stations located immediately downstream of the steel mill outfalls (Stations 2, 3, and 7). In contrast, *Gammarus* sp. had relatively low densities at these three stations. In addition, at Station 5 where the trichopterans were relatively more abundant, the usually very abundant oligochaetes and amphipods were at a minimum. The five major chironomid taxa consistently contributed relatively low numbers of individuals, although the numbers of total chironomid taxa were much higher and varied greatly between stations. At the three stations below the steel mill outfalls, the abundance of the chironomids was lowest.

The macroinvertebrate community in the upper Ohio River changes by station; the results of a two-way ANOVA indicated that there were significant differences (P = 0.0001) between the number of taxa per station (Table C-15). However, there were no significant differences between depths. The total number of taxa at Station 3 was lowest and is significantly different (P  $\leq$  0.05) from those at Stations 1, 5, 6, and 8.

### 7. Comparison Between Laboratory Toxicity Tests and Instream Biological Response

### 7.0 Background

The comparison between toxicity measured in the laboratory on a few species and the impact occurring in the stream on whole communities must compensate for a very limited database from which to predict. The sensitivity of the test species relative to that of species in the community is almost never known and certainly not in these toxicity tests. Therefore, when toxicity is found, there is no method to predict whether many species in the community, or just a few, will be adversely affected at similar concentrations, since the sensitivity of the species in the community is not known. For example, at a given waste concentration, if the test species has a toxic response and if the test species is very sensitive, then only those species in the community of equal or greater sensitivity would be adversely affected by direct toxic effects. Conversely, if the test species is tolerant of the waste, then many more species in the community would be affected at the concentration which begins to cause toxic effects to the test species. It is possible that no species in the community is as sensitive as the most sensitive test species, but since there are so many species composing the community, this is unlikely. It is more likely that a number of species in the community will be more sensitive than the test species. The highest probability is that the test species will be near the median sensitivity of organisms in the community if the test species is chosen without knowledge of its sensitivity (as was the case here).

In a special case, where toxicants remain the same and the species composing the community remain the same, the number of species in the community having a sensitivity equal to or greater than the test species also will remain the same. As a result, there should be a consistent relationship between the degree of toxicity as measured by the toxicity test and the reduction in the number of species in the community. In this special case, there should be a tight correlation between degree of toxicity and the number of species. If the toxic stress is great enough to diminish the production of offspring by a test species, it should also be severe enough to diminish the reproduction of some species within the community of equal or greater sensitivity. This should ultimately lead to elimination of the more sensitive species if the reduction is large enough. Therefore, a lower number of taxa should be a predictable response of the community. For example, there should be a relationship between the number of young per female *Ceriodaphnia* or the growth of fathead minnows (or other test species) and the number of species in the community. Obviously, the test species must have a sensitivity, such that at ambient concentrations to which the community has responded, a partial effect is produced in the toxicity test. However, unless the special case described above exists, the correlation between toxicity and species richness will not be a tight one.

Effluents differ from single chemicals in some important respects. We know from the literature on single chemicals that there usually are large differences in the relative sensitivity of species to a chemical and that the relative sensitivity changes with different chemicals. For example the fathead minnow may be more sensitive to effluent A and *Ceriodaphnia* more sensitive to effluent B. We also know that effluents vary in their composition from time to time and often within a few hours. We should not be surprised therefore to find fathead minnows being more sensitive to an effluent on one day and daphnids more sensitive on another day.

Effluents begin changing in composition as soon as they are discharged. Fate processes such as bacterial decomposition, oxidation and many others change the composition. In addition various components will change at different rates. For example ammonia would be expected to disappear more rapidly than PCBs. If so, then the composition of the effluent is ever changing as it moves through the receiving water. Note that this change is not just a lessening concentration as a result of dilution but also a change in the relative concentrations of the components. In reality the aquatic organisms at some distance from the outfall are exposed to a different toxicant than those near the discharge point! Therefore it is logical to expect that sometimes one test species would be more sensitive to the effluent as it is discharged and another species more sensitive after fate processes begin altering the effluent. To be sure the source of the effluent is the same but it is certainly not the same "effluent" in regard to its composition. If these statements are true then one should also expect that species in the community in the receiving water may

be affected at one place near the discharge and a different group of species may be affected from the same effluent at another location.

An effluent cannot be viewed as just diluting as it moves away from the outfall. In fact it is a "series of new effluents" with elapsed flow time. If so, there are important implications for interpretation of toxicity and community data. One should not expect the various test species to respond similarly to water collected from various ambient stations. We should expect one species to be more sensitive at one station and another species to be more sensitive at the next. The affected components of the community should vary in a like manner.

An even bigger implication is that the surrogate species concept is invalid in such a situation. As one examines the community data in the report of Mount et al., 1984; Mount et al., 1985; and in the studies in press, it is clear that there is no one community component that is consistently sensitive. Sometimes the benthic invertebrates and the periphyton have similar responses and both are different from the fish. Sometimes the fish and periphyton have similar responses and these are unlike the benthic invertebrates.

The same is true of the test species. Sometimes the *Ceriodaphnia* respond like the periphyton and other times like the fish. The important point is that a careful analyses of our knowledge of toxicology, effluent decay, and relative sensitivity tells us that we *cannot* expect:

- 1. Ceriodaphnia toxicity to always resemble toxicity to benthic invertebrates
- 2. Fathead minnow toxicity to always resemble toxicity to fish
- 3. Fathead minnows and other fish to display the same relative sensitivity to different effluents.

Any test species should have a sensitivity representative of some components of the community. The important distinction is that one never can be sure which components they will represent.

In comparing toxicity test results to community response, comparison must be made with the above in mind. Certainly those community components that are most sensitive will be most impacted and/or lost. The response of the most sensitive test species should therefore be used to compare to the response of the most sensitive of the community.

A weakness in using the number of species as the measure of community response is that species may be severely affected yet not be absent. The density of various species is greatly influenced by competition for available habitat, predation, grazing, and/or secondary effects which may result from changing species composition. Density is more subject to confounding causes, other than direct toxicity, and is not as useful as the species richness in the community to compare community response to measured toxicity.

Several measures of community structure are based on number of species, e.g., diversity and community loss index. Since diversity measures are little affected by changes in the number of species (or taxa) that are in very low densities in the community, diversity is an insensitive measure for some perturbations which can be measured by toxicity tests. The community loss index is based only on the presence or absence of specific species relative to a reference station and would be useful except that habitat differences between stations heavily effect this measure. There are several problems when using the number of (taxa) species measured. The foremost is that the mere presence or absence of species is not a comprehensive indicator of community health, especially if the species are ecologically unimportant. Secondly, a toxic stress may not eliminate species but yet have a severe effect on density; presence or absence does not consider such partial reductions. The presence or absence of species as the measure of community impact is influenced by the chance occurrence of one or a few individuals due to either drift, immigration, or some catastrophic event when in fact that species is not actually a part of the community where it is found. Effects other than toxicity, such as habitat, will always confuse such comparisons to toxicity data to some extent. Use of artificial substrates should reduce habitat effects compared to natural substrates. They cannot be eliminated. Identification of taxa to different levels can reduce the sensitivity of species richness

Even though species richness has numerous sources of error as a representative measure of community health, it remains the best measure for comparison with toxicological data. Species sensitivity will respond in the most direct way to toxic response of the community with the least interference.

## 7.1 Comparison of Toxicity Test Results and Field Data

Only the benthic macroinvertebrate data were used for comparison to the ambient toxicity test data. The number of species/taxa composing the periphyton community were not determined and so these data were not available. The zooplankton community, while sufficient in number of taxa, is not useful because of the turbulence and the short distance in the river and resulting rapid time-of-travel from Station 1 to Station 8. Only if an effluent was instantaneously lethal to zooplankton, would there likely be a measurable effect on the population sampled in this study because the stressed animals would remain suspended due to turbulence and would not die and decompose in the time required to travel through the study area. A survey of fish species was not conducted. Furthermore, since the study area was so small, effects on the fish species need to be dramatic to be detected in such a large river. An effect on the fish population would most likely have to be an avoidance response to be measurable. The statistical analysis of the number of macroinvertebrate taxa indicated no significant differences between 0.6 m and 1.5 m samples, so the data for each depth were averaged (Table 7-1). Survival of fathead minnows was not significantly different between stations except Station T-7, but there were significant differences in weights between stations. However, these differences are no larger than those between duplicates in 0.6 m samples for Stations 1 and 4 water. Therefore, the data have been averaged across depths since the differences are likely due to experimental variation (Table 7-2). None of the stations were significantly different for Ceriodaphnia young reproduction or survival and so they, too, were averaged (Table 7-3). Using the station with the least toxicity or the most species as zero percent impact, all

Table 7-1. Number of Macroinvertebrate Taxa Collected from the Ohio River

Station	∕Depth	Total Number of Taxa	Mean Number of Taxa Per Station (+ SD)	Percent of Reductions <sup>ta</sup>
1	0.6	24	20.0 71	0
	1.5	34	29.0 ± 7.1	
2	0.6	16	15.0 ± 1.4	48
	1.5	14	15.0 ± 1.4	
3	0.6	13	13.5 ± 0.7	53
	1.5	14	13.5 ± 0.7	
4	0.6	26	25.0 ± 1.4	14
	1.5	24	25.0 ± 1.4	
5	0.6	26	28.5 ± 3.5	2
	1.5	31	20.0 ± 3.9	
6	0.6	26	25.5 ± 0.7	12
	1.5	25	25.5 ± 0.7	
7	0.6	19	23.0 ± 5.7	21
	1.5	27	23.0 ± 5.7	
8	0.6	25		12
	1.5	26	25.5 ± 0.7	

Table 7-2. Fathead Minnow Growth in Ambient Station Water.

Station	Mean Weight (mg)	Station Mean	Percent Increase of Toxicity
T-1 T-1A B-1	0.402 0.259 0.400	0.354	7
T-2 B-2	0.365 0.353	0.359	6
T-3 B-3	0. <b>38</b> 6 0.377	0.382	0
Т-4 Т-4А В-4	0.379 0.356 0.377	0.337	12
T-5 B-5	0.2 <b>93</b> 0.274	0.284	26
T-6 B-6	0.307 0.277	0.292	24
T-7 B-7	0.270 0.344	0.307	20
T-8 T-8A B-8	0.328 0.365 0.406	0.366	4

Source: Table 3-4.

Table 7-3.	Ceriodaphnia Reproduction in Ambient Station
	Water

Station	Mean Number of Young Per Female	Station Mean	Percent Increase of Toxicity
T-1 T-1A B-1 B-1A	28.1 25.7 22.4 25.4	25.4	6
T-2 B-2	19.7 23.1	21.4	21.
Т-3 В-3	20.5 21.7	21.1	22
T-4 B-4	27.5 26.6	27.1	0
Т-5 В-5	24.9 23.8	24.4	10
Т-6 В-6	24.3 24.9	24.6	9
т-7 В-7	23.9 28.8	26.4	3
T-8 B-8	24.6 24.8	24.7	9

<sup>(a)</sup>Using Station 1 as the maximum.

Source: Tables C-2, C-3, C-4, and C-5.

Source: Table 3-5.

other stations are calculated as a percent of that value. Because there were many potential sources of toxicity upstream of Station 1, that station could not be considered free from toxicity nor could any other. Therefore, the station with the least toxicity or the most number of taxa, was considered least impacted and was used as zero impact for comparative purposes.

The percent impact at all other stations was then calculated from that value and each measurement (fathead minnow toxicity, daphnid toxicity and reduced species richness) used a different reference station as zero percent impact. Tables 7-1, 7-2, and 7-3 show these values. Table 7-4 was then constructed as follows. For each station, if the highest toxicity percentage and species richness percentage were each below 20 percent or each was 20 percent or more, a correct prediction was scored. This number of correct predictions was entered into the upper left column of Table 7-4 as a percent value. Similar calculations were done for each column of the matrix substituting the appropriate percent values for each. The 20 percent incremental categories are arbitrarily selected. The percent correctly predicted stations is 75 percent using the 20-100 percent for the toxicity data and the 20-100 field data. It was 63 percent for the 20-100 percent toxicity data and the 40-100 percent field data. One hundred percent are correctly predicted using 40-100, 60-100, and 80-100 percent for the toxicity data and for the field data. The prediction of these higher impact levels are predictions of no effect because the reductions in both filed and toxicity data were of severe enough to cause that much impact. These data are not sufficient to judge what percent is the best predictor. After all eight study site reports are completed, an overall assessment can be made to ascertain which reduction level is the best predictor of instream biological response.

Figure 7-1 shows the profiles of toxicity, based on daphnid data, and the percent change in macroinvertebrate taxa at the eight stations. The profiles are very similar. If the increased toxicity at Stations 5 and 6 evidenced by the fathead minnow (Table 7-2) is real and not experimental variation, whether it would

#### Table 7-4. Percent of Stations Correctly Predicted Using Four Categories of Percent Impact

Toxicity	_	Field	Data	
Data	20-100	40-100	60-100	80-100
20-100	75	63	75	75
40.100	75	75	100	100
60-100	75	75	100	100
80-100	75	75	100	100

Source Tables 7-1, 7-2, and 7-3.

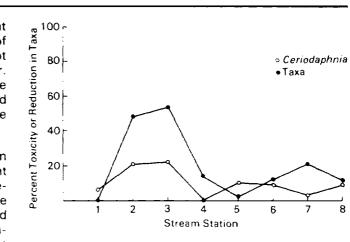


Figure 7-1. Percent toxicity and percent reduction in macroinvertebrate taxa for eight ambient stations.

be evidenced by other groups of organisms not enumerated in this study cannot be judged. The profiles of fathead minnow data and macroinvertebrates are not similar.

The much higher river flows (about 2 times) during the toxicity testing period probably substantially lessened the effluent exposure in the toxicity tests compared to the effluent exposure the macroinvertebrate substrates received during the last 10 days they were in the river.

There is no evidence of gross toxicity in either the field or the laboratory data. The *Ceriodaphnia* data show the most toxicity at Stations 2 and 3 and the macroinvertebrates show the greatest reductions there as well. The fathead minnow data show the most toxicity at Stations 5 and 6. Considering the limited field data for comparison and the large river size, the ambient toxicity data are reasonable estimates of instream biological response, where the toxic effects, if present, are not dramatic.

### References

- Hamilton, M. A. 1984. Statistical Analysis of the Seven-Day Ceriodaphnia reticulata Reproductivity Toxicity Test. EPA Contract J3905NASX-1. 16 January. 48 pp.
- Mount, D. I. and T. J. Norberg. 1984. A seven-day life cycle cladoceran toxicity test. Environ. Toxicol. Chem. 3(3):425-434.
- Mount, D. I. and T. J. Norberg-King, ed. 1985. Validity of Effluent and Ambient Toxicity Tests for Predicting Biological Impact, Scippo Creek, Circleville, Ohio. EPA/600/3-85/044.
- Mount, D. I., A. E. Steen, and T. J. Norberg-King, ed. 1985. Validity of Effluent and Ambient Toxicity Tests for Predicting Biological Impact in Five Mile Creek, Birmingham, Alabama. EPA/600/8-85/ 015.
- Mount, D. I., N. A. Thomas, T. J. Norberg, M. T. Barbour, T. H. Roush, and W. F. Brandes. 1984. Effluent and Ambient Toxicity Testing and Instream Community Response on the Ottawa River, Lima, Ohio. EPA/600/3-84/080. August. Various pagings.
- Norberg, T. J. and D. I. Mount. 1985. A new fathead minnow (*Pimephales promelas*) subchronic toxicity test. Environ. Toxicol. Chem. 4(5):711-718.
- Rogers, J. 1984. University of Wisconsin at Superior, Wisconsin, and EPA Environmental Research Laboratory at Duluth, Minnesota. July. Personal communication.
- Sokal, R. R. and F. J. Rohlf. 1981. Biometry. W. H. Freeman and Company, New York.
- Steele, G. R. and J. H. Torrie. 1960. Principles and Procedures of Statistics, a Bio-Metrical Approach. 2nd Edition. McGraw-Hill, New York. 633 pp.
- Stemberger, R. S. 1979. A Guide to Rotifers of the Laurentian Great Lakes. EPA/600/4-79/021. 186 pp.
- Weber, C. I. 1973. Recent developments in the measurements of the response of plankton and periphyton to changes in their environment. *In:* Bioassay Techniques and Environmental Chemistry (G. E. Glass, ed.), pp. 119-138. Ann Arbor Science Publishing, Ann Arbor, Michigan.

### Appendix A Toxicity Test and Analytical Methods

Each of eight ambient stations along the Ohio River was sampled at depths of 0.6 m and 1.5 m. All samples were collected as daily grab samples using an electric pump and collected in 1-gal collapsible polyethylene containers. Samples were collected daily between 0900 hours and 1500 hours. On 18 July samples could not be collected due to mechanical problems on the boat.

Samples were filtered through a plankton net to remove zooplankton. Temperature and dissolved oxygen (DO) concentrations of the ambient samples were between 24-26°C and 7.9-8.2 mg/liter, respectively. The testing was conducted by the EPA Wheeling Office, Region III, West Virginia.

### A.1 Ceriodaphnia Test Methods

Adult *Ceriodaphnia dubia* from ERL-Duluth which were 10 days old were used as brood stock. They were transported by air to Wheeling and immediately transferred to fresh Ohio River water. These animals had been cultured in Ohio River water at ERL-D for seven days prior to test initiation.

The test method generally followed that of Mount and Norberg (1984) with the exceptoin that 1-oz plastic portion cups were used instead of glass beakers. The cups were discarded after use.

Ten replicates were run from each ambient sample and each cup contained 15 ml of sample. Less than six-hour-old *Ceriodaphnia* were placed in each replicate cup; except for five replicates from Stations T-1 through T-6 at 0.6-m and Stations B-1A and B-4 through B-8 at 1.5 m, where animals less than 24 hours old were used to initiate the tests. Temperature throughout the test was maintained at  $25 \pm 1^{\circ}$ C in thermostatically controlled incubators. Initial DO, pH, and conductivity measurements were taken from the 2-liter sample for the fathead minnow test and were used as initial values for both test organisms.

Test solutions were renewed daily and young, if present, were counted and discarded. Final DO and pH were measured in one of ten cups from each ambient station after each renewal. Samples were not renewed on 18 July. However, survival observations were recorded for this date. A food formulation was used which consisted of three parts: (1) 5 g/liter of dry yeast; (2) 5 g/liter of Cerophyl®\* stirred overnight and filtered through a plankton net; and (3) 5 g/liter of trout chow, aerated vigorously for seven days, settled, and decanted. The yeast suspension and the supernatant from the Cerophyl® and trout chow were mixed in equal parts every seven days. The mixture was kept refrigerated as were the Cerophyl<sup>®</sup> and yeast components, but the trout chow supernatant was kept frozen until the mixture was made. This food is suitable for a wide variety of water types, including reconstituted water. This mixture is fed 0.1 ml per day of Ceriodaphnia rather than 0.05 ml as was recommended for yeast diet (Mount and Norberg 1984). The suspended solids concentration in this food is  $\sim$ 1,800 mg/liter.

Groups of five replicatees from each station and depth were randomized daily on test boards, but maintained the same shelf position in the incubators throughout the test.

### A.2 Fathead Minnow Tests

The methods for the fathead minnow tests followed those described by Norberg and Mount (1985). Larval fathead minnows were less than 24 hours old and were air shipped from the USEPA Newtown Fish Toxicology Station. The fish were assigned one to four at a time to replicate compartments until each had 10 fish (or 40 fish per station).

Newly-hatched brine shrimp were fed three times daily. The uneaten brine shrimp were removed daily during the renewal process by siphoning the tanks to a depth of approximately one centimeter, after which two liters of new test solution were added. To aid in the renewal, a rubber foot made from a Tygon Y-tube and attached to the siphon was used during the renewal.

Before the test solutions were renewed, final DO and pH measurements were recorded. Room temperature was maintained between 22-28°C. There was a 16hour light, 8-hour dim photoperiod throughout the testing period. Chamber locations were randomized daily.

<sup>\*</sup>Cerophyle was obtained from Agri-Tech, Kansas City, Missouri. As of January 1985, Cerophyle was no longer being produced by that manufacturer. Use of trade names does not constitute endorsement.

On 18 July no river water was collected. However, survival observations were recorded and the test solutions were siphoned down to approximately one liter and excess brine shrimp were removed. This was done to improve the surface-to-volume ratio and prevent possible BOD stress effects on the fish.

After seven days, the fish were preserved in 4 percent formalin. Upon returning to Duluth, they were rinsed with distilled water, oven-dried for 18 hours in preweighed aluminum weighing boats, and weighed on a five-place analytical balance.

### A.3 Quantitative Analyses

### A.3.1 Ceriodaphnia

The statistical analyses were performed using the procedure of Hamilton (1984) as modified by Rogers (personal communication). In this procedure the young production data were analyzed to obtain the mean number of young per female per treatment. Daily means were calculated and these means were summed to derive the 7-day mean young value. By this method, any young produced from females that die during the test are included in the mean daily estimate (all data method). Using this procedure, mortalities of the original females affect the estimate minimally, but the mortality of the adult is used along with the young production to determine overall toxicity. Confidence intervals are calculated for the mean reproductivity using a standard error estimate calculated by the bootstrap procedure. The bootstrap procedure subsamples the original data set (1,000 times) by means of a computer to obtain a robust estimate of standard error.

Tukey's Honestly Significant Difference test (Sokal and Rohlf, 1981) is used to determine significant differences in survival and young production between stations.

### A.3.2 Fathead Minnows

The mean weights are statistically analyzed with the assumption that the four test chamber compartments behave as replicates. The method of analysis assumes that the variability in the mean treatment response is proportional to the number of fish per treatment. MINITAB (copyright Pennsylvania State University 1982) was used to estimate a t-statistic for comparing the mean treatment and control data using weighted regressions with weights equal to the number of measurements in the treatments. The t-statistic is then compared to the critical t-statistic for the standard two-tailed Dunnett's test (Steele and Torrie 1960). The survival data are arcsine-transformed prior to the regression analyses to stabilize variances for percent data.

### Appendix B. Biological Sampling and Analytical Methods

### **B.1** Plankton Survey

Plankton were collected from eight stations on the Ohio River near Wheeling, West Virginia, on 23 July 1984, Samples were collected at 0.6- and 1.5-m depths by pumping 10 liters of water through an 80-m mesh net. No sampling replication was conducted. Samples were preserved in 10 percent formalin. In the laboratory, the samples were concentrated by allowing the contents of the sample container to settle, and siphoning from the top as much liquid as possible without disturbing the plankton. The entire sample was enumerated by placing approximately 5 ml at a time on a Ward zooplankton counting wheel and identifying to the lowest possible taxon. Identifications were made using a dissecting scope at 25X magnification, and those organisms which could not be identified at that power were mounted and viewed under a compound scope at a higher magnification.

The crustaceans, rotifers, and total zooplankton were analyzed by Analysis of Variance (ANOVA) on the untransformed and natural log-transformed data. A two-way ANOVA was performed on the densities of these three groups to determine if there are differences between stations and depths. In addition, a two-way ANOVA was performed on the number of taxa per station. Tukey's Honestly Significant Difference tests were conducted to determine which stations were different, when a significant difference was detected using the ANOVAs.

### **B.2** Periphyton Survey

The periphytic community was sampled quantitatively using clear acetate strips suspended in the Ohio River at the same locations as the 1.5-m artificial substrates for the benthic macroinvertebrates. Triplicate strips were placed in the river at eight stations on 5 July 1984 and retrieved on 2 August 1984 for a 28-day colonization period.

The strips were preserved in formalin until analysis. The strips were scraped and the material was analyzed for chlorophyll *a* and biomass (ash-free dry weight, AFDW).

For AFDW, samples were dried at 105°C to a constant weight and ashed at 500°C. Distilled water then was added to replace the water of hydration lost from clay and other minerals. Samples were redried at 105°C before final weighing, and biomass was expressed in  $g/m^2$ . Filters for chlorophyll *a* analysis were macerated in a 90 percent acetone solution, then centrifuged and analyzed spectrophotometrically. A chlorophyll *a* standard (Sigma Chemicals) extracted in a 90 percent acetone solution was used for instrument calibration. Chlorophyll *a* standing crop was expressed as mg/m<sup>2</sup>. The biomass and chlorophyll *a* data were used to calculate the Autotrophic Index (Weber 1973), which indicates the relative proportion of heterotrophic and autotrophic (photosynthetic) components in the periphyton.

The chlorophyll *a* and AFDW data were statistically examined by one-way ANOVA using SAS and MINITAB to detect differences between sampling locations. The ANOVAs were performed on all data and again with Station 8 omitted. (Station 8 had the highest value and only one of the three replicate substrates was recovered.)

### **B.3** Benthic Macroinvertebrate Survey

Aquatic macroinvertebrates were sampled from the Ohio River during July and August 1984 utilizing Hester-Dendy artificial substrates. The Ohio River was sampled at eight locations from RK 100 to RK 113 near Wheeling, West Virginia. Samplers were placed in the river on 5 July 1984 and retrieved on 2 August 1984, resulting in a 28-day colonization period. Three replicate Hester-Dendy samplers were suspended from permanent structures along the shoreline at 0.6- and 1.5-m depths at each location. The samplers at the 1.5-m depth were round-plate substrates as described by Weber (1973) which have an effective surface area of 0.13 m<sup>2</sup>. The samplers at the 0.6-m depth were square-plate substrates (individual plate =  $7.5 \times 7.5 \text{ cm}$  constructed by the Wheeling, West Virginia office of the USEPA. The square-plate samplers were constructed to conform with the round-plate samplers; however, they had an effective surface area of 0.16 m<sup>2</sup>. The samplers were preserved upon retrieval with 10 percent formalin with rose bengal stain added to aid in sorting.

Macroinvertebrates and debris were scraped and brushed free of the artificial substrate upon receipt in the laboratory. The residue and organisms collected on each sampler were sieved in the laboratory on a U.S. Standard No. 30 mesh sieve and preserved in 10 percent formalin. All samples were analyzed utilizing procedures outlined in EA's Macroinvertebrate Quality Control and Procedures Manual. Prior to analysis, each sample was rinsed on a U.S. No. 60 mesh sieve to remove preservative.

The sample material was then sorted, a small portion at a time, under a dissection microscope at 10X magnification. All organisms (except chironomids) were identified under 10X magnification. The chironomids were mounted on glass slides in a nonresinous mounting media for examination under a compound binocular microscope at 40-1,000X magnification. Oligochaeta (segmented worms) were not identified beyond the familial level. All other organisms were identified to the lowest taxonomic level practicable (usually genus or species) using state-of-the-art taxonomic keys. Abundance was standardized to number per m<sup>2</sup> for density comparisons.

The macroinvertebrate data were analyzed using two-way ANOVAs on the numbers of organisms for selected taxa: unidentified Naididae, *Gammarus* sp., *Hydropsyche orris, Cyrnellus fraternus, Cricotopus cylindraceus, Dicrotendipes* sp., *Polypedilum convictum* type, unidentified Chironomidae pupa, and total Chironomidae. The ANOVAs were performed to detect any differences between stations or depths. Tukey's Honestly Significant Difference test was performed when a significant difference was detected using the ANOVAs to determine which stations were different. In addition, a one-way ANOVA and Tukey's test were performed on the total number of taxa per station.

### Appendix C Additional Biological Data

### Table C-1. Numbers of Plankton Collected from the Ohio River Near Wheeling, West Virginia, August 1984

	Stat	ion 1	Stati	on 2 <sup>(a)</sup>	Stat	ion 3	Stati	ion 4
Taxa	0.6 m	1.5 m	0.6 m	1.5 m	0.6 m	1.5 m	0.6 m	1.5 m
Crustaceans								
Cyclopoid copepods	З	11	14	16	12	16	10	14
Calanoid copepods		3	2		7	4	1	4
Nauplii	1	12	19	20	30	25	17	11
Bosmina sp.	2	1	6	2	9	9	7	9
Daphnia sp.		2			4	2		
<i>Eubosmina</i> sp.					1			
<i>Diaphanosoma</i> sp.								1
Total crustaceans	6	29	41	38	63	56	35	39
Rotifers								
Brachionus budapestinensis		3	22	12	9	27	7	14
B. calyciflorus	46	95	193	194	358	378	196	213
B. caudatus	6	36	161	162	98	133	169	128
B. angularis	63	102	172	204	229	212	272	316
B. urceolaris	1	5	30	9	26	29	21	29
B. quadridentatus	1	2		2	10		12	
B. havanaensis			2	-	3		1	-
B. bidentata		1	_	1	-		1	
B. variabilis					3	4		
Keratella sp.	87	24	168	312	165	188	361	275
Polyarthra sp.		1	11	6	7	3	6	1
Trichocerca sp.		1	3	6		10	2	3
Kellicottia sp	1			2			3	1
Platyias sp.					3		1	
Filinia sp.		2	1		4			
Monostyla sp.			2		1			
Euchlanis sp.								1
Total Rotifers	205	272	765	910	916	993	1,052	986
Algae								
Ceratium sp.	1	3	4	74	40	29	102	46
Closterium sp.			41			1		2
Total Algae	1	3	45	74	40	30	102	48
Total Zooplankton	212	304	851	1,022	1,019	1,079	1,189	1,073
	Stati	on 5	Stati		Stati	on 7	Stati	on 8
Таха	0.6 m	1.5 m	0.6 m	1.5 m	0.6 m	1.5 m	0.6 m	1.5 m
Crustaceans								
Cyclopoid copepods	18	27	7	16	8	12	Э	24
Calanoid copepods	3	3	2	2	2	1	Ŭ	3
Nauplii	16	24	12	19	13	20	17	21
Basmina sp	5	7	2	2	6	-0	2	1
Daphnia sp	ĩ	-	-	-	-	-	_	
Eubosmina sp.	·							
Diaphanosoma sp.								
Total Crustaceans	43	61	23	39	29	39	22	49

#### Table C-1. (continued)

	Stat	ion 5	Stat	ion 6	Stat	on 7	Station 8	
Таха	0.6 m	15 m	0.6 m	15 m	0.6 m	1.5 m	06 m	15 m
Rotifers								
Brachionus budapestinensis	23	11	25	11	23	17	18	2
B. calyciflorus	154	153	290	184	185	219	222	195
B. caudatus	148	106	231	131	218	166	328	243
B. angularis	191	251	368	238	293	320	358	245
B. urceolaris	4	15	21	28	27	19	14	27
B. guadridentatus	5	5	4	9	4	5	4	4
B. havanaensis	5 2	4	4	Ť	1	1	2	
B bidentata			1	1			1	
B. variabilis		9					1	
Keratella sp.	180	548	340	200	218	282	406	367
Polyarthra sp.	6	4	1	2	8	2	6	2
Trichocerca sp	2	4	2		0		0	10
Kellicottia sp.	1	2					2	2
Platylas sp.		1	1	1	1			
Filinia sp								
Monostyla sp.	4				1	1		1
Euchlanis sp.		2						
Total Rotifers	720	1,115	1,288	806	980	1.032	1,367	1,098
Algae								
Ceratium sp.	37	82	153	29	123	106	126	46
Closterium sp.	0,	1	100	20	1	100	120	40
Total Algae	37	83	153	29	124	106	126	46
Total Zooplankton	800	1,259	1,464	874	1,133	1,177	1,515	1,193

'a Density estimates are based on one sample from each location.

## Table C-2. Density (No./m²) and Percent Occurrence of Macroinvertebrates Collected at Stations 1 and 2 in the Ohio River, Wheeling, West Virginia, July-August 1984

		Stat	ion 1		Station 2 <sup>ra</sup>					
	0.0	5 m	1.5	5 m	0.6	3 m	1.5	5 m		
Таха	Mean No. m²	Percent	Mean No ´m²	Percent	Mean No ∕m²	Percent	Mean No.: m²	Percent		
Coelenterata										
Hydra sp	2.1	< 0.1			~-					
Platyhelmintnes Planariidae										
<i>Dugesia</i> sp.	66.7	27	48.7	2.1	21.9	0. <b>9</b>	115	0.7		
Annelida Oligochaeta Naididae Unid Na:didae	150 0	6.1	138.4	5.9	1,153.1	49.5	538 3	34 5		
Crustacea Amphipoda Gammandae										
<i>Gammarus</i> sp	839.6	34.1	1,481.6	62.7	765.6	32.8	822 8	52.7		
Acarı Hydracarına	2.1	< 0.1	2 6	01			-			

#### Table C-2. (continued)

		Stat	ion 1	Station 2 <sup>1a</sup>						
	0.6			ò m	0.€		1 E           Mean           rcent         No            3.8                                    3.8                3.8           0.3            2.3         3.8           0.7            0.8                5.1         15.4           0.1                5.1         15.4           0.1                5.1         15.4           0.1                5.1         15.4           0.1                    5.1         15.4           0.1                <	15 m		
Taxa	Mean No. / m²	Percent	Mear No. m <sup>.</sup>	Percent	Mean No m²	Percent		Percent		
nsecta										
Ephemeroptera										
Heptageniidae										
Stenonema sp.	4.2	0.2	26	0.1						
S. integrum	~-		5.1	0.2			3.8	02		
S. terminatum	4.2	0.2	2.6	0.1			-			
Caenidae										
Caenis sp.	2.1	<0.1					~-			
Idonata										
Libellulidae										
Perithemis sp			2.6	0.1						
richoptera										
Hydropsychidae										
Hydropsyche orris	16.7	0.7	10.3	0.4						
H. simulans	2.1	<0.1	2.6	0.1						
Polycentropodidae										
Cyrnellus fraternus	6.3	0.3	38.5	1.6			3.8	0.2		
Neureclipsis sp.		-+	5.1	0.2				0.2		
liptera										
Empididae										
Unid. Empididae	6. <b>3</b>	0.3			6.3	0.3		~-		
Chironomidae										
Chironomus sp.			2.6	0.1						
Cricotopus bicinctus group	33.3	1.4	2.6	0.1	6.3	0.3		~-		
C. cylindraceus group	79.2	3.2	7.7	0.3	53.1	2.3	3.8	0.2		
C. intersectus group	4.2	0.2		-~	15.6	0.7				
C. tremulus group	62.5	2.5	2.6	0.1	18.8	0.8		~		
Dicrotendipes sp.	95. <b>8</b>	3.9	61.5	2.6	50.0	2.1	77	0.5		
Harnischia sp.			5.1	0.2						
Micropsectra sp.	41.7	1.7	17.9	0.8	3.1	0.1				
M. curvicornis			2.6	0.1						
Nanocladius sp.	75.0	3.0	30.8	1.3	15.6	0.7	30.8	2.0		
Parametriocnemus sp.			12.8	0.5						
Paratanytarsus sp.	4.2	0.2	10.3	0.4						
Polypedilum convictum type	83.3	3.4	17.9	0.8	118.8	51	15.4	1.0		
P. fallax group			2.6	0.1	3.1					
P. scalaenum type	42	0.2	17.9	0.8	6.3			1.5		
Pseudochironomus sp.			5.1	0.2						
Rheotanytarsus sp.	66.7	27.1	158.9	6.7		~-				
Stenochironomus sp.			33.3	1.4	~-		46.1	3.0		
Tanytarsus sp.			5.1	0.2						
Thienemannimyia series	8.3	0.3	17.9	0.8	68.8			2.2		
Unid. Chironomidae pupa	202.1	8.2	133.3	5.6	21.9	0.9		0.7		
follusca										
Gastropoda										
Ancylidae										
Ferrissia sp.			2.6	0.1						
Physidae										
Physa sp.			5.1	021						
Pelecypoda										
Corbiculidae										
Corbicula fluminea			66.6	2.8	3.1	0.1	3.8	0.2		
Total Benthos	2,462.9	100	2,363.5	100	2,331.4	100	1,560.8	100		

<sup>1a</sup>One replicate substrate was not recovered.
 <sup>tb</sup>There were highly significant differences between stations (P = 0.0001). The number of taxa at Station 3 was different than at Stations 1, 6, and 8 (P = 0.05).
 NOTE: Total Taxa = distinct taxa; does not include pupa of included taxa.

		Stat	ion 3		Station 4					
	0.6	δm	1.5	i m	0.6	3 m	1	.5		
Taxa	Mean No∵m²	Percent	Mean No.∠m²	Percent	Mean No.∕m²	Percent	Mean No.∕m²	Percen		
Platyhelminthes							·			
Planariidae										
<i>Dugesia</i> sp.	2.1	0.2	7.7	0.4		-				
Annelida										
Oligochaeta										
Naididae										
Unid. Naididae	297.9	22.6	889.5	51.0	618.8	45.3	256.3	27.0		
Crustacea										
Amphipoda										
Gammaridae										
Gammarus sp.	735.4	55.9	569 1	32.6	185.4	13.6	241.0	25.4		
Decapoda	,00.	00.0	000	02.0		.0.0	247.0	20.4		
Astacidae										
Immature Astacidae		~-	2.6	0.1						
immature Astacidae		~-	2.0	0.1						
Cari										
Hydracarina	~-		12.8	0.7		~ -	5.1	0.5		
nsecta										
Ephemeroptera										
Baetidae										
Baetis sp.					~		2.6	0.3		
Heptageniidae										
Stenacron interpunctatum		. ~			6.3	0.5	12.8	1.3		
Stenonema integrum	- ~		2.6	0.1	2.1	0.2				
Immature Keptageniidae			5.1	0.3						
Caenidae										
Tricorythodes sp.				~-	2.1	0.2				
Trichoptera										
Hydropsychidae										
Hydropsyche orris					18.8	1.4	23.1	2.4		
Polycentropodidae					10.0	1.4	20.7	2.4		
Cyrnellus fraternus	29.2	2.2	23.1	1.3			15.4	1.6		
Neureclipsis sp.	20.2	~-			6.3	0.5	2.6	0.3		
Medieciipsis sp.					0.5	0.5	2.0	0.3		
Diptera										
Empididae Unid. Empididae					<b>-</b> -	~-	5.1	0.5		
•						~-		0.5		
Chironomidae Ablabaamula an					2.1	0.2	2.6	0.3		
Ablabesmyia sp.		0. <b>2</b>				0.2				
Cricotopus bicinctus group	2.1	1.7			2.1 18.8		7.7			
C. cylindraceus group	22.9					1.4		0.8		
C. intersectus group	2.1	0.2		~-	37.5	2.7				
C. tremulus group	12.5	0.9			12.5	0.9	5.1	0.5		
Dicrotendipes sp.	1375	10.4	138.4	7.9	122.9	9.0	872	9.2		
Endochironomus sp.				~ -			2.6	0.3		
Glyptotendipes sp				~-			26	0.3		
Micropsectra sp.				~-	8.3	0.6	7.7	0.8		
Nanocladius sp.	2.1	0.2		~ -	33 3	2.4	128	13		
Orthocladius sp.					2.1	0.2		~-		
Parachironomus sp			~		4.2	0.3				
Parametriocnemus sp.		-			2.1	0.2	2.6	0.3		
Paratanytarsus sp.					10.4	0.8	-	··-		
Phaenopsectra sp.	·		2.6	0.1	2.1	0.2				
Polypedilum convictum type	35.4	2.7	26	01	1104	8.1	17.9	1.9		
P. fallax group		~ -				·-	5.1	0.5		
P. scalaenum type	83	0.6	17.9	1.0	14.6	1.1	97.4	10.3		
Pseudochironomus sp.					2.1	0.2				
					10.4	0.8				

# Table C-3. Density (No./m²) and Percent Occurrence of Macroinvertebrates Collected at Stations 3 and 4 in the Ohio River, Wheeling, West Virginia, July-August 1984

#### Table C-3. (continued)

		Stat	ion 3		Station 4					
	0.6 m		1.5	1.5 m		5 m	1 5			
Taxa	Mean No. m²	Percent	Mean No_m²	Percent	Mean No m	Percent	Mean No m·	Percent		
Stenochironomus sp.	_		-	-		-	30.8	3.2		
Tanytarsus sp.					8.3	0.6	2.6	0.3		
Thienemannimyia series	16.7	1.3	17.9	1.0	37.5	2.7	53.8	5.7		
Unid. Chironomidae pupa	12 5	09	10.3	06	85.4	6.2	46.1	4.9		
Mollusca Pelecypoda Corbiculidae <i>Corbicula fluminea</i>			410	2.4						
Total Benthos	1,316.7	100	1,743.2	100	1,366.9	100	948 6	100		
Total Taxa'a'	13		14		26		24			

<sup>ta</sup> There were highly significant differences between stations (P = 0.0001). The number of taxa at Station 3 was different than at Stations 1, 6, and 8 (P = 0.05).

Note: Total Taxa " distinct taxa, does not include pupa of included taxa.

## Table C-4. Density {No./m²} and Percent Occurrence of Macroinvertebrates Collected at Stations 5 and 6 in the Ohio River, Wheeling, West Virginia, July-August 1984

		Stat	ion 5		Station 6					
	0.6	S m	1.5	5 m	0.6	3 m	1.5			
Таха	Mean No.∕m²	Percent	Meari No.∕m²	Percent	Mean No.∠m²	Percent	Mean No.∠m²	Percent		
Platyhelminthes Planariidae <i>Dugesia</i> sp.	2.1	0.2	7.7	0.5	2.1	0.1	15.4	0.7		
Annelida Oligochaeta Naididae Unid. Naididae	112.5	8.8	53.8	3.5	560.4	28.1	169.2	7.6		
Crustacea Amphiboda Gammaridae <i>Gammarus</i> sp.	254.2	19.9	461.4	29.9	506.3	25.4	853.6	38.2		
Acarı Hydracarina			15.4	1.0	6. <b>3</b>	0.3	2.6	01		
Insecta Ephemeroptera Heptageniidae										
Stenacron interpunctatum	2.1	0.2						· -		
Stenonema integrum Odonata Libellulidae	2.1	0.2	5.1	0.3	2.1	0.1				
Perithemis sp			2.6	0.2		· -	2.6	O. 1		
Trichoptera Hydropsychidae										
Cheumatopsyche sp			10.3	0.7	4.2	0.2				
Hydropsyche orris	83.3	6.5	105.1	6.8	33.3	1.7	89.7	4.0		
H. Orris pupa					4.2	0.2				
H. simulans			5.1	0.3		~	10.0	0.0		
Potamyia flava	6.3	0.5			2.1 2.1	0.1 0 1	12.8	0.6		
Symphitopsyche morosa					2.1	01				

#### Table C-4. (continued)

		Stat	ion 5			Stat	ion 6	
	06	im	1 5	i m	0 €	3 m	1	5
Таха	Mean No m:	Percent	Mean No m²	Percent	Mean No m²	Percent	Mean No m∙	Percent
Polycentropodidae								
Cyrnellus fraternus	31.3	2.5	176.9	11.5	6.3	0.3	128.2	5.7
Neureclipsis sp.	4.2	0.3	5.1	0.3	6.3	0.3	28.2	1.3
Leptoceridae								
Oecetis sp.			2.6	0.2	~-		~-	
Diptera								
Empididae								
Unid. Empididae	~-		5.1	0.3			5.1	0.2
Chironomidae								
Ablabesmyia sp.	~		5.1	0.3				
Cricotopus bicinctus group	6.3	0.5			70.8	3.6	28.2	1.3
C. cylindraceus group	212.5	16.7	69.2	4.5	72.9	3.7	25.6	1.1
C. intersectus group	56.3	4.4	17.9	1.2	8.3	0.4		
C. tremulus group	54.2	4.2	17.9	1.2	56.3	2.8	25.6	1.1
Dicrotendipes sp.	85.4	6.7	135.9	8.8	97.9	4.9	102.5	4.6
Glyptotendipes sp.			5.1	0.3				
Micropsectra sp.	8.3	0.7			2.1	0.1	20.5	0.9
M. curvicornis	2.1	0.2					2.6	0.1
Microtendipes sp.	2.1	0.2	33.3	2.2				
Nanocladius sp.	77.1	6.0	41.0	2.7	37.5	1.9	89 7	4.0
Parametriocnemus sp.		0.0	2.6	0.2			20.5	0.9
Paratanytarsus sp.	6.3	0.5	2.6	0.2				-~
Phaenopsectra sp.	4.2	0.3	2:0			-~		
Polypedilum convictum type	52.1	4.1	71.B	4.7	154.2	7.7	76.9	3.4
P. fallax group				-~	2.1	0.1	/0.3	
P. scalaenum type	12.5	1.0	53.8	3.5	20.8	1.0	53.8	2.4
Pseudochironomus sp.	2.1	0.2	2.6	0.2				Z.4 
Rheotanytarsus sp.	64.6	5.1	35.9	2.3	122.9	6.2	220.4	9.9
Stenochironomus sp.		J.1	25.6	1.7	2.1	0.1	53.8	9.9 2.4
Tanytarsus sp	10.4	0.8	5.1	0.3	12.5	0.6	7.7	0.3
Thienemannimyla series	29.2	2.3	69.2	4.5	31.3	1.6	115.4	
Unid. Chironomidae pupa	91.7	7.2	89.7	4.5 5.8	160.4	8.1	79.5	5.2 3.6
Mollusca								
Gastropoda								
Physidae								
Physa sp.					4.2	0.2		
Pelecypoda					<b>₩.</b> ∠	0.2		
Corbiculidae								
Corbicula fluminea			2.6	0.2			2.6	0.1
Total Benthos	1.275.5	100	1.543.1	100	1.992.0	100	2.232.7	100
	,	100		100		100	_,	100
Total Taxa'"	26		31		26		25	

<sup>ei</sup>There were highly significant differences between stations (P = 0.0001). The number of taxa at Station 3 was different than at Stations 1, 6, and 8 (P = 0.05).
 Note: Total Taxa = distinct taxa; does not include pupa of included taxa.

		Stat	ion 7			Stat	ion 8	
	0.6	i m	1.5	ō m	0.6	ŝ m	1.8	ō m
Таха	Mean No.∕m²	Percent	Mean No.∕m²	Percent	Mean No.∕m²	Percent	Mean No.∕m²	Percent
Vematoda	8.3	0.2						
Platyhelminthes								
Planariidae							11.5	0.8
<i>Dugesia</i> sp.							11.5	0.0
Annelida								
Oligochaeta								
Naididae								
Unid. Naididae	3,229.2	81.3	297.3	25.3	837.5	28.4	138.4	9.1
Crustacea								
Amphipoda								
Gammaridae								
Gammarus sp.	152.1	3.8	376.8	32.1	368.8	12.5	615.2	40.6
Acari								
Hydracarina		- <b>-</b>	5.1	0.4	6.3	0.2	7.7	0.5
nsecta								
Ephemeroptera								
Heptageniidae <i>Stenonema</i> sp.		~~	2.6	0.2	6.3	0.2		
S. femoratum							3.8	0.3
S. integrum			2.6	0.2			3.8	0.3
S. terminatum	4.2	0.1						
Immature Heptageniidae			2.6	0.2				
Caenidae								
Caenis sp.					3.1	0.1		
Baetidae						<b>-</b> .		
<i>Baetis</i> sp.					3.1	0.1		
frichoptera								
Hydropsychidae								
Cheumatopsyche sp.					6.3	0.2		
Hydropsyche sp.			5.1	0.4				
H. orris	6.3	0.2	74.3	6.3	12.5	0.4	69.2	4.6
H. orris pupa	2.1	<0.1	7.7	0.7				
H. valanis			2.6 5.1	0.2	6.3	0.2	 7.7	0.5
Potamyia flava			5.1	0.4	0.5	0.2	1.1	0.5
Polycentropodidae Cyrnellus fraternus		~-	5.1	0.4	15.6	0.5	34.6	2.3
Neureclipsis sp.							7.7	0.5
Coleoptera Elmidae								
Stenelmis sp. adult	2.1	<0.1						
Distore								
Diptera Ceratopogonidae								
Unid. Ceratopogonidae							3.8	0.3
Empididae								
Unid. Empididae	2.1	<0.1	2.6	0.2	6.3	0.2		
Chironomidae							<b>_</b> .	_
Cricotopus bicinctus group	33.3	0.8	17.9	1.5	40.6	1.4	3.8	0.3
C. cylindraceus group	43.8	1.1	35.9	3.1	218.8	7.4	19.2	1.3
C. intersectus group	8.3	0.2			37.5	1.3	11.5	0.8 1.0
C. tremulus group	41.7	1.0	20.5	1.7	159.4	5.4	15.4 50.0	3.3
Dicrotendipes sp.	16.7	0.4	38.5	3.3 0.7	262.5 43.8	8.9 1.5	30.8	2.0
<i>Micropsectra</i> sp. <i>Nanocladius</i> sp.	133.3	3.4	7.7 33.3	2.8	43.8 181.3	6,1	19.2	1.3
WATCH TACHTS WIT	133.3	3.4	33.3	∡.o	101.0	0.1		·

# Table C-5. Density (No./m²) and Percent Occurrence of Macroinvertebrates Collected at Stations 7 and 8 in the Ohio River, Wheeling, West Virgina, July-August 1984

#### Table C-5. (continued)

		Stat	ion 7			Stat	tion 8	
	0.6	δm	1 5	5 m	0.6	3 m	15	5 m
Taxa	Mean No. m	Percent	Mean No m⁻	Percent	Mean No m	Percent	Mean No m	Percent
Paratanytarsus sp.					18.8	0.6	3.8	03
Phaenopsectra sp	2.1	√<0.1		-			_ ·	
Polypedilum convictum type	18.8	05	17.9	1.5	234.4	7.9	42.3	2.8
P fallax group	2.1	< 0.1	5.1	04			-	
P. scalaenum type	25.0	0.6	30.8	2.6	53.1	1.8	142.3	94
Pseudochironomus sp	-		-	-	3.1	0.1		-~
Rheotanytarsus sp			5.1	04	78 1	26	115	0.8
Stenochironomus sp			33.3	2.8	-	-	69.2	4.6
Tanytarsus sp.					12.5	0.4	3.8	0.3
Thienemannimyia series	156.3	39	89.7	7.6	140 6	48	76.9	51
Unid Chironomidae pupa	83.3	2.1	28.2	2.4	193.8	6.6	103.8	6.9
Mollusca								
Gastropoda								
Physidae								
Physa sp.	21	<0.1	12.8	1.1				-
Pelecypoda								
Corbiculidae								
Corbicula fluminea			2.6	0.2			·	
Total Benthos	3,973.2	100	1,173.9	100	2,950.4	100	1,514.6	100
Total Taxa <sup>la:</sup>	19		27		25		26	

<sup>ta</sup>There were highly significant differences between stations (P = 0.001). The number of taxa at Station 3 was different than at Stations 1, 6, and 8 (P = 0.05).

Note: Total Taxa = distinct taxa; does not include pupa of included taxa.

## Table C-6. Numbers of Macroinvertebrates for Each Replicate Sample Collected at Stations 1 and 2 in the Ohio River, Wheeling, West Virginia, July-August 1984

			Stat	tion 1		Station 2						
		0.6 m			1.5 m			0.6 m			1.5 m	<b>-</b>
Таха	A	В	С	A	В	С	A	В	C <sup>a;</sup>	A	В	C (a)
Coelenterata												
Hydra sp.		1		-		-	-	-				
Platyhelminthes												
Planariidae												
<i>Dugesia</i> sp.	11	15	6	2	7	10		7		3		-
Annelida												
Oligochaeta												
Naididae												
Unid, Naididae	17	18	37	29	15	10	120	249		42	98	-
Crustacea												
Amphipoda												
Gammaridae												
Gammarus sp.	105	120	178	260	161	157	40	205		158	5 <b>6</b>	
Acarı												
Hydracarina	-	1				1						
Insecta												
Ephemeroptera												
Heptageniidae												
Stenonema sp.	2	· _		1								
S. integrum					2						1	-
S. terminatum	_		2		1			_	-			

#### Table C-6. (continued)

			Sta	tion 1					Sta	ition 2		
		0 6 m			15 m			0 6 m			1.5 m	)
Taxa	A	В	C	A	8	С	A	в	C 1	A	В	C'4'
Caenidae												
Caenis sp.		1				·						
Odonata												
Libellulidae												
Perithemis sp.		-~	·-	-	1							
Trichoptera												
Hydropsychidae												
Hydropsyche orris	5	1	2			4						~-
H. simulans			1			1	· -					
Polycentropodidae												
Cyrnellus fraternus		1	2	1	4	10					1	
Neureclipsis sp					1	1				t		~-
Diptera												
Empididae												
Unid. Empididae		2	1				2					
Chironomidae												
Chironomus sp.					1	~ -						
Cricotopus bicinctus group	8	2	6			1	1	1				
C. cylindraceus group	10	4	24			3	2	15		-	1	
C intersectus group		2		~~			3	2	~-	-	•	
C. tremulus group	12	6	12	1			4	2				
Dicrotendipes sp.	22	18	6	4	10	10	11	5		1		
Harnischia sp.					2					~_		
Micropsectra sp.	12	6	2	3	1	3	1			_		
M. curvicornis				ĩ		~-						
Nanocladius sp.	12	14	10		7	5	2	3		6	2	
Parametriocnemus sp.					2	š						
Paratanytarsus sp	-		2	1	2	1						
Polypedilum convictum					-							
type	16	14	10	2	2	3	3	35		2	2	
P. fallax group				1			1				~~	
P scalaenum type		2		4	3			2			6	
Pseudochironomus sp.						2						
Rheotanytarsus sp.	88	108	124	4	9	49						
Stenochironomus sp.				3	7	3				2	10	
Tanytarsus sp.						2.		a. 🛥				
Thienemannimyia series	2	2			1	6	16	6		1	8	
Unid. Chironomidae pupa	37	20	40	6	14	32	3	4		1	2	
Mollusca												
Gastropoda												
Ancylidae												
Ferrissia sp.	_					1						
Physidae						I						~-
Physa sp				2			_	_		<i></i>		
Pelecypoda	-			4				~-				
Corbiculidae												
Corbicula fluminea				10	10	6		1			1	
								'				
Total Number of Taxa <sup>ter</sup>	14	20	17	17	21	23	13	13	··-	9	12	

<sup>'at</sup>One replicate substrate was not recovered. <sup>tet</sup>Total taxa values are for distinct taxa and do not include pupa.

	Station 3					Station 4						
		0.6 m			1.5 m			0.6 m		1.5 m		
Taxa	A	В	С	A	В	C	A	В	С	A	В	С
latyhelminthes												
Planariaidae												
Dugesia sp.		~	1	3						~		
Annelida												
Oligochaeta												
Naididae												
Unid. Naididae	24	10	109	16	55	276	41	169	87		62	38
									•			
Crustacea												
Amphipoda												
Gammaridae												
Gammarus sp.	108	85	160	62	44	116	2	87		48	36	10
Decapoda												
Astacidae												
Immature Astacidae	~-			1	~				~-			
Acari						-						
Hydracarina	~					5					1	1
nsecta												
Ephemeroptera												
Baetidae												
Baetis sp.		~ -								1		
Heptageniidae										•		
Stenacron interpunctatum			~					1	2		1	4
Stenonema integrum						1	~	1				
Immature Heptageniidae			~	2								
Caenidae												
Tricorythodes sp.							1					~-
Trichoptera												
Hydropsychidae												
Hydropsyche orris				~			4	1	4	4	1	4
Polycentropodidae												
Cyrnellus fraternus	13	1		7		2				3	1	2
Neureclipsis sp.							2	1	~-	1		
N:-+												
Diptera Empididae												
Unid. Empididae												
Chironomidae						~-					1	1
Ablabesmyia sp.		~-					1					1
Cricotopus bicinctus group		~	1			~	1			~~		
C. cylindraceus group	7		4				1	7	1	1	1	1
C. intersectus group	1							5	13		~-	
C. tremulus group	2		4				1	2	3			2
Dicrotendipes sp.	43	2	21	23	17	14	15	24	20	15	13	6
Endochironomus sp.			~				~					1
Chuntestandines an											1	~
Glyptotendipes sp.							1	3			2	1
Micropsectra sp.				~-		-~	2	9	5	1	2	2
<i>Micropsectra</i> sp. <i>Nanocladius</i> sp.	1								1			
<i>Micropsectra</i> sp. <i>Nanocladius</i> sp. <i>Orthocladius</i> sp.				~								
Micropsectra sp. Nanocladius sp. Orthocladius sp. Parachironomus sp.	1			~			1		1			
Micropsectra sp. Nanocladius sp. Orthocladius sp. Parachironomus sp. Parametriocnemus sp.	1 						1 1					
Micropsectra sp. Nanocladius sp. Orthocladius sp. Parachironomus sp. Parametriocnemus sp. Paratanytarsus sp.	1   						1	3	2			
Micropsectra sp. Nanocladius sp. Orthocladius sp. Parachironomus sp. Parametriocnemus sp. Paratanytarsus sp. Phaenopsectra sp.	1   				 	 	1	3	2	 	1  	
Micropsectra sp. Nanocladius sp. Orthocladius sp. Parachironomus sp. Parametriocnemus sp. Paratanytarsus sp. Phaenopsectra sp. Polypedilum convictum type	1    15		2		  1	  	1  19	3	2 1 23	  	1  3	  4
Micropsectra sp. Nanocladius sp. Orthocladius sp. Parachironomus sp. Parametriocnemus sp. Paratanytarsus sp. Phaenopsectra sp. Polypedilum convictum type P. fallax group	1   15		  2		  1	  	1  19 	3	2 1 23	  	1  3 1	  4 1
Micropsectra sp. Nanocladius sp. Orthocladius sp. Parachironomus sp. Parametriocnemus sp. Paratanytarsus sp. Phaenopsectra sp. Polypedilum convictum type P. fallax group P. scalaenum type	1   15  1	    	  2  3	 1  2	  1 	   5	1  19  1	3  11 	2 1 23  5	   9	1  3 1 23	 4 1 6
Micropsectra sp. Nanocladius sp. Orthocladius sp. Parachironomus sp. Parametriocnemus sp. Paratanytarsus sp. Phaenopsectra sp. Polypedilum convictum type P. fallax group P. scalaenum type Pseudochironomus sp.	1   15  1		2	1	  1 	  5	1  19  1 1	3  11  1	2 1 23 5	  9	1  3 1 23	 4 1 6
Micropsectra sp. Nanocladius sp. Orthocladius sp. Parachironomus sp. Parametriocnemus sp. Paratanytarsus sp. Phaenopsectra sp. Polypedilum convictum type P. fallax group P. scalaenum type	1   15  1	    	  2  3	 1  2	  1 	   5	1  19  1	3  11 	2 1 23  5	   9	1  3 1 23	  4 1

# Table C-7. Numbers of Macroinvertebrates for Each Replicate Sample Collected at Stations 3 and 4 in the Ohio River, Wheeling, West Virginia, July-August 1984

#### Table C-7. (continued)

	Station 3						Station 4						
		0.6 m			1.5 m			0.6 m			1.5 m		
Taxa	A	В	С	A	В	С	A	В	С	A	В	С	
<i>Thienemannimyia</i> series Unid. Chironomidae pupae	4 2	1 1	3 3	1	2	6 2	4 6	7 15	7 20	5 3	13 8	3 7	
Mollusca Pelecypoda Corbiculidae <i>Corbicula fluminea</i>				3	6	7							
Total Number of Taxa <sup>®</sup>	11	5	10	11	5	9	20	18	15	11	19	19	

# Table C-8. Numbers of Macroinvertebrates for Each Replicate Sample Collected at Stations 5 and 6 in the Ohio River, Wheeling, West Virginia, July-August 1984

	Stat	Station 5				Station 6						
		0.6 m			1.5 m			0.6 m			1.5 m	
Таха	A	В	С	A	В	С	A	В	С	A	8	С
Platyhelminthes												
Planariidae												
<i>Dugesia</i> sp		1			2	1			1			6
Ectoprocta												
Plumatellidae												
Hyalinella punctata										+(#/		
Annelida												
Oligochaeta												
Naididae												
Unid. Naididae		16	38	6	11	4	227	38	4	43	17	6
				-		•			·			•
Crustacea												
Amphipoda												
Gammaridae												
<i>Gammarus</i> sp.	47	36	39	66	56	58	17	66	160	153	71	109
Acari												
Hydracarina				5		1		2	1		1	
.,												
Insecta												
Ephemeroptera												
Heptageniidae												
Stenacron interpunctatum			1									
Stenonema integrum			1		2				1			~-
Odonata												
Libellulidae												
Perithemis sp.					1						1	
Trichoptera												
Hydropsychidae												
Cheumatopsyche sp.				1	3			2				
Hydropsyche orris	9	11	20	7	14	20	1	12	3	28	1	6
<i>H. orris</i> pupa								2				
H. simulans				1		1			÷			
Potamyia flava	2	1						1		4		1
Symphitopsyche morosa									1			
Polycentropodidae		_							_	_		
Cyrnellus fraternus	7	3	5	23	33	13		1	2	6	17	27
Neureclipsis sp.	1		1	1	1	<del>~</del>		3		6	3	2
Leptoceridae												
Oecetis sp.				1								

#### Table C-8. (continued)

			Stat	ion 5					Sta	tion 6		
		06 m			1.5 m			06 m			1.5 m	
Таха	A	B	С	A	В	C	A	В	С	A	В	С
Diptera										_		
Empididae												
Unid. Empididae		-			1	1	-				2	
Chironomidae												
Ablabesmyia sp.				1	1			<del>~</del> ·	··			
Cricotopus bicinctus group	2	1			~ -	~~	6	18	10	4	5	2
C. cylindraceus group	70	2	30	12	7	8	6	16	13	6	3	1
C intersectus group	10	7	10	2	3	2	1	<b>-</b>	3		-	-
C. tremulus group	8	11	7	4	2	1	4	22	1	4	3	3
Dicrotendipes sp.	18	11	12	18	11	24	5	28	14	18	10	12
Glyptotendipes sp.				1	1			- ·				
Micropsectra sp.		1	3	. –				1	-	4	4	
M curvicornis		1	·				· -			_ ·		1
Microtendipes sp.			1	6	2	5					-	
Nanocladius sp.	14	15	8	11	2	3	1	8	9	18	5	12
Parametriocnemus sp.				1	· <del>-</del>		~ -		-	6	1	1
Paratanytarsus sp	2		1	1			~			_		-
Phaenopsectra sp.	-	1	1		. –					- ~		-
Polypedilum convictum type	12	5	8	3	18	7	5	50	19	12	14	4
P. fallax group				_		~-	~ -		1			
P scalaenum sp.	2	3	1	5	12	4	7	2	1	14	7	~
Pseudochironomus sp.		1	· _		1							
Rheotanytarsus sp.	10	4	7	6	3	5		32	27	20	33	33
Stenochironomus sp.	~		~	4	1	5	1			14	6	1
Tanytarsus sp.	2	3	~	2				1	5			3
Thienemannimyia series	2	6	6	5	16	6	11		4	32	10	3
Unid Chironomidae pupa	15	11	18	11	15	9	7	52	18	11	10	10
Mollusca												
Gastropoda												
Physidae							-					
Physa sp.							2	-		~-	-	
Pelecypoda												
Corbiculidae												
Corbicula fluminea				1			~-			·		1
Total Number of Taxa <sup>157</sup>	16	21	20	26	24	19	14	19	20	18	20	20

<sup>tal</sup>Colonial organisms present, not included in total taxa count. <sup>ID</sup>Total taxa values are for distinct taxa and do not include pupa.

			Stat	ion 7					Sta	tion 8		
	<u> </u>	0.6 m			1.5 m			0.6 m			1.5 m	
Таха	A	B	С	А	В	С	А	B	С, в,	A	В	C'ai
Vematoda	1	3									~	
Platyhelminthes												
Planariidae												
<i>Dugesia</i> sp.										~	3	
Annelida												
Oligochaeta												
Naididae Unid, Naididae	612	265	673	49	33	34	193	77		10	10	
Offici Naturdae	012	205	073	49	33	34	193	//		18	18	
Crustacea												
Amphipoda												
Gammaridae												
Gammarus sp.	21	16	36	35	50	62	98	20		96	64	
Acari							~			~		
Hydracarina					1	1	2			2		
nsecta												
Ephemeroptera												
Heptageniidae												
Stenonema sp.					1		1	1				
S. femoratum											t	
S. integrum						1				1		
S. terminatum	1		1									
Immature Heptageniidae				1								
Caenidae												
<i>Caenis</i> sp.							1					
Baetidae												
<i>Baetis</i> sp. Trichoptera							1					
Hydropsychidae												
Cheumatopsychie sp.								2				
Hydropsyche sp						2						
H. Orris	1	1	1	14	6	9	3	1		7	11	
H. orris pupa	1				1	2						
H. valanis				1								
Potamyia flava				1	1		1	1		1	1	
Polycentropodidae												
Cyrnellus fraternus					2			5		5	4	
Neureclipsis sp									~-		2	
Coleoptera												
Elmidae Stenelmis sp. adult	1									_		
Diptera	•											
Ceratopogonidae												
Unid. Ceratopogonidae											1	
Empididae												
Unid. Empididae		1				1	2					
Chironomidae												
Cricotopus bicinctus group		3	9	1	2	4	12	1			1	
C. cylindraceus group	10	2	9	11	2	1	50	20		3	2	
C. intersectus group	~	1	3				12				3	
C. tremulus group	7	5	8	4	2	2	38	13		3	1	
Dicrotendipes sp.	2	3	3	6 2	8 1		76	8		8 5	5 3	
<i>Micropsectra</i> sp. <i>Nanocladius</i> sp.	20	22	22	6	3	4	10 40	4 18		5	3	
ivanociauius sp. Parametriocnemus sp.						2	40	··-		1	1	
Paratanytarsus sp.							6			1		
Phaenopsectra sp.	1											
Polypedilum convictum	-											
type	3	4	2	3		4	58	17		8	3	

## Table C-9. Numbers of Macroinvertebrates for Each Replicate Sample Collected at Stations 7 and 8 in the Ohio River, Wheeling, West Virginia, July-August 1984

#### Table C-9. (continued)

			Stat	ion 7					Sta	ition 8		
		06 m			15 m			06 r	n		15 m	
Taxa	Α	В	С	A	В	С	А	В	С "	А	В	C *
P fallax group	1			2		~-			-	-		
P scalaenum type	9	2	1	6	4	2	12	5		28	9	
Pseudochironomus sp					-	~ -	. –	1	-	~		
Rheotanytarsus sp.	-			1		1	10	15		1	2	
Stenachironomus sp.				12		1		-		12	6	
Tanytarsus sp.	-				~		2	2			1	
Thienemannimyia series	41	15	19	8	17	10	32	13		13	7	
Unid. Chironomidae pupa	16	5	19	3	5	3	41	21		13	14	
Mollusca												
Gastropoda												
Physidae												
Physa sp.	1			1	2	2		. –	.~	-		~ ·
Pelecypoda												
Corbiculidae												
Corbicula fluminea				1			-	~	-	-		
Total Number of Taxa' <sup>b</sup>	18	14	13	20	17	20	22	19	-	19	23	

<sup>rai</sup>One replicate substrate was not recovered. <sup>IET</sup> otal taxa values are for distinct taxa and do not include pupa.

#### Table C-10. Analysis of Variance and Tukey's Studentized Range Test for Zooplankton, Ohio River<sup>(a)</sup>

#### Crustaceans

Dependent variable: In count

Source	df	Sum of Squares	Mean Square	F Value	PR > F
Model	8	27.89	3.49	4.68	0.0283
Error	7	5.22	0.74		
Corrected total	15	33.11			
Station	7	23.05		4.42	0.0343
Depth	8	4.84		6.49	0.0382
	Tukey's	Studentiz	ed Range	Test	

Station	З	5	2	4	8	7	6	1
Mean	5. <del>9</del> 5	5.20	3.95	3.70	3.55	3.40	3.10	1.75

#### Rotifers

Dependent variable: count

Source	df	Sum of Squares	Mean Square	F Value	PR > F
Model Error Corrected total	8 7 15	3.59 0.27 3.87	0.45 0.04	11.3	0.0022
Station Depth	7 1	3.59 0.004		12.90 0.11	0.0016 0.7535

#### Tukey's Studentized Range Test

Station	8	6	4	7	З	5	2	1
Mean	4.82	4.63	4.63	4.62	4.57	4.50	4.44	<b>3</b> .20

#### Total Zooplankton

Dependent variable: In count

Source	df	Sum of Squares	Mean Square	F Value	<b>PR</b> > F
Model	8	3.48	0.43	11.17	0.0023
Error	7	0.27	0.04		
Corrected total	15	3.75			
Station	7	3.47		12.73	0.0017
Depth	1	0.008		0.23	0.6496
	Tukey's	s Studentiz	ed Rang	e Test	
Station	8	4 6	7	35	2 1
Mean	4.85	4.67 4.67	4.65 4	.63 4.56	4.48 3.27

""SAS PROC GLM.

#### Table C-11. Analysis of Variance and Confidence Interval-Overlap Results of Clorophyll a and Biomass Measurements of Periphyton, Ohio River '\*

#### Chlorophyll a

Dependent variable: Chla (all stations)

Source	df	Sum of Squares		F Value	PR >F
Station	5	26,104	5,221	6.48	0.008
Error	9	7,250	806		
Corrected total	14	33,354			

95 Percent Confidence Interval Overlap

Station	2	3	4	7	6	8
Mean	40.1	31.2	29.1	73.1	122.5	151.6

#### Dependent variable: Chia (Stations 1-7)

Source	df	Sum of Squares	Mean Square	F Value	PR > F
Station	4	18,371	4,593	5.70	0.014
Error	9	7,250	806		
Corrected total	13	25,621			
95 P	ercent	Confidenc	e Interval	Overlap	

Station	2	3	4	7	6	
Mean	40.1	31.2	29.1	73.1	122.5	

#### Biomass

Dependent variable: In AFDW (all stations)

Source	df	Sum of Squares		F Value	PR > F
Station	5	3.12	0.62	3 70	0.043
Error	9	1.52	0.17		
Corrected total	14	4.64			

#### 95 Percent Confidence Interval Overlap

Station	3	4	2	7	8	6	
Mean	1.26	1.14	1.70	1.69	2.41	2.32	

#### Dependent variable: In AFDW (Stations 1-7)

Source	df	Sum of Squares	Mean Square	F Value	
Station	4	2.54	0.64	3.76	0.046
Error	9	1.52	0.17		
Corrected total	13	4.06			
95 F	Percent	Confidenc	e Interva	l Overlap	
Station Mean	3 1.27	4 1.14	2 1 70 1.	7 6 69 2.32	2

'<sup>a'</sup>MINITAB.

#### Table C-12. Analysis of Variance and Tukey's Studentized Range Test Results for Oligochaetes and Amphipods, Ohio River

Source	df	Sumo	Sum of Squares		Mean Square		Value	PR .> F
Model	15	682	,986.22		45,532.41		6.38	0.0001
Error	28	199	,858.50		7,137.80			
Corrected total	43	882	,844.72					
Station	7	292	.953.14				5.86	0.0003
Depth	1	104	500.00			4	14.64	0.0007
Station Depth	7	285	,561.81				5.72	0.0004
		Tukey	s Studentize	d Range Tes	t			
Station	7	2	3	8	4	6	1	5
Mean	277.67	127.25	81.67	76.50	66.17	55.83	21.00	12.50

Oligochaete (unidentified Naididae)

#### Dependent variable counts

#### Amphipod (Gammarus sp.)

#### Dependent variable: counts

df	Sum of Squares			Mean Square		F Value	
15	92	,800.56		6,186.70		2.97	0.0062
28	58	.231.16		2,079 68			
43	151	,031.72					
7	81	.327.47				5 59	0.0004
1	1	,530.67				0.74	0.3982
7	9	,665.72				0.66	0.7002
	Tukey	s Studentize	d Range Tes	t			
1	2	6	3	8	5	7	4
163.50	114.75	96.00	95.83	69.50	50.33	36.67	30.50
	15 28 43 7 1 7	15 92 28 58 43 151 7 81 1 1 7 9 Tukey 1 2	15         92,800.56           28         58,231.16           43         151,031.72           7         81,327.47           1         1,530.67           7         9,665.72           Tukey's Studentize           1         2         6	15         92,800.56           28         58,231.16           43         151,031.72           7         81,327.47           1         1,530.67           7         9,665.72           Tukey's Studentized Range Tes           1         2         6         3	15         92.800.56         6,186.70           28         58.231.16         2,079.68           43         151.031.72         2           7         81.327.47         1           1         1,530.67         7           7         9,665.72         Tukey's Studentized Range Test           1         2         6         3         8	15         92,800.56         6,186.70           28         58,231.16         2,079.68           43         151,031.72         7           7         81,327.47         1           1         1,530.67         7           7         9,665.72         7           Tukey's Studentized Range Test           1         2         6         3         8         5	15         92,800.56         6,186.70         2.97           28         58,231.16         2,079.68         2           43         151,031.72         559         0.74           7         81,327.47         559         0.74           7         9,665.72         0.66         0.66           Tukey's Studentized Range Test           1         2         6         3         8         5         7

### Table C-13. Analysis of Variance and Tukey's Studentized Range Test Results for Chironomidae Taxa, Ohio River

### All Chironomid Taxa

Dependent variable: counts

Source	df	Sum of Squares			Mean Square	F	Value	PR > F
Model	15	166	,349.01		11,089.93		4.47	0 0003
Error	28	69	,444.17		2,480.15			
Corrected total	43	235	,793.18					
Station	7	90	,264.93				5.20	0.0007
Depth	1	44	,118.37				7.79	0.0002
Station · Depth	7	36	5,723.72				2.12	0 0750
		Tukey	's Studentize	d Range Tes	t			
Station	8	1	6	5	7	4	2	3
Mean	174.00	144.50	128.17	105.00	69.17	69.17	41.75	32.50

#### Table C-13. (continued)

#### Dicrotendipes sp.

Dependent variable: In counts

Source	df	f Sum of Squares			Mean Square		F Value	PR > F	
Model	15	. <u> </u>	17.17		1.14		2.79	0.0093	
Error	28		11.51		0.41				
Corrected total	43		28.68						
Station	7		12.22				4.25	0.0026	
Depth	1		1.50				3.65	0.0664	
Station Depth	7		4.06				1.41	0.2399	
		Tukey	's Studentize	d Range Te	st				
Station	3	5	4	8	6	1	7	2	
Mean	2.79	2.77	2.73	2.63	2.62	2.40	1.45	1.41	

### Polypedilum convictum type

Dependent variable: In count

Source	df	Sum (	of Squares		Mean Square	F	Value	PR > F
Model	15		32.07		2.14		3.79	0.0011
Error	28		15.79		0.56			
Corrected total	43		47.86					
Station	7		16.33				4.14	0.0031
Depth	1		12.05				21.38	0.0001
Station · Depth	7		4.30				1.09	0.3955
		Tukey	's Studentize	d Range Tes	st			
Station	8	6	5	4	1	2	7	3
Mean	2.64	2.60	2.16	1.94	1.92	1.79	1.18	0.76

### Rheotanytarsus sp.

#### Dependent variable: counts

Source	df	Sum o	of Squares		Mean Square	F	Value	$PR \ge F$
Model	15	31	,479 13		2,098.61		22.48	0.0001
Error	28	2	,613.67		93.34			
Corrected total	43	34	,092.80					
Station	7	20	,129.63				30.81	0.0001
Depth	1	1	,390.29				14.89	0.0006
Station · Depth	7	9	,777.48				14.96	0.0001
		Tukey	s Studentize	d Range Te	st			
Station	1	6	8	5	4	7	3	2
Mean	63.67	24.17	7.00	5.83	0.83	0.33	0.00	0.00

#### Table C-13. (continued)

Source	df	Sum of Squares			Mean Square	F	F Value		
Nodel	15	3.962.26			264.15		3 4 2	0 0024	
rror	28	2	2,164.17		77 29				
Corrected total	43	6	6,126.63						
tation	7	-	2,71935				5.03	0.0009	
lepth	1		836.23				10.82	0.0027	
tation Depth	7	396 35					0.73	0.6462	
		Tukey	's Studentize	d Range Te	st				
Station	1	8	6	5	4	7	2	3	
Mean	24.83	22.25	18.00	1317	9.83	8 50	2.50	1.67	

#### Unidentified Chironomidae Pupae

### Cricotopus cylindraceus

Dependent variable: In count

df	Sum of Squares Mean S		Mean Square	F	F Value		
15	37.33			2.49	3 90		0.0009
28		17.86		0.64			
43		55.19					
7		18.55				4.15	0.0030
1		15.88				24 90	0.0001
7		4.06				0.91	0.5141
	Tukey	's Studentizer	l Range Tes	t			
5	8	6	7	1	2	4	3
2.61	2.36	1.91	1.68	1.43	1 14	0.92	0.61
-	15 28 43 7 1 7 5	15 28 43 7 1 7 Tukey 5 8	15 37.33 28 17.86 43 55.19 7 18.55 1 15.88 7 4.06 Tukey's Studentized 5 8 6	15 37.33 28 17.86 43 55.19 7 18.55 1 15.88 7 4.06 Tukey's Studentized Range Tes 5 8 6 7	15       37.33       2.49         28       17.86       0.64         43       55.19       0.64         7       18.55       1         1       15.88       7         7       4.06       Tukey's Studentized Range Test         5       8       6       7	15       37.33       2.49         28       17.86       0.64         43       55.19       0.64         7       18.55       1         1       15.88       2         7       4.06       2         Tukey's Studentized Range Test         5       8       6       7       1       2	15       37.33       2.49       3.90         28       17.86       0.64         43       55.19       0.64         7       18.55       4.15         1       15.88       24.90         7       4.06       0.91         Tukey's Studentized Range Test         5       8       6       7       1       2       4

#### Table C-14. Analysis of Variance and Tukey's Studentized Range Test Results for Trichoptera, Ohio River

			HUTOPSYCH					
Dependent variable: count								
Source	df	Sum	of Squares		Mean Square	F	<sup>-</sup> Value	PR > F
Model	15	1	,060.97		70.73		2.79	0.0091
Error	28		708.67		25.31			
Corrected total	43	1	1,769.64					
Station	7		836.30				4.72	0.0013
Depth	1		73.50				2.90	0.0994
Station · Depth	7		153.39				0.87	0.5449
		Tukey	's Studentize	d Range Te	st			
Station	5	6	8	7	4	1	3	2
Mean	13.50	8.50	5.50	5.33	3.00	2.00	0.00	0.00

#### Hydropsyche orris

#### Cyrnellus fraternus

Dependent variable: In count

Source	df	Sum	of Squares		Mean Square	í	<sup>-</sup> Value	PR > F
Model	15		36.76		2.45		5.22	0.0001
Error	28		13.15		0.47			
Corrected total	43		49.91					
Station	7		23.13				7.03	0.0001
Depth	1		8.13				17.30	0.0003
Station · Depth	7		4.72				1.44	0.2310
		Tukey	's Studentize	d Range Tes	st			
Station	5	6	8	3	۱	4	7	2
Mean	2.43	1.66	1.30	1.08	1.08	0.53	0.18	0.17

## Table C-15. Analysis of Variance and Tukey's Studentized Range Test Results for the Benthic Macroinvertebrate Taxa, Ohio River

Dependent variable:

Source	df	Sum of Squares Mea			Mean Square	F	F Value		
Model	8		764.62		95.58	1	12.58		
Error	35		266.02		7.60				
Corrected total	43	1,	030.64						
Station	7		746.80			1	4.04	0.0001	
Depth	1		17.82				2.34	0.1347	
		Tukey	's Studentize	d Range Tes	ι				
Station	5	8	1	6	4	7	2	3	
Mean	21.17	20.75	18.67	18.50	17.00	16.33	11.75	8.33	