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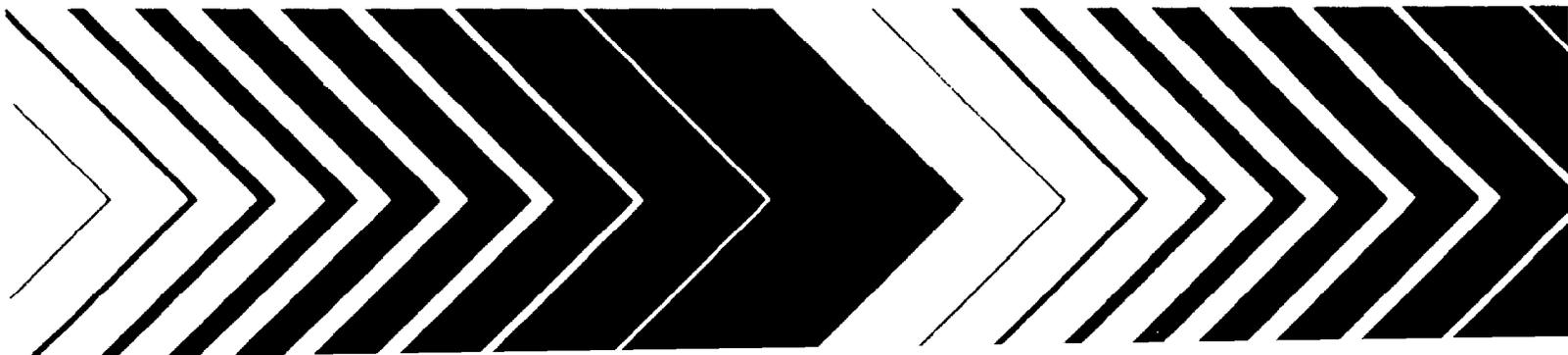
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Research and Development



Validity of Effluent and Ambient Toxicity Tests for Predicting Biological Impact, Kanawha River, Charleston, West Virginia



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Validity of Effluent and Ambient Toxicity Tests for Predicting Biological Impact, Kanawha River, Charleston, West Virginia

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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;
2. 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 discharges in receiving water; and
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 quality 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 is 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.

The following study was on the Kanawha River near Charleston, West Virginia, and was conducted in August and September, 1984.

To date, eight sites involving municipal and industrial dischargers have been investigated. 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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COMPARISON OF LABORATORY TOXICITY DATA AND
RECEIVING WATER BIOLOGICAL IMPACT
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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 eighth in a series of eight and was conducted on the Kanawha River near Charleston, West Virginia, which receives discharges from many industrial and municipal facilities. The study area comprises about 125 km of the Kanawha River, from the London Pool downstream to the Winfield Pool. The Kanawha River is an inland waterway and is navigable throughout the study area. Ambient toxicity tests using both the *Ceriodaphnia dubia* and fathead minnow 7-d tests were conducted on samples from 34 river stations. Because of the nature of the site, a comparison of ambient toxicity to community impact only, was attempted. Effluent dilution toxicity tests using *Ceriodaphnia* were conducted on samples from 11 discharges and fathead minnow effluent dilution toxicity tests were run on four discharges. These effluent tests were not a planned part of the study to meet the objective but were done to provide data to the West Virginia DNR. Biological studies conducted at the ambient stations included plankton, periphyton, and benthic macroinvertebrates.

From 60 to 100% correct predictions of community impact were made by the toxicity tests, depending on the levels of effect compared. There was a high ($P \leq 0.005$) correlation between *Ceriodaphnia* toxicity measured and impact on zooplankton over 125 kilometers of river, evidence that the ambient test is an accurate predictor of water quality effects on the instream biota. Impacts on macroinvertebrates was underestimated by the ambient tests. The toxicity values derived from the effluent dilution tests do not suggest that the effluents should cause toxicity after mixing.

Quality Assurance

Coordination of the study was done by the principal investigator preceding the field work. A reconnaissance trip was made to the site prior to onsite work to obtain the necessary details regarding each discharge and to make a cursory evaluation of the river. Following that trip, details were delineated for setting the sampling and testing dates and the specific sampling sites, as well as the specific measurement to be made for each stream station. Upon arrival a meeting was held with West Virginia's Department of Natural Resources, the principal investigator, and the contract laboratory people to make final arrangements for sampling of effluents and river sites. Also, the selection of effluents to be tested was done. Following the meeting, a boat trip to identify the sampling stations and to select where the artificial substrates would be placed was made. The principal investigator was responsible for all the quality assurance related decisions. All instrumentation used during the study were calibrated daily according to manufacturers specifications.

1. Introduction

The study site was the Kanawha River near Charleston, West Virginia. The study area receives many discharges from various industrial facilities. The study site was chosen to provide an opportunity to determine if ambient and effluent toxicity test results would correspond to the response of the biota in a large river. Toxicity was evaluated using two test species. The response of the community was measured using artificial substrates, as well as periphyton and zooplankton collection. The effluent dilution and ambient toxicity tests were run on site where the substrates were placed in the river, and another set of ambient toxicity tests were run with river water collected when the substrates were removed.

Several of the stations were located in the zone of effluent mixing. The discharges and dilution volumes were so large that dye studies were too expensive for the funds available. The Kanawha River is channelized for ship and barge traffic and, without elaborate dye studies, the effluent concentrations at various stations cannot be approximated. Therefore, the effluent dilution test results cannot be used to predict where impact should occur because the instream waste concentrations of each or any effluent are not known. However, using effluent flow discharge data and river flow, instream waste concentrations can be calculated. The river flow variation was large when the substrates were in place, and again there was no information as to how the flow affected the effluent concentrations at the sample stations where mixing was not complete. Thus, the effluent exposure those substrates experienced before and after the toxicity test period may have been the same as, or quite different from, the exposure concentrations during the two periods that ambient toxicity test samples were collected.

Determining the impact of individual discharges to rivers as large as the Kanawha is very difficult unless the impact is dramatic. However, the combined effects of many discharges could be quite large, even though any single discharge might have unmeasurable effects on the aquatic community. Thus, the value of any method that can estimate such individually immeasurable impacts is obvious.

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 8. Methods and supporting data are included in the appendixes for reference.

2. Study Design

Study components included (1) 7-day *Ceriodaphnia dubia* toxicity tests using samples from 34 ambient river stations, (2) 7-day larval fathead minnow growth tests using samples from 34 ambient river stations, (3) effluent tests of both species on selected effluents, and (4) assessment of the zooplankton and benthic macroinvertebrate communities. Two separate sets of toxicity tests were conducted. The first set of tests was done on site and included both effluent dilution and grab ambient toxicity tests. The second set of tests was done off site on shipped grab samples only on the same ambient stations as were tested while on site. These tests were run in the mobile trailer at the Environmental Research Laboratory-Duluth, Minnesota. The on site ambient tests were done using 7 different daily grab samples while the off site tests used a single grab sample for the entire test. In some instances insufficient sample was available for the latter series.

2.1 Toxicity Testing Study Design

Toxicity tests were performed on the effluents to measure subchronic effects on the growth of larval fathead minnows and chronic reproductive effects on *Ceriodaphnia* (Chapter 4). A range of effluent concentrations was used so that acute mortality could be measured as well as chronic mortality. The objective of these tests was to estimate the minimum concentration of each effluent that would cause acute mortality or chronic effects.

In addition to the effluent tests, ambient river stations were selected and samples collected from them were used to measure ambient toxicity to *Ceriodaphnia* and fathead minnows (Chapter 4). These tests measured the loss of toxicity from the effluents after mixing, dilution from other inputs, degradation, and other losses such as sorbtion. These test results would also provide data for the prediction of ecological impact for comparison with the biological survey data, without having to know the effluent concentration.

The off site ambient toxicity tests were conducted using samples collected during a period of low river flows. These tests were done to see if the fungus problem in the first set of tests had subsided and to examine changes in toxicity due to lower river flow.

2.2 Biological Survey Study Designs

The field surveys included a quantitative assessment of the zooplankton, periphyton, and artificial substrate macroinvertebrate communities. Artificial substrates were used to collect both periphytic and macroinvertebrate organisms. The zooplankton data are summarized in Chapter 5. Chlorophyll and biomass were measured on periphyton (Chapter 6) and the number of taxa and abundance were measured on the macroinvertebrates (Chapter 7).

2.3 Approach To Integration of Laboratory and Field Efforts

The final component of this study was to integrate the ambient toxicity predictions with the measured community impact. The results of the ambient toxicity tests can be used to predict community impact regardless of whether instream waste concentrations are known. The effluent tests were done to provide data to the West Virginia DNR and the data were not used to predict effluent effects.

3. Site Description

The Kanawha River flows northwesterly from its origin at the confluence of the Gauley River and New River in West Virginia to the Ohio River. The study area covers 125 km of the river length encompassing rural and urban areas. All but two sampling stations were located in the London, Marmet and Winfield Pools (Figure 3-1). Stations 20.1 and 25.7 were in a pool formed by a dam on the Ohio River. River flow is controlled at each of these three locks and dams.

Eleven dischargers were included in the study from river kilometer (RK) 67.1 to RK 143.5. The discharges were from diverse chemical and industrial facilities (Table 3-1). Ambient river stations for community surveys and toxicity testing were located from RK 20.1 to RK 145.0. They were selected based on their relationship to effluent discharges in the river and were situated in near shore areas generally away from barge traffic. Table 3-2 contains a listing of river

Figure 3-1. Study area and station locations on the Kanawha River, August and September 1984.

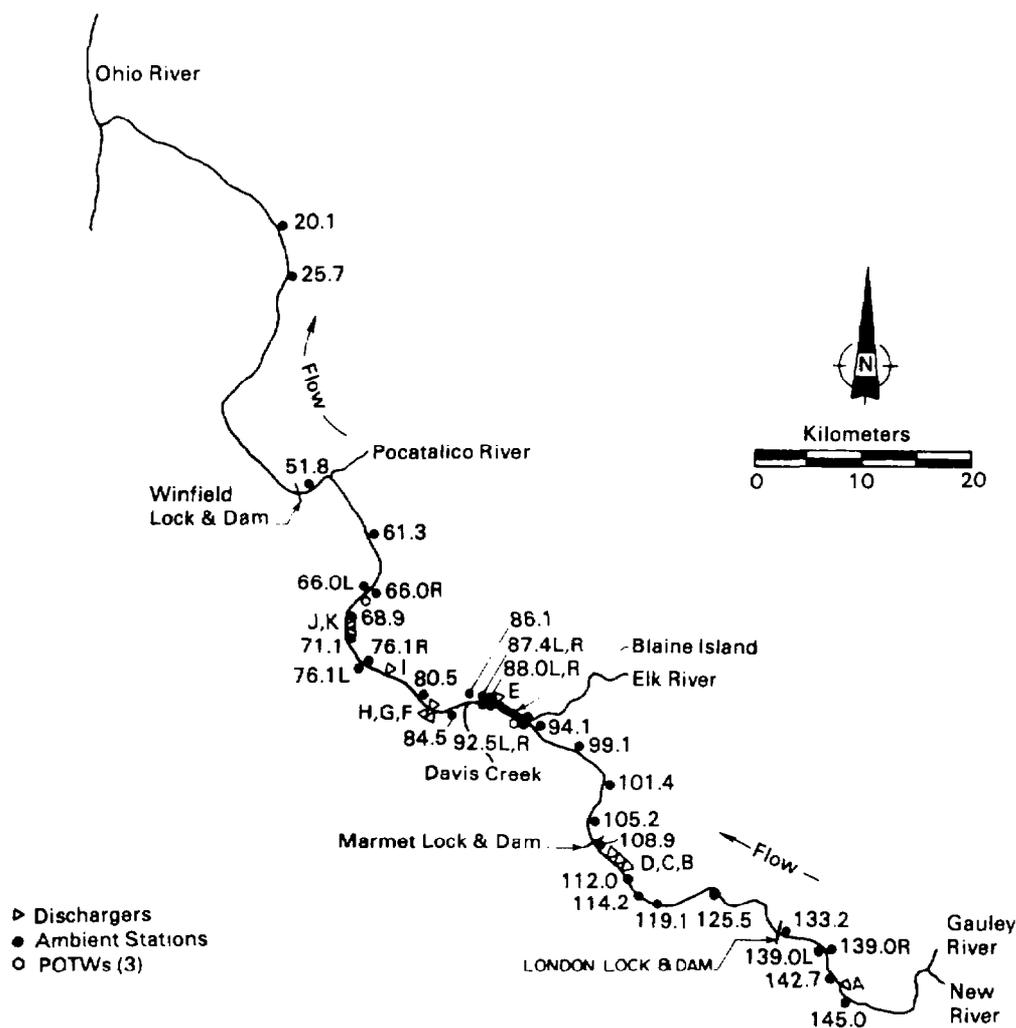


Table 3-1. Effluent Discharges to the Kanawha River

Effluent	River Kilometer	Secondary Industrial Category Codes	Category Description
A	143.5	3313	Electrometallurgical products
B	111.0	2869, 2879	Industrial organics, agricultural chemicals
C	111.0	2869, 2879	Industrial organics, agricultural chemicals
D	110.2	2819	Industrial inorganic chemicals
E	88.2	2812, 2819	Alkalies and chlorine, industrial inorganic chemicals
F	85.8	2812, 2891	Alkalies and chlorine adhesives and solvents
G	85.8	2819, 2869, 2879	Alkalies and chlorine, industrial organics, agricultural chemicals
H	85.8	2819, 2869, 2879	Alkalies and chlorine, industrial organics, agricultural chemicals
I	77.4	2869, 2879	Industrial organics, agricultural chemicals
J	68.9	2819, 2869	Industrial inorganic chemicals, industrial organics
K	67.1	2861, 2869, 2879, 7391	Gum and wood chemicals, industrial organics, agricultural chemicals, research and development laboratory

kilometers, dischargers and sampling stations. Only those dischargers whose effluents were tested are listed but there were others. In addition, some of the ambient stations were pairs located on opposite banks of the river.

The navigational channels in the Kanawha River are maintained by dredging, so that shallow waters are only found very near shore. The locks and dams along the river influence the habitat as does flow regulation.

During the on site testing in August, 1984, the river was quite high due to intense rain in the upper watershed. Ambient testing was delayed several days to allow the flow to return to a more normal one.

Table 3-2. Kanawha River Station Locations and Descriptions

River Kilometer	Station Description
20.1	Biological sampling station; ambient station
25.7	Biological sampling station; ambient station
51.8	Biological sampling station; ambient station
61.3	Biological sampling station; ambient station
66.0L	Biological sampling station; ambient station
66.0R	Biological sampling station; ambient station
66.8	POTW 3 discharge
67.1	K discharger
68.4	Dilution water obtained for effluents J, K, and POTW 3; biological sampling station; ambient station
68.9	J discharger
71.1	Biological sampling station; ambient station
76.1L	Biological sampling station; ambient station
76.1R	Biological sampling station; ambient station
77.4	I discharger
80.5	Dilution water obtained for effluent I; biological sampling station; ambient station
83.5	Ambient station
84.5	Biological sampling station; ambient station

Table 3-2. (Continued)

River Kilometer	Station Description
85.8	H, G, F discharger; Davis Creek
86.1	Dilution water obtained for effluents G and F
87.4L	Biological sampling station; ambient station
87.4R	Biological sampling station; ambient station
88.0L	Biological sampling station; ambient station
88.0R	Biological sampling station; ambient station
88.2	E discharger
89.6	POTW 2 discharge
90.4	POTW 1I and POTW 1M discharges, Dilution water obtained for effluents E, H, POTW 2, POTW 1M, and POTW 1I; biological sampling station; ambient station
92.5L	Biological sampling station
92.5R	Biological sampling station; ambient station
94.1	Biological sampling station; ambient station
99.1	Biological sampling station; ambient station
101.4	Biological sampling station; ambient station
105.2	Biological sampling station; ambient station
108.9	Biological sampling station; ambient station
110.2	D discharger
111.0	C, B discharger
112.0	Biological sampling station; ambient station
114.2	Dilution water obtained for effluents D, C, and B; biological sampling station; ambient station
118.4	Biological sampling station
119.1	Ambient station
125.5	Biological sampling station; ambient station
133.2	Biological sampling station; ambient station
139.0L	Biological sampling station; ambient station
139.0R	Biological sampling station; ambient station
142.7	Biological sampling station; ambient station
143.5	A discharger
145.0	Dilution water obtained for effluent A; biological sampling station; ambient station

4. Laboratory Toxicity Tests

The purpose of the toxicity tests was to determine the ambient toxicity of water samples collected near the artificial substrates and to compare that toxicity with the response of the substrate community at each station. The ambient stations were chosen based on their location to an industry or group of industries to determine the effects on the river. Since flows were large and the number of discharges were too numerous to allow dye studies for estimating instream waste concentrations, there was no critical need to do effluent dilution tests. Therefore, the selection of effluents for testing was left to the West Virginia Division of Water Resources and the choice was based on the needs of West Virginia's staff.

Because the river was at a very high water stage during the August testing period, a set of ambient samples was collected in September and shipped to ERL-D to obtain measures of ambient toxicity at lower flows (Table 4-11). During the onsite testing, a problem with a fungal growth on the *Ceriodaphnia* affected the effluent dilution tests but not the ambient tests. This fungus was clearly not parasitic because the animals molted regularly and after molting, their appearance was normal until time passed and the fungus grew again. The effect was apparently only a physical one; the fungus weighed down the animals until they could not remain in the water column. In another project being done simultaneously in the same mobile laboratory, and using aliquots of the same samples and test animals from the same culture, no fungus was observed. The only obvious difference was a 24-48 hour storage of the sample before use. Also, one industry split the effluent and dilution water samples collected for the on site tests and had the identical *Ceriodaphnia* 7-d tests done by a contract laboratory. That laboratory had no problem with fungus, and again, the sample had aged a few hours during transit before the tests were started. Even the one percent effluent test solution greatly reduced or eliminated the fungus growth. For example, the mean survival for the *Ceriodaphnia* dilution water controls for the 15 effluent dilution tests was 41.5 percent (21.9 S.D.), whereas the survival for the 1 percent treatments for the 15 effluents was 83.1 percent (16.6 S.D.). The mean young per female and standard deviation was 16.1 (3.7) and 21.3 (2.9) for the dilution water control and 1 percent treatment, respectively. The fungus was definitely not caused by the effluents tested in this study.

A similar problem was encountered in the Scippo Creek Study (Mount and Norberg, 1985); and again in water that was shipped to the offsite lab (and, therefore, was 24 hours older), there was no fungal growth.

On two occasions previous to the site study, ambient samples were shipped from the Kanawha River to a remote laboratory and no fungus problem was encountered. Possibly, the fungus was associated with the runoff following the rainfall which occurred just before the study began. Because of the rains, the ambient tests were started two days after the effluent dilution tests and the fungus problem was minor substantiating that fungus was associated with the high flow.

Brood size did not seem to be much affected by the fungus. If the *Ceriodaphnia* did not get so "over-weighted" that they died from struggling to free themselves, they produced normal broods. When test solutions were changed, those adults that were severely laden were killed rather than allowing them to die and the death being attributed to toxicity. These were stop-gap measures in order to obtain something from the tests. In the September study using shipped river water samples, reproduction and survival was excellent and no sign of the fungus problem could be seen. Whether that was due to the delay caused by shipping or lower flow of the river is not known.

The fathead minnows (*Pimephales promelas*) did very well in both studies. During the onsite testing, the final dissolved oxygen (DO) in the fathead minnow chambers was about one third to one half the concentrations of final DO in the September tests. Both the ambient and the effluent dilution tests showed this low DO in August and there appears to be no major difference between upstream and downstream stations. Apparently there was an increased oxygen demand in the river associated with the high flows.

4.1 Chemical/Physical Conditions

4.1.1 Onsite Tests

Table C-1 contains the initial chemistry data for effluent dilution tests for both test species, although effluent dilution tests with the fathead minnows were done only on four effluents. The final DO values are

for the *Ceriodaphnia* tests only while the final DO values for the fathead minnow effluent tests are in Table C-2. Table C-3 contains initial chemistry data for the ambient tests and both species. Since the effluents were diluted with water from various ambient stations throughout the study area, initial values were not taken on all stations after the first day. The river velocity and turbulence was high, the stations used for dilution water were close to one another and a decision was made to reduce the workload since differences between stations under such conditions were unlikely. Table C-4 contains the final DO values for ambient tests on both species.

All values for the *Ceriodaphnia* tests are in acceptable ranges. The final DO values for the fathead minnows are low and below the normally accepted range. Poor growth was expected but, as will be shown later, growth was excellent. The most probable reason is that the DO was measured with a probe 1 cm or more below the surface, whereas the fatheads were living in the oxygen-rich surface film where DO was much higher. Temperature for the *Ceriodaphnia* test was $25 \pm 1^\circ\text{C}$ and for the fathead minnow tests it was $25 \pm 3^\circ\text{C}$.

4.1.2 Offsite Tests

Table C-5 contains the initial pH, DO, and conductivity of the samples used for the ambient tests done offsite. Since the entire test was done on the same sample and the samples were refrigerated between changes, initial chemistry was done only once. Final DO values for both species, done daily, are also in Table C-5. For only two stations and only for the fathead minnow tests, are the DO ranges below 5.0 mg/L and then only 4.6 mg/L at the minimum. Nearly all values are in the acceptable range. Temperatures were $25 \pm 1^\circ\text{C}$ for the *Ceriodaphnia* and $25 \pm 2^\circ\text{C}$ for the fathead minnow tests.

4.2 Toxicity Test Results

Table 4-1 contains the results of the effluent dilution tests using *Ceriodaphnia* and Tables 4-2 and 4-3 contain the data for the fathead minnow effluent

dilution tests. In Table 4-1, the last column ("Number of Test Animals") is the number of original animals upon which the percent survival is based. Some of the animals that were heavily fungused were intentionally removed to avoid their deaths being attributed to toxicity. Almost without exception, young production per female was near or at the normal 20 young per adult expected (Mount and Norberg, 1984) for the lowest two or three effluent concentrations.

Fathead minnow survival and growth was normal and there was no fungal problem encountered. The 3 percent concentration of discharge A had poor growth and survival for unknown reasons (Table 4-3). Only in the effluents POTW11 and POTW2, 100 percent concentrations, were there statistically lower growth rates of the fathead minnows.

Table 4-10 contains the point estimates of acceptable effluent concentrations (AEC) for both species and all effluents tested. These AEC values are the geometric mean of the no observed effect concentration (NOEC) which causes no adverse effect and the lowest observed effect concentration (LOEC) which causes an adverse effect. Only three effluents had effect concentrations below 10 percent while all other effluent AEC's were higher. None of the effluent concentrations reached the AEC after complete mixing at the river flows existing during the study because river flows were above normal and the effluent flows were not nearly large enough to produce instream waste concentrations (IWC's) approaching the AEC's.

Table 4-4 contains the on site ambient test data for the *Ceriodaphnia* and Tables 4-5 and 4-6 contain the fathead minnow data. Stations at RK 145.0, 92.5R, and 88.0L had statistically lower young production than the station with the highest young production value, RK 112.0. In addition, a small effluent-laden tributary, Davis Creek, at RK 85.8 had statistically lower young production. For the fathead minnows, survival was not lower ($P < 0.05$) at any station, but growth was reduced at RK 99.1, 87.4R, 87.4L, and 76.1R as did Davis Creek (RK 85.8) as compared to the station which had the the highest weight value,

Table 4-1. Young Production and Adult Survival of *Ceriodaphnia* Exposed to Various Concentrations of Fifteen Effluents, Kanawha River, August 1984

Effluent (RK) ^{a1}	Percent Effluent (v/v)	Mean Number of Young per Female	Confidence Interval	Percent Survival	Number of Test Animals
POTW 11 (90.4)	100	2.2 ^{b1}	0.0-4.8	50	10
	30	20.9	19.2-22.5	75	8
	10	20.0	9.0-31.0	100	4
	3	22.5	20.4-24.6	100	4
	1	24.7	22.4-27.0	90	10
Dilution Water (90.4)		21.8	17.7-25.9	60	10

Table 4-1. (continued)

Effluent (RK) ^(a)	Percent Effluent (v/v)	Mean Number of Young per Female	Confidence Interval	Percent Survival	Number of Test Animals	
POTW 1M (90.4)	100	-- ^(b)	--	0 ^(b)	10	
	30	-- ^(b)	--	0 ^(b)	10	
	10	22.0	17.6-26.1	80	10	
	3	19.5	16.8-22.2	89	9	
	1	21.1	18.2-24.0	100	10	
	Dilution Water (90.4)		17.3	12.0-22.7	75	8
POTW 2 (89.6)	100	-- ^(b)	--	0 ^(b)	10	
	30	-- ^(b)	--	0 ^(b)	10	
	10	24.2 ^(b)	20.8-27.7	90	10	
	3	21.4	16.8-26.0	100 ^(b)	10	
	1	21.5	17.7-25.4	89	9	
	Dilution Water (90.4)		13.9	8.4-19.4	50	10
POTW 3 (66.8)	100	-- ^(b)	--	0	10	
	30	14.1	11.3-16.8	50	10	
	10	14.1	10.2-18.1	70	10	
	3	17.6	12.2-23.0	56	9	
	1	19.0	10.8-27.0	43	7	
	Dilution Water (68.4)		16.2	8.4-23.7	33	9
A (143.5)	100	28.1 ^(b)	25.7-30.5	100	8	
	30	21.0	12.5-29.3	67	9	
	10	24.8	19.2-30.3	100	8	
	3	18.8	15.1-22.6	63	8	
	1	20.2	15.0-25.4	71	7	
	Dilution Water (145.0)		18.3	13.0-23.7	89	9
B (111.0)	100	-- ^(b)	--	0	9	
	30	18.6	16.3-20.9	89	9	
	10	18.9	15.6-22.2	100 ^(b)	9	
	3	19.2	15.7-22.7	80	10	
	1	21.1	17.9-24.4	100 ^(b)	8	
	Dilution Water (114.2)		20.9	11.1-30.6	33	9
C (111.0)	100	-- ^(b)	--	0	10	
	30	5.3	1.5-9.1	100	3	
	3	20.6	18.8-22.4	80	10	
	1	18.9	13.8-24.3	78	9	
	Dilution Water (114.2)		14.8	4.8-25.0	33	9
	D (110.2)	100	18.5	12.1-24.8	40	10
30		16.9 ^(b)	13.8-19.9	78	9	
10		19.3 ^(b)	16.6-21.9	89 ^(b)	9	
3		16.0 ^(b)	12.9-19.2	60	10	
1		16.6 ^(b)	13.3-19.7	90 ^(b)	10	
Dilution Water (114.2)			8.5	3.7-13.3	20	10
E (88.2)	100	-- ^(b)	--	0	10	
	30	-- ^(b)	--	0	10	
	10	-- ^(b)	--	0	10	
	3	19.4	15.2-23.7	100 ^(b)	9	
	1	18.9	16.0-21.8	100 ^(b)	10	
	Dilution Water (90.4)		11.5	4.6-18.3	44	9

Table 4-1. (continued)

Effluent (RK) ^{a1}	Percent Effluent (v/v)	Mean Number of Young per Female	Confidence Interval	Percent Survival	Number of Test Animals
F (85.8)	100	-- ^(b)	--	0	10
	30	-- ^(b)	--	0	10
	10	18.4	16.8-19.9	100 ^(b)	8
	3	20.3	15.8-24.7	89	9
	1	20.9	17.9-23.9	89	9
	Dilution Water (90.4)	18.3	6.3-29.7	33	9
G (85.8)	100	-- ^(b)	--	0 ^(b)	10
	30	15.1	8.4-22.1	30	10
	10	20.5	17.1-23.8	90	10
	3	20.2	17.7-22.7	90	10
	1	20.9	16.7-25.0	60	10
	Dilution Water (86.1)	17.4	10.2-24.8	40	10
H (85.8)	100	-- ^(b)	--	0 ^(b)	10
	30	-- ^(b)	--	0	10
	10	-- ^(b)	--	0	10
	3	16.6	13.0-20.0	89	9
	1	19.1	15.7-22.6	89	9
	Dilution Water (86.1)	12.2	7.9-16.4	50	10
I (77.4)	100	-- ^(b)	--	0	10
	30	-- ^(b)	--	0	10
	10	22.2	20.4-24.0	80	10
	3	23.6	19.0-28.1	100 ^(b)	9
	1	24.6	22.2-26.9	78	9
	Dilution Water (80.5)	14.5	0.0-57.2	22	9
J (68.9)	100	--	--	0	10
	30	--	--	0	10
	10	14.2 ^(b)	8.7-19.8	50 ^(b)	10
	3	21.8 ^(b)	16.2-27.3	80 ^(b)	10
	1	25.7 ^(b)	24.8-26.6	70 ^(b)	10
	Dilution Water (68.4)	--	--	0	10
K (67.1)	100	-- ^(b)	--	0	10
	30	-- ^(b)	--	0	10
	10	8.3	4.8-11.8	70	10
	3	23.2	20.3-26.1	100 ^(b)	10
	1	26.8 ^(b)	24.9-28.7	100 ^(b)	10
	Dilution Water (68.4)	19.4	15.2-23.8	40	10

^{a1}River kilometer of the discharge, see Tables 3-1, 3-2.

^(b)Significantly different (P < 0.05).

Table 4-2. Mean Individual Weight of Larval Fathead Minnows After Seven Days Exposure to Various Concentrations of Four Effluents in Upstream Water, Kanawha River, August 1984

Effluent (RK) ^{a1}	Replicate	Percent Effluent (v/v)					Dilution Water
		100	30	10	3	1	
POTW 11 ^(b) (90.4)	A	0.245	0.570	0.648	0.510	0.651	0.550
	B	0.294	0.599	0.641	0.646	0.640	0.731
	C	0.310	0.512	0.591	0.595	0.328	0.538
	D	0.309	0.594	0.660	0.624	0.608	--
	Weighted Mean	0.289 ^(c)	0.570	0.635	0.594	0.559	0.601
	SE	0.044	0.039	0.038	0.038	0.042	0.048

Table 4-2. (continued)

Effluent (RK) ^(a)	Replicate	Percent Effluent (v/v)					Dilution Water
		100	30	10	3	1	
POTW 1M ^(b) (90.4)	A	0.430	0.563	0.489	0.521	0.563	0.589
	B	0.481	0.583	0.520	0.643	0.544	0.734
	C	0.409	0.457	0.526	0.535	0.597	0.431
	D	0.504	0.458	0.584	0.517	0.678	0.538
	Weighted Mean	0.459	0.516	0.527	0.548	0.596	0.571
	SE	0.036	0.035	0.036	0.037	0.036	0.036
POTW 2 ^(b) (89.6)	A	0.422	0.478	0.383	0.542	0.469	0.724
	B	0.367	0.511	0.501	0.651	0.690	0.565
	C	0.306	0.426	0.517	0.539	0.550	0.546
	D	0.367	0.515	0.559	0.608	0.618	0.602
	Weighted Mean	0.369 ^(c)	0.481	0.486	0.582	0.578	0.609
	SE	0.035	0.033	0.032	0.033	0.034	0.035
A ^(b) (143.5)	A	0.649	0.467	0.615	0.357	0.584	0.553
	B	0.793	0.711	0.736	0.390	0.644	0.628
	C	0.540	0.511	0.556	0.424	0.701	0.558
	D	0.598	0.692	0.650	0.495	0.677	0.640
	Weighted Mean	0.645	0.587	0.639	0.413 ^(c)	0.648	0.596
	SE	0.041	0.041	0.041	0.050	0.043	0.041

^(a)River kilometer of the discharge.

^(b)POTWs, 1I, 1M, and 2 were diluted with RK 90.4 water; A was diluted in RK 145.0 water.

^(c)Significantly lower from each test's dilution water weights ($P \leq 0.05$)

Table 4-3. Seven-Day Percent Survival of Larval Fathead Minnows to Various Concentrations of Four Effluents in Upstream Water, Kanawha River, August 1984

Effluent (RK) ^(a)	Replicate	Percent Effluent (v/v)					Dilution Water
		100	30	10	3	1	
POTW 1I ^(b) (90.4)	A	70	90	100	100	80	80
	B	90	100	100	100	80	80
	C	50	90	100	100	80	100
	D	80	100	100	100	80	100
	Mean	72.5	95	100	100	82.5	90
POTW 1M ^(b) (90.4)	A	90	90	100	90	90	100
	B	100	100	90	70	90	90
	C	80	100	100	80	100	100
	D	100	90	80	100	90	80
	Mean	92.5	95	92.5	85	92.5	92.5
POTW 2 ^(b) (89.6)	A	90	90	100	100	80	80
	B	100	80	100	100	70	70
	C	70	100	100	100	100	90
	D	70	100	80	60	90	80
	Mean	82.5	92.5	95	90	85	80
A ^(b) (143.5)	A	80	100	100	70	90	90
	B	100	90	90	70	100	100
	C	100	100	90	50	80	90
	D	100	80	100	60	70	90
	Mean	95	92.5	95	62.5	85	92.5

^(a)River kilometer of the discharge.

^(b)POTWs, 1I, 1M, and 2 were diluted with RK 90.4 water; A was diluted in RK 145.0 water.

Note: No significantly lower differences for any effluents were found ($P \leq 0.05$).

Table 4-4. Results of Ambient Toxicity Tests with *Ceriodaphnia*, Kanawha River, Charleston, West Virginia, August 1984

Ambient Station (RK)	Mean Number Young Per Female	Confidence Intervals	Mean Percent Survival
145.0	15.9 ^(a)	13.1-18.6	80
142.7	18.0	14.9-21.1	100
139.0R	19.6	14.5-24.6	70
139.0L	21.3	16.8-25.6	80
133.2	19.0	12.3-25.6	78
125.5	17.1	13.6-20.6	88
119.1	17.9	14.9-20.9	89
114.2	18.7	16.0-21.4	100
112.0	24.8	22.3-27.3	90
108.9	16.7	12.4-21.1	70
105.2	17.9	12.4-23.3	70
101.4	16.0	10.7-21.6	90
99.1	18.5	14.3-22.8	80
94.1	21.1	16.1-26.0	80
92.5R	15.0 ^(a)	12.5-17.5	90
90.4	19.0	17.0-21.0	90
88.0R	22.7	19.7-25.7	100
88.0L	-- ^(a)	--	0
87.4R	19.9	15.3-24.5	80
87.4L	20.6	18.2-23.0	100
Davis Creek, 85.8	6.13 ^(a)	2.7-9.4	60
83.3	24.3	22.5-26.1	80
84.5	16.5	12.1-20.9	100
80.5	19.6	15.2-24.0	100
76.1R	19.2	14.6-23.8	100
76.1L	19.8	16.2-23.4	90
71.1	19.5	14.4-24.6	100
68.4	21.6	17.0-26.2	90
66.0R	23.4	20.3-26.7	90
66.0L	17.2	11.9-22.5	100
61.3	16.4	14.3-18.4	89
51.8	17.4	12.8-22.0	89
25.7	19.3	16.2-22.4	100
20.1	20.5	17.6-23.4	100

^(a)Significantly different ($P \leq 0.05$).

RK 88.0L. However, none of those stations were greatly different from the highest value they were compared to even though they were statistically different. Such differences could be due to other causes such as enriched water with more food. For the *Ceriodaphnia* at RK 88.0L and Davis Creek (RK 85.8), differences in feeding level would not be expected to cause such low numbers. The food used will consistently produce 20 young in reconstituted water (Mount, unpublished data), where the food added is the only food available. The differences in *Ceriodaphnia* production at Stations 145.0, 92.5R, and 61.3 could be the result of experimental variations or food level. Tables 4-7, 4-8, and 4-9 contain the ambient test data for the September testing on single grab samples shipped to Duluth. There were no statistically significant differences in survival and growth or reproduction for either species when the station with the highest value is used for comparison. Survival of *Ceriodaphnia* was notably low at RK

stations 125.5, 108.9, and 84.5, although not statistically significant. The method of analyses for young per female (Appendix A) essentially excludes effect of adult mortality on young production estimates. In general, the reproduction of *Ceriodaphnia* and growth of the fathead minnows was uniform and at slightly above levels normally obtained in unenriched water.

4.3 Discussion

Since the low concentration of effluents, as well as the higher ones, eliminated the fungus problem, the data can be used but with some caution. Quality control sets should have been included in which a known water of good quality was used. Since samples had been tested previous to this study and good performance was obtained, they were not thought to be needed. Since the effect of the fungus would be to overestimate toxicity, and the AEC values obtained are all higher than the instream waste concentrations (IWCs) (for the fathead minnows as well as *Ceriodaphnia*), one can conclude that the effluents should not cause toxicity at the flows existing during the study.

The on site ambient tests with *Ceriodaphnia*, in general, had acceptable survival and, whereas there were five stations with significantly reduced young production, only Station 88.0L and Davis Creek (RK 85.8) are below the normal range usually obtained. The values that are significantly lower for the fathead minnows are also within the normal range. Therefore, the toxicity, if any, is certainly not very great. Several spills were reported during the study period by various plants in the study area and, since the test animals were exposed to a new sample every day, some effect of these spills could be evidenced by these data.

No statistical differences were found in the September study for either species. The overall impression from both testing periods is that the effluents tested are not causing toxicity after dilution at the flows prevailing during the two study periods. If the reduced growth of the fathead minnow and young production of the *Ceriodaphnia* is due to toxicity, it is minimal.

The oxygen demand of the ambient water during the August period of high flow, and the widespread fungus problem associated with it, perhaps should be further investigated. Fungal growths were also found on the artificial substrates providing some field evidence as well that the problem observed in the tests was not just an artifact.

Table 4-5. Mean Individual Weights of Larval Fathead Minnows After Seven Days From Ambient Toxicity Tests of the Kanawha River, August 1984.

Ambient Station (RK)	Replicate				Weighted Mean	SE
	A	B	C	D		
145.0	0.527	0.595	0.432	0.528	0.521	0.038
142.7	0.503	0.593	0.336	0.490	0.492	0.038
139 OR	0.526	0.566	0.419	0.485	0.489	0.036
139.0L	0.431	0.536	0.521	0.441	0.485	0.036
133.2	0.547	0.671	0.462	0.536	0.554	0.038
125.5	0.462	0.512	0.532	0.601	0.523	0.037
119.1	0.542	0.524	0.386	0.775	0.504	0.041
114.2	0.503	0.575	0.502	0.623	0.546	0.038
112.0	0.415	0.533	0.583	0.450	0.501	0.036
108.9	0.462	0.537	0.528	0.463	0.494	0.035
105.2	0.548	0.579	0.420	0.622	0.553	0.036
101.4	0.551	0.584	0.565	0.451	0.542	0.034
99.1	0.492	0.456	0.500	0.322	0.443 ^(a)	0.036
94.1	0.574	0.674	0.454	0.517	0.554	0.033
92.5R	0.491	0.578	0.575	0.458	0.523	0.034
90.4	0.507	0.467	0.430	0.588	0.498	0.033
88.0R	0.586	0.505	0.493	0.522	0.526	0.034
88.0L	0.610	0.659	0.536	0.623	0.603	0.036
87.4R	0.382	0.490	0.432	0.460	0.441 ^(a)	0.033
87.4L	0.520	0.465	0.271	0.484	0.435 ^(a)	0.033
Davis Creek, 85.8	0.388	0.443	0.457	0.393	0.420 ^(a)	0.033
84.5	0.486	0.550	0.563	0.546	0.536	0.033
83.3	0.580	0.543	0.498	0.442	0.516	0.033
80.5	0.498	0.572	0.361	0.479	0.478	0.033
76.1R	0.610	0.441	0.374	0.393	0.455 ^(a)	0.033
76.1L	0.490	0.548	0.517	0.479	0.509	0.033
71.1	0.552	0.561	0.576	0.594	0.571	0.033
68.4	0.599	0.424	0.497	0.438	0.490	0.034
66.0R	0.682	0.492	0.456	0.644	0.569	0.034
66.0L	0.452	0.490	0.562	0.490	0.499	0.034
61.3	0.520	0.445	0.439	0.574	0.495	0.034
51.8	0.568	0.467	0.496	0.504	0.509	0.035
25.7	0.572	0.536	0.572	0.493	0.543	0.035
20.1	0.532	0.546	0.607	0.689	0.594	0.034

^(a)Station 88.0L was used for comparisons, significantly lower ($P \leq 0.05$).

Table 4-8. Mean Individual Weights (mg) of Larval Fathead Minnows After Seven Days from Ambient Toxicity Tests, Kanawha River, Charleston, West Virginia, September 1984

Ambient Station (RK)	Replicate				Weighted Mean	SE
	A	B	C	D		
145.0	0.606	0.615	0.580	0.517	0.581	0.023
142.7	0.575	0.630	0.675	0.544	0.608	0.023
139.0R	0.588	0.533	0.550	0.625	0.571	0.024
139.0L	0.669	0.567	0.450	0.550	0.560	0.025
133.2	0.590	0.615	0.539	0.610	0.590	0.023
125.5	0.622	0.622	0.564	0.564	0.597	0.025
119.1	0.606	0.630	0.656	0.705	0.649	0.023
112.0	0.569	0.550	0.567	0.525	0.552	0.023
108.9	0.635	0.539	0.560	0.515	0.563	0.023
101.4	0.585	0.544	0.644	0.694	0.616	0.033
94.1	0.633	0.678	0.645	0.644	0.649	0.023
92.5L	0.505	0.570	0.750	0.695	0.620	0.038
90.4	0.578	0.550	0.469	0.531	0.534	0.037
88.0L	0.645	0.689	0.750	0.461	0.637	0.038
87.4L	0.550	0.675	0.656	0.714	0.648	0.038
84.5	0.617	0.600	0.522	0.520	0.565	0.037
76.1	0.572	0.494	0.520	0.680	0.567	0.036
68.4	0.645	0.681	0.528	0.556	0.605	0.037
66.0R	0.500	0.629	0.605	0.511	0.539	0.044
61.3	0.450	0.689	0.550	0.658	0.577	0.034
51.8	0.611	0.600	0.605	0.570	0.596	0.033
25.7	0.620	0.717	0.517	0.611	0.619	0.033
20.1	0.617	0.595	0.670	0.557	0.614	0.033

Note: No significantly lower differences for any stations were found ($P \leq .05$). Station 94.1 was used for comparison.

Table 4-9. Seven-Day Percent Survival of Larval Fathead Minnows Exposed to Various Ambient Stations of the Kanawha River, Charleston, West Virginia, September 1984

Ambient Station (RK)	Replicate				Mean Survival
	A	B	C	D	
145.0	100	100	100	90	98
142.7	100	100	100	90	98
139.0R	89	90	100	80	90
139.0L	80	90	80	70	80
133.2	100	10	90	100	98
125.5	90	90	70	70	80
119.1	90	100	90	90	93
112.0	80	100	100	100	95
108.9	100	90	100	100	98
101.4	100	90	90	90	93
94.1	100	90	100	90	95
92.5L	100	100	80	80	90
90.4	100	100	100	80	95
88.0L	100	90	90	90	93
87.4L	90	100	90	78	90
84.5	90	100	90	100	95
76.1	100	90	100	100	98
68.4	100	100	100	80	95
66.0R	90	88	90	90	90
61.3	100	90	73	60	81
51.8	90	80	100	100	93
25.7	89	100	90	90	92
20.1	90	91	100	70	88

Note: No significantly lower differences for any stations were found ($P \leq .05$). Station 94.1 was used for comparison.

Table 4-10. Acceptable Effluent Concentration (AEC)^{1a} of 15 Effluents for the Kanawha River

Effluent	AEC (Percent Effluent [v/v])	
	<i>Ceriodaphnia</i>	Fathead Minnow
POTW 1	54.8	54.8
POTW 1	17.3	>100
POTW 2	17.3	54.8
POTW 3	54.8	
A	>100	>100
B	54.8	
C	17.3	
D	>100	
E	5.5	
F	17.3	
G	54.8	
H	5.5	
I	17.3	
J	17.3	
K	5.5	

^{1a}Geometric mean of the no observed effect concentration (NOEC) which causes no adverse effect and the lowest observed effect concentration (LOEC) which causes an adverse effect.

Table 4-11. Kanawha River Flows, Charleston, West Virginia. Source of Data is the U.S. Geological Survey, Charleston, West Virginia

Date	Flows (m ³)
August 13	750.5
August 14	1243.3
August 15	1081.8
August 16	589.1
August 17	382.3
August 18	259.4
August 19	317.2
August 20	253.2
August 21	185.5
August 22	184.4
August 23	244.4
August 24	230.5
August 25	175.9
August 26	140.5
August 27	113.3
August 28	91.5
August 29	104.5
August 30	130.3
August 31	235.6
September 1	869.4
September 2	55.1
September 3	266.8
September 4	303.0
September 5	262.2
September 6	253.8
September 7	188.6
September 8	172.2
September 9	182.1
September 10	124.3
September 11	99.7
September 12	92.3
September 13	109.3
September 14	102.8
September 15	103.7
September 16	127.2
September 17	122.0
Mean flow during onsite testing	301.6
Mean flow during substrate exposure	202.1
Mean flow during zooplankton sampling	115.5

5.0 Zooplankton Community Survey

The zooplankton community was sampled using the methods described in Appendix B. The current of the Kanawha River was fast (even though the river is totally in pools as a result of navigation dams) during the August onsite study as a result of rain upstream. Flows were from 1243 to 253 m³/sec during the sampling period (August 14-20), about four to 20 times above normal low flow (see Table 4-11). Samples for zooplankton were collected in August when the substrates were placed in the river, but upon examination no further counts were made because there were insufficient densities to be valid. Samples were taken again from 12-17 September when the artificial substrates were removed and after the flow had been at more normal summer values.

5.1 Zooplankton Populations

Table 5-1 lists the taxa and density of organisms for the three replicate samples at each station. Density at RK 20.1, 25.7, 51.8 and 61.3 are up to three times greater than some of the other upstream stations and there is an abrupt drop in density at the stations just upstream of RK 61.3. This change is not reflected in the number of taxa which is the same and at the

maximum for RK 51.8, RK 61.3, RK 66R and RK 68.4. There is no conspicuous reason for a drop in density. RK 66 is over 40 kilometers downstream of the Marmet Dam with no obvious change in the river in that reach. The Pocatalico River enters between stations 61.3 and 66.0.

The trend in number of taxa is shown in Figure 5-1 which shows a slight downward trend from downstream to upstream, excepting the two lower most stations (these two had a higher density). Tributary inputs to the mainstream Kanawha River are relatively small, the stream is totally in pool between the dams and a decreasing stream size or shorter residence time does not seem a likely cause. The 50 percent or more decrease in number of taxa from Stations 51.8, 61.3, 66L and 66R and 68.4 to Station 87.4L and 87.4R to 108.9 is certainly not a result of stream size or residence time.

While the change in density from downstream to upstream is bigger than in number of taxa, both show a similar trend.

Table 5-1. Density of Zooplankton Collected from Various Ambient Stations of the Kanawha River, Charleston, West Virginia, September 1984 (Number per 100 ml)

	20.1			25.7			51.8			61.3		
	A	B	C	A	B	C	A	B	C	A	B	C
<i>Brachionus calycifloris</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus quadridentatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euchlanis</i> sp.	0	0	0	0	0	0	0	0	0	0.5	0	0
<i>Platyras quadricornis</i>	0	0	0	0	0	0	0.5	0	0	0	0	0
<i>Lecane</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna</i> sp.	0	0	0	0	0	0	0	0	0.5	1.5	0	0
<i>Diaphanosoma</i> sp.	10.5	8.5	1.0	8.5	5.0	3.5	21.0	20.5	15.0	4.5	1.5	5.0
<i>Daphnia</i> sp.	2.0	2.0	1.0	0.5	1.0	0.5	0.5	0.5	1.0	0.5	0	0
<i>Ceriodaphnia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina</i> sp.	29.5	25.5	12.5	3.5	8.5	5.5	3.5	2.5	4.0	8.5	2.0	1.0
<i>Ilyocryptus</i> sp.	0.5	0.5	1.0	1.0	0.5	0.5	0	0	0.5	8.0	1.0	0
<i>Chydorinae</i>	0	0	0	0.5	0	0	0	0	0	0	0	0
<i>Camptocercus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alona</i> sp.	1.5	0.0	1.5	2.0	0.5	1.0	0.5	0.5	0	4.5	1.0	0.5
<i>Diaptomus</i> sp.	76.0	31.5	18.5	7.5	27.5	16.0	6.0	5.0	3.0	4.0	2.0	3.0
<i>Cyclops</i> sp.	12.5	15.5	9.0	7.0	8.5	9.0	6.5	5.5	2.5	24.5	1.5	0.5
<i>Encyclops</i> sp.	0	0	0	0	0	0	2.5	1.5	1.0	12.0	4.5	2.5
<i>Leptodora kindtii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Branchiura</i>	0	0	0	0	0	0	0	0	0	0	0	0
Total taxa per station		7		7			10			10		

Table 5-1. (Continued)

	66.0L			66.0R			68.4			71.1		
	A	B	C	A	B	C	A	B	C	A	B	C
<i>Brachionus calycifloris</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus quadridentatus</i>	0	0	0	0	0	0	0.5	0	0	0	0	0
<i>Euchlanis</i> sp.	1.0	0.5	0.5	0	2.0	0.5	1.0	0.5	0.5	0	0	0
<i>Platylas quadricornis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna</i> sp.	0	0	0	0	0	0.5	0	0	0	0	0	0
<i>Diaphanosoma</i> sp.	1.0	2.0	0.5	1.0	5.0	1.0	0.5	1.0	1.0	0.5	0	0
<i>Daphnia</i> sp.	0	0	0	0	0.5	0	0	0	0.5	0	0	0
<i>Ceriodaphnia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina</i> sp.	0.5	1.5	1.5	2.0	1.5	0	2.0	0.5	3.5	1.0	0	0
<i>Ilyocryptus</i> sp.	0	1.0	0	0	0	0	0	0	0	0	0	0.5
<i>Chydorinae</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Camptocercus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alona</i> sp.	1.0	0	3.0	0	0	0.5	0	0	0.5	2.0	0.5	0.5
<i>Diaptomus</i> sp.	1.5	1.0	0.5	2.5	2.0	2.5	1.5	1.5	3.0	1.5	1.0	0.5
<i>Cyclops</i> sp.	2.5	2.5	1.5	0	1.5	0	1.0	1.5	1.5	0.5	0	0
<i>Eucyclops</i> sp.	2.5	1.0	1.5	1.5	2.5	1.5	2.0	1.5	1.5	3.5	2.5	2.0
<i>Leptodora kindtii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Branchiura</i>	0	0.5	0	0.5	0	0.5	0	0.5	0	0	0	0
Total taxa per station		9			10			10		7		

Table 5-1. (Continued)

	76.1L			76.1R ¹			80.5			84.5		
	A	B	C	A	B	C	A	B	C	A	B	C
<i>Brachionus calycifloris</i>	0	0	0	0	0		0	0	0	0	0	0
<i>Brachionus quadridentatus</i>	0	0	0	0	0		0	0	0	0	0	0
<i>Euchlanis</i> sp.	0.5	0	0	0.5	0		0	0.5	0	0	0	0
<i>Platylas quadricornis</i>	0	0	0	0	0		0	0	0	0	0	0
<i>Lecane</i> sp.	0	0	0	0	0		0	0	0	0	0	0
<i>Asplanchna</i> sp.	0	0	0	0	0		0	0	0	0	0	0
<i>Diaphanosoma</i> sp.	0.5	0	0.5	0	0		0	0	0.5	0.5	0.5	0
<i>Daphnia</i> sp.	0	0	0	0	0		2.5	2.0	3.5	0	0	0
<i>Ceriodaphnia</i> sp.	0	0	0	0	0		0	0	0	0	0	0
<i>Bosmina</i> sp.	1.0	0	0	0.5	0.5		0	0	0	0	0	0
<i>Ilyocryptus</i> sp.	0	0	0.5	0.5	0		0	0	0	0	0.5	0
<i>Chydorinae</i>	0	0	0	0	0		0	0	0	0	0	0
<i>Camptocercus</i> sp.	0	0	0	0	0		0	0	0	0	0	0
<i>Alona</i>	1.5	0	0	0.5	0		0	0	0	0.5	0	0
<i>Diaptomus</i> sp.	1.5	2.0	1.0	1.0	2.5		2.0	0.5	0	0	0.5	0.5
<i>Cyclops</i> sp.	0	0	0	0	0		0	0.5	0	1.0	1.5	0.5
<i>Eucyclops</i> sp.	2.5	1.5	4.0	2.5	0.5		1.5	0.5	0	3.0	0.5	0.5
<i>Leptodora kindtii</i>	0	0	0	0	0		0	0	0	0	0	0
<i>Branchiura</i>	0	0	0	0.5	0		0	0.5	0	0	0	0
Total taxa per station		7			7			7		7		

¹Replicates A and C were totaled together and listed under A. due to error in counting

Table 5-1. (Continued)

	87.4L			87.4R			88.0L			88.0R		
	A	B	C	A	B	C	A	B	C	A	B	C
<i>Brachionus calycifloris</i>	0	0	0	0.5	0	0	0	0	0	0	0	0
<i>Brachionus quadridentatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euchlanis</i> sp.	0	0	0	0.5	1.0	0	0	0	0	0.5	0.5	1.0
<i>Platylas quadricornis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> sp.	0	0.5	0	0	0	0	0.5	0	0.5	0	0	0
<i>Daphnia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina</i> sp.	1.0	0	0	0	0	0	0	0	0	0	0	0
<i>Ilyocryptus</i> sp.	0.5	0.5	0	0	0	0	0	0	0	0	0.5	1.0
<i>Chydorinae</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Camptocercus</i> sp.	0	0	0	0	0	0	0	1.0	0	0	0	0
<i>Alona</i> sp.	0	0.5	0	0	0	0	1.0	0.5	0.5	0	1.0	2.5
<i>Diaptomus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclops</i> sp.	0	0	0	0	0	0	0	0	0.5	0	0	0
<i>Eucyclops</i> sp.	1.0	1.0	1.5	1.0	0.5	0	0	0.5	0	1.5	1.0	2.5
<i>Leptodora kindtii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Branchiura</i>	0	0	0	0	0	0	0	0	0	0	0	0
Total taxa per station		5			3			5			4	

Table 5-1. (Continued)

	90.4			92.5L			92.5R			94.1		
	A	B	C	A	B	C	A	B	C	A	B	C
<i>Brachionus calycifloris</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus quadridentatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euchlanis</i> sp.	0	0.5	0.5	0.5	1.0	0	0	0.5	1.0	1.0	0.5	1.0
<i>Platylas quadricornis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> sp.	0	0	0	0.5	0	0	0	0	0	0	0	0
<i>Daphnia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ilyocryptus</i> sp.	0	0	0	1.0	0	0	2.0	0	1.5	0	0	0.5
<i>Chydorinae</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Camptocercus</i> sp.	0	0	0	0	0	0	0	0	0.5	0	0	0
<i>Alona</i> sp.	0	0	0.5	3.0	0	0	2.0	0.5	0	0.5	0.5	0.5
<i>Diaptomus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclops</i> sp.	0	0.5	0	0	0	0	0	0	0	0	0	0
<i>Eucyclops</i> sp.	0	0	2.0	2.0	0	1.5	1.5	1.0	1.0	1.5	0.5	1.5
<i>Leptodora kindtii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Branchiura</i>	0	0	0	0	0	0	0	0	0	0	0	0
Total taxa per station		4			5			5			4	

Table 5-1. (Continued)

	99.1			101.4			105.2			108.9		
	A	B	C	A	B	C	A	B	C	A	B	C
<i>Brachionus calycifloris</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus quadridentatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euchlanis</i> sp.	0	0	0	0	0	0	0	0.5	1.5	0.5	0	0.5
<i>Platylas quadricornis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> sp.	0	0	0	0.5	0	0	0	0	0	5.5	1.5	2.5
<i>Daphnia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina</i> sp.	0	0	0	0	0	0	1.0	2.0	0.5	0.5	1.5	1.0
<i>Ilyocryptus</i> sp.	0.5	1.5	0	0	0	0	0	0.5	0	0	0.5	0
<i>Chydorinae</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Camptocercus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alona</i> sp.	2.5	0	1.0	0	1.0	1.0	0	0.5	0.5	2.5	1.5	1.5
<i>Diaptomus</i> sp.	0	0	0	0	0.5	0.5	0	0	0	0.5	0	0
<i>Cyclops</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eucyclops</i> sp.	0.0	0.5	1.5	1.0	1.5	0	0.5	1.0	1.0	1.5	1.0	1.5
<i>Leptodora kindtii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Branchiura</i>	0.5	0	0	0	0	0	0	0	0	1.5	1.5	0.5
Total taxa per station		4			4			5			8	

Table 5-1. (Continued)

	112.0			114.2			118.4			125.5		
	A	B	C	A	B	C	A	B	C	A	B	C
<i>Brachionus calycifloris</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus quadridentatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euchlanis</i> sp.	0.5	0	0.5	1.0	0.5	0	1.5	0	0	0	0	0.5
<i>Platylas quadricornis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> sp.	0.5	0	0	0	0	0	0	0	0	0	0.5	1.5
<i>Daphnia</i> sp.	0	0	1.0	0	0	0	0	0	0	0.5	1.5	1.0
<i>Ceriodaphnia</i> sp.	1.0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina</i> sp.	0	0	0	0	0	0	0.5	0	0.5	0	0	0
<i>Ilyocryptus</i> sp.	2.5	1.0	1.0	0	0	0.5	0.5	0.5	0.5	0.5	0	0.5
<i>Chydorinae</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Camptocercus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alona</i> sp.	3.0	1.5	1.5	1.0	0	0.5	1.5	1.0	8.0	0.5	1.0	0
<i>Diaptomus</i> sp.	0	0	0	0.5	0	0	0	0	0	0	0	0
<i>Cyclops</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0.5
<i>Eucyclops</i> sp.	0	0	1.5	1.0	0.5	0.5	0.5	0	1.0	0.5	0.5	0.5
<i>Leptodora kindtii</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Branchiura</i>	0	0	0	0	0	0	0	0	0	0	0	0
Total taxa per station		7			5			5			7	

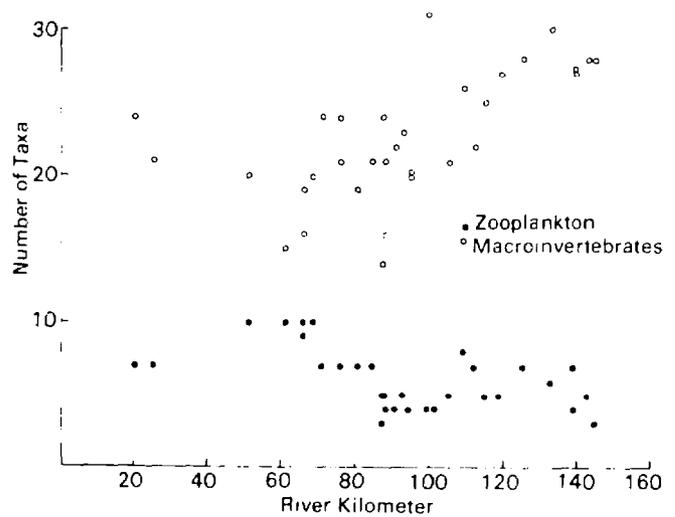
Table 5-1. (Continued)

	133.2			139.0L			139.0R			142.7		
	A	B	C	A	B	C	A	B	C	A	B	C
<i>Brachionus calycifloris</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus quadridentatus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euchlanis</i> sp.	0	0.5	1.0	0	0	0	0.5	0.5	0	0	0	0
<i>Platyias quadricornis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane</i> sp.	0	0	0	0	0	0.5	0	0	0	1.0	0.5	1.0
<i>Asplanchna</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> sp.	5.0	1.5	2.0	0	0	0	0	0	0	0	0	0
<i>Daphnia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina</i> sp.	0	0	0	0.5	0	0	0	0	0	0	0	0
<i>Ilyocryptus</i> sp.	0	0.5	0	0	0	0.5	0.5	0	0	0.5	0	0.5
<i>Chydorinae</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Camptocercus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alona</i> sp.	0	0.5	0	0	0	0	0	0	0	1.0	0	0
<i>Diaptomus</i> sp.	0	0	0	0	2.5	0	0.5	0.5	0.5	0	0.5	0
<i>Cyclops</i> sp.	0	0	0	0.5	0	0	0	0	0	0	0	0
<i>Eucyclops</i> sp.	0.5	0.0	0.5	0	0.5	1.5	1.0	0.5	0.5	0.5	0	0.5
<i>Leptodora kindtii</i>	0	0	1.0	0	0	0	0	0	0	0	0	0
<i>Branchiura</i>	0	0	0	0	0	0	0	0	0	0	0	0
Total taxa per station		6			7			4			5	

Table 5-1. (Continued)

	145.0		
	A	B	C
<i>Brachionus calycifloris</i>	0	0	0
<i>Brachionus quadridentatus</i>	0	0	0
<i>Euchlanis</i> sp.	0.5	0	0
<i>Platyias quadricornis</i>	0	0	0
<i>Lecane</i> sp.	0.5	1.0	0
<i>Asplanchna</i> sp.	0	0	0
<i>Diaphanosoma</i> sp.	0	0	0
<i>Daphnia</i> sp.	0	0	0
<i>Ceriodaphnia</i> sp.	0	0	0
<i>Bosmina</i> sp.	0	0	0
<i>Ilyocryptus</i> sp.	0	0	0
<i>Chydorinae</i>	0	0	0
<i>Camptocercus</i> sp.	0	0	0
<i>Alona</i> sp.	0	0	0
<i>Diaptomus</i> sp.	0	0	0
<i>Cyclops</i> sp.	0	0	0
<i>Ducyclops</i> sp.	0	1.5	1.5
<i>Leptodora kindtii</i>	0	0	0
<i>Branchiura</i>			
Total taxa per station		3	

Figure 5-1. Number of zooplankton and macroinvertebrate taxa at various stations, Kanawha River.



6. Periphyton Community Survey

6.1 Chlorophyll *a* and Biomass Measurements

Samples for chlorophyll *a* and biomass were collected on 12-16 September 1984. The artificial substrates had been submerged at varying water depths due to large changes in river stage caused by the early rains. Appendix B describes the sampling techniques.

Variability was large both between replicates at a station and between stations (Table 6-1). In general, higher values for chlorophyll *a* were obtained at

Station 99.1 and upstream except for Stations 20.1 and 25.7. Mean concentrations were $> 1.6 \text{ mg/m}^2$ chlorophyll *a* in this reach of the Kanawha River, whereas downstream of Station 99.1, the mean concentration for 40 percent of the stations was $\leq 0.5 \text{ mg/m}^2$ chlorophyll *a* (Table 6-2).

Periphyton biomass, measured as ash-free dry weight (AFDW), varied from 0.051 to 8.577 g/m^2 (Table 6-1). Similar to the chlorophyll *a* data, lowest values occurred at Station 88.0L and highest values occurred at Station 20.1. Such similarities are expected since chlorophyll *a* and AFDW measure algal biomass.

Table 6-1. Replicate Chlorophyll *a*, Biomass, and Autotrophic Index Values for Periphyton Collected from Artificial Substrates in the Kanawha River, West Virginia, September 1984

Station (RK)	Chlorophyll <i>a</i> (mg/m^2)			Biomass (g m^{-2})			Autotrophic Index		
	A	B	C	A	B	C	A	B	C
20.1	73.168	13.794	8.208	8.577	4.107	3.121	117	298	380
25.7	0.352*	49.281	5.825	0.577	5.755	1.844	1,639	117	317
51.8	0.402	1.325	0.084*	0.551	0.991	0.695	1,371	748	8,274
61.3	2.465	1.349	0.996	1.165	0.908	0.904	473	673	908
66.0L	0.692	--	--	1.709	--	--	2,470	--	--
66.0R	4.641	2.626	0.789	1.910	1.876	1.948	412	714	2,469
68.4	0.818	0.143*	0.147*	1.020	1.434	1.746	1,247	10,028	11,878
71.1	0.957	15.919	0.352*	2.096	2.734	1.201	2,190	172	3,412
76.1L	0.085*	0.137*	0.335*	1.107	1.881	2.550	13,024	13,730	7,612
76.1R	3.678	2.373	2.640	1.720	1.233	1.220	468	520	462
80.5	0.554	0.623	0.363	1.143	0.873	1.086	2,063	1,401	2,992
84.5	0.048*	0.057*	0.068*	0.311	0.689	0.444	6,479	12,088	6,529
87.4L	0.044*	0.051*	--	0.362	0.519	--	8,227	10,176	--
87.4R	4.778	0.089*	0.258*	0.458	0.314	0.052	96	3,528	202
88.0R	0.253*	0.175*	--	0.661	0.261	--	2,613	1,491	--
88.0L	0.013*	0.007*	0.022*	0.102	0.051	0.150	7,846	7,286	6,818
90.4	0.211*	0.226*	--	0.261	0.198	--	1,237	876	--
92.5L	0.106*	0.550	0.024*	0.100	0.360	0.054	943	655	2,250
92.5R	0.486	0.093*	0.103*	0.273	0.351	0.198	562	3,744	1,922
94.1	0.851	1.216	--	0.425	0.606	--	499	498	--
99.1	0.869	1.519	12.508	0.921	1.326	2.289	1,060	873	183
101.4	--	--	--	--	--	--	--	--	--
105.2	22.026	--	--	2.187	--	--	99	--	--
108.9	8.189	8.873	9.872	1.430	1.297	1.568	175	146	159
112.0	2.308	3.325	1.370	0.508	0.995	0.666	220	299	486
114.2	16.270	8.967	24.994	1.971	1.600	5.039	121	178	202
118.4	3.326	2.299	3.073	1.060	0.803	0.766	319	349	249
125.5	1.240	3.112	0.457*	0.876	0.825	0.486	706	265	1,063
133.2	4.021	5.943	2.874	1.193	1.263	1.024	297	213	356
139.0L	3.629	8.669	7.807	0.905	2.113	1.287	249	244	165
139.0R	1.231	2.499	4.421	0.645	0.779	0.898	524	312	203
142.7	15.538	11.262	7.320	3.404	2.081	1.447	219	185	284
145.0	14.096	4.172	7.674	2.563	0.976	1.538	182	234	200

Notes. Asterisk (*) indicates chlorophyll *a* value based on fluorometric analysis. Dash (--) indicates artificial substrate(s) missing.

Mean values of AFDW vary from 0.101 to 5.268 g/m²; however, no trends are observed.

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 AI values were ≥ 160 at all stations except Station 105.2. Such high AI values indicate that the periphyton community is dominated by either non-algal (heterotrophic) taxa or nonliving organic matter.

6.2 Evaluation of the Periphytic Community

High mean chlorophyll *a* values were found at the two most downstream stations (20.1 and 25.7) and above Station 94.1 (Table 6-2). Very high AI values for the stations located between Stations 25.7 and 94.1 indicate that the periphyton community was either non-algal or nonliving. The material causing the high AI values could be the same as the fungus-like material observed in the toxicity tests.

Table 6-2. Mean Chlorophyll *a* and Biomass Standing Crops and Autotrophic Index Values for Periphyton Collected from Artificial Substrates in the Kanawha River, West Virginia, September 1984

Station (RK)	Chlorophyll <i>a</i> (mg · m ⁻²)	Biomass (g · m ⁻²)	Autotrophic Index
20.1	31.723	5.268	265
25.7	18.486	2.725	691
51.8	0.604	0.746	3,464
61.3	1.603	0.992	685
66.0L	0.692	1.709	2,470
66.0R	2.685	1.911	1,198
68.4	0.369	1.400	7,718
71.1	5.743	2.010	1,925
76.1L	0.186	1.846	11,455
76.1R	2.897	1.391	483
80.5	0.513	1.034	2,152
84.5	0.058	0.481	8,365
87.4L	0.048	0.440	9,226
87.4R	1.708	0.275	1,309
88.0R	0.214	0.461	2,052
88.0L	0.014	0.101	7,317
90.4	0.218	0.230	1,056
92.5L	0.227	0.171	1,283
92.5R	0.227	0.274	2,086
94.1	1.034	0.516	498
99.1	4.965	1.512	705
101.4	--	--	--
105.2	22.026	2.187	99
108.9	8.978	1.432	160
112.0	2.334	0.723	335
114.2	16.744	2.870	167
118.4	2.899	0.876	306
125.5	1.603	0.729	678
133.2	4.279	1.160	289
139.0L	6.702	1.435	219
139.0R	2.717	0.774	346
142.7	11.373	2.311	229
145.0	8.647	1.692	205

Note: Dash (--) indicates artificial substrate was missing.

7. Macroinvertebrate Community Survey

The macroinvertebrates were measured using artificial substrate samples suspended for approximately four weeks. The water samples for zooplankton and toxicity testing were taken in close proximity to the substrate samplers. The substrates were placed between 14-20 August and were recovered from 12-17 September. They were located out of barge traffic lanes and typically 5-20 meters from shore. Where discharges occurred but were not obviously fully mixed, stations on each side of the river were established. The collection techniques are described in Appendix B.3.

7.1 Macroinvertebrate Populations

The number of taxa (Table 7-1 and Figure 5-1) show a rather clear increase from downstream to upstream. The station with the highest (99.1) and lowest number of taxa (87.4L) are less than 12 kilometers apart, however. The station (RK 87.4L) with the lowest number of taxa, 14, is located downstream of Blaine's Island, an area of a high concentration of discharges. The total number of organisms collected at RK 87.4 was much lower compared to Station 99.1.

The use of artificial substrates reduces habitat effects. Therefore, decreasing numbers downstream would seem to be water quality caused. This is reinforced by the close proximity of stations with high and low numbers of taxa. In contrast to the zooplankton, the four most downstream stations are not markedly higher than the stations just upstream of these four stations.

The trends shown by the macroinvertebrates are definitely different than the trend of the zooplankton.

Table 7-1. Numbers of Macroinvertebrates From Artificial Substrates in the Kanawha River, Charleston, West Virginia, August 1984

	20.1			25.7			51.8			61.3		
	A	B	C	A	B	C	A	B	C	A	B	C
Stenonema	5	20	15	19	26	14	4	20	12	10	8	14
Caenis	4	1	4	2		6	3	4	1			
Tricorythodes	17	5	5	1	3	3	4	2		11	2	3
Isonychia												
Baetis												
Neureclipsis	54	170	58	21	54	43	38	47	89	56	157	56
Hydropsyche												
Cheumatopsyche												
Polycentropsis	2											
Hydroptila	13	3	2									
Small cased caddis												
Heleidae												
Atherix	1											
Hemerodromia												
Chironomus							1		2			
Pseudochironomus												
Tribelos	3		7	78	67	14	19	7	35			
Dicrotendipes	158	165	125	226	135	272	148	181	135	45	99	43
Glyptotendipes	90	22	118				11	32	25	15	12	2
Polypedium	42	12	10	3	6	8	6	5	11	5	4	4
Micropsectra	6		3		3		15	12	4	8	51	8
Rheotanytarsus	22	8		4	3	2		2	1	3		1
Tanytarsus	21	13	9	1		4	5	10	8	3	4	2
Cricotopus	18	2										
Psectrocladius	20	54	12	1	10	2	16	15	14	8	26	
Corynoneura	4	4	1		1		1			2		
Nanocladium												
Ablabesmyia	18	33	19	10	19	28	24	39	23	7	15	10
Labrundinia												
Tanypus		1										
Neohermes												
Acroneuria												
Optioservus												
Argia				1								
Didymops				1		1						
Ostracoda	2		1					3	7		1	
Hyalella							1					
Gammarus	5	2	2		1	1					5	
Physa												
Gyrinus												
Spherium		7			1							
Ferrissima							1					
Hydracarina												
Hydra												
Hirudinae												
Oligochaete	127	18	14	12	7	5	14	23	28	3	14	5
Planaria				2	2	2	1					
Nematoda		1				2		2	1			
Cryptochironomus					1							
Procladius												
Metrionemus												
Total taxa per station		24			21			20			15	

Table 7-1. (Continued)

	66.0L			66.0R			68.4			71.1		
	A	B	C	A	B	C	A	B	C	A	B	C
<i>Stenonema</i>	4			3	2	2	2	3	7	1	5	3
<i>Caenis</i>	2			3	2	3		4	7	6	5	1
<i>Tricorythodes</i>	2			8	8	9	3	1	6	7	5	5
<i>Isonychia</i>												
<i>Baetis</i>											1	
<i>Neureclipsis</i>	55			44	35	84	162	66	59	63	74	89
<i>Hydropsyche</i>												
<i>Cheumatopsyche</i>						1						
<i>Polycentropsis</i>	1					1						
<i>Hydroptila</i>												
Small cased caddis												
Heleidae							1					
<i>Atherix</i>										1	1	
<i>Hemerodromia</i>												
<i>Chironomus</i>												
<i>Pseudochironomus</i>												
<i>Tribelos</i>				2				26	17	21	2	1
<i>Dicrotendipes</i>	64			83	79	80	24	107	48	127	93	23
<i>Glyptotendipes</i>				21		3		11	28	19	6	4
<i>Polypedilum</i>	13			18	24	7	5	2	6	23	10	4
<i>Micropsectra</i>	22			30	40	16	8	6	5	4	4	9
<i>Rheotanytarsus</i>	4			11	15	6				3	2	
<i>Tanytarsus</i>	3			38	50	20		13	10	20	87	5
<i>Cricotopus</i>				65	18	5			6		10	
<i>Psectrocladius</i>	33			26	23	20	7	1	2	18	42	17
<i>Corynoneura</i>	3			3	4	2				1	6	2
<i>Nanocladius</i>											1	
<i>Ablabesmyia</i>	17			11	10	11	17	27	15	34	28	35
<i>Labrundinia</i>	1						1					
<i>Tanytus</i>												
<i>Neohermes</i>												
<i>Acroneuria</i>												
<i>Optioservus</i>												
<i>Argia</i>										1	1	
<i>Didymops</i>				1								
Ostracoda										1	2	
<i>Hyalella</i>												
<i>Gammarus</i>												
<i>Physa</i>												
<i>Gyrinus</i>												
<i>Spherium</i>							2	1				
<i>Ferrissima</i>												
<i>Hydracarina</i>												
<i>Hydra</i>										1		
Hirudinae												
<i>Oligochaete</i>	19			125	61	41	30	23	28	32	96	60
<i>Planaria</i>	1								1			1
<i>Nematoda</i>							1				1	
<i>Cryptochironomus</i>												
<i>Procladius</i>												
<i>Metriemus</i>												
Total taxa per station		16			19			20			24	

Table 7-1. (Continued)

	76.OL			76.OR			80.5			84.5L		
	A	B	C	A	B	C	A	B	C	A	B	C
Stenonema	6	7		6	9	4	7	6	12	3	2	5
Caenis	5	3	1	3	3		3	3	4	12	5	8
Tricorythodes	2	1	1	1	11	4	6	5	9	1	4	12
Isonychia												
Baetis												
Neureclipsis	50	96	43	32	28	94	46	34	30	23	23	33
Hydropsyche												
Cheumatopsyche												
Polycentropis												
Hydroptila						1						
Small cased caddis												
Heleidae						1						
Atherix										2	1	1
Hemerodromia										1		
Chironomus			2									
Pseudochironomus												
Tribelos	34	6	36	1	1			4	4	3		16
Dicrotendipes	81	36	49	89	151	50	16	25	33	26	9	30
Glyptotendipes				9	5	8		12	2			
Polypedium	4	4	6	17	16	15	13	28	28	33	36	9
Micropsectra	5	5	9	7	15	8	40	4	46	32	42	23
Rheotanytarsus		1	2	28	3	8	34	22	13	14	21	14
Tanytarsus	15	19	33	2	39	16	7	87	8	11	21	25
Cricotopus	2	1	2	3	8		3	16	4	8	2	7
Psectrocladius	8	18	8	11	22	19	32	15	33	5	8	5
Corynoneura		1		2	9	1	8	7	6		5	4
Nanocladius												
Ablabesmyia	33	41	36	16	18	33	14	19	14	6	11	12
Labrundinia							1		2		1	
Tanypus												
Neohermes												
Acroneuria												
Optioservus												
Argia	1			1								
Didymops	2			1		1				1	1	
Ostracoda	1		1									
Hyalella		1	1									
Gammarus								1				
Physa												
Gyprinus												
Spherium												
Ferrissima		2										
Hydracarina												
Hydra												
Hirudinae								2				
Oligochaete	25	68	52	64	148	83	75	119	151	20	57	34
Planaria		3	3	3	2	2						
Nematoda		3									1	
Cryptochironomus			1								1	
Procladius												
Metrionemus												
Total taxa per station		24			21			19			21	

Table 7-1. (Continued)

	87.4L			87.4R			88.0R			88.0L		
	A	B	C	A	B	C	A	B	C	A	B	C
Stenonema	1	1		7	24	16	58	38			1	
Caenis	1	1		7	6	14	11	3		2	3	
Tricorythodes	1			11	21	8	16	13				
Isonychia												
Baetis					1							
Neureclipsis	24	43		24	25	8	40	20		16	17	16
Hydropsyche						1						
Cheumatopsyche				3	2	3		1				
Polycentropis						1	1	1				
Hydroptila												
Small cased caddis												
Heleidae												
Atherix					1			1				
Hemerodromia						1		1				
Chironomus												
Pseudochironomus												
Tribelos	12	1			6		8	1				
Dicretendipes	50	12		5	21	17	15	4		17	2	12
Glyptotendipes	2	2		3		3	2	1		98	53	77
Polypedilum	5	6		99	46	70	14	28		9		
Micropsectra	22	5		100	16	22	75	47			5	2
Rheotanytarsus				6	33	25	18	10		2	6	18
Tanytarsus	16	4		75	15	48	6	28				
Cricotopus	12	1		5	21	12	2	8		16	9	19
Psectrocladius				8			5			1		
Corynoneura				7	12	7	5	6		1	1	
Nanocladius						2						
Ablabesmyia	7	10		4	6	8	9	2				
Labrundinia										5	16	4
Tanypus												
Neohermes												
Acroneuria												
Optioservus												
Argia		1										
Didymops										1		
Ostracoda										1	1	
Hyalella										1		
Gammarus												
Physa												
Gyprinus												
Spherium												
Ferrissima												
Hydracarina												
Hydra												
Hirudinae												
Oligochaete	29	8		18	24	22	2	8		7	16	18
Planaria												
Nematoda				1	1		1					
Cryptocnironomus												
Procladius												
Metriionemus												
Total taxa per station		14			24			21			16	

Table 7-1. (Continued)

	90.4			92.5L			92.5R			94.1		
	A	B	C	A	B	C	A	B	C	A	B	C
Stenonema	55	36		8	45	62	14	10	9	7	14	
Caenis	10	6		12	15	6	44	22	12	9	9	
Tricorythodes	21	12		17	23	16	22	12	7	6	8	
Isonychia												
Baetis							2					
Neureclipsis	33	14		16	23	24	13	25	36	20	23	
Hydropsyche												
Cheumatopsyche						1						
Polycentropis					1							
Hydroptila												
Small cased caddis												
Heleidae									1			
Atherix												
Hemerodromia	2											
Chironomus		1										
Pseudochironomus												
Tribelos				1			4	30	45	9	6	
Dicrotendipes				10	1	1	37	31	53	49	34	
Glyptotendipes	25	24		42	45	19				5	1	
Polypedilum							11	10	12	9	15	
Micropsectra	21	34		12	30	68	18	12	53	27	43	
Rheotanytarsus	37	41		47	77	43	21		13	12	12	
Tanytarsus	67	8		27	16	14	122	26	9	69	28	
Cricotopus	16	48		35	61	73	13	1	5	4	3	
Psectrocladius	3	21		1	11	9	14	4	4	11	5	
Corynoneura	23	2		3	14	14	6		8	3	1	
Nanocladius	6	17		12	19	12						
Ablabesmyia		5				3	9	18	21	7	9	
Labrundinia	18	3		18	7	1						
Tanytus	1					1						
Neohermes												
Acroneuria												
Optioservus								4				
Argia	1									2		
Didymops							1				1	
Ostracoda	3	1		1					1	1	2	
Hylella				1								
Gammarus												
Physa												
Gyprinus												
Sphertum												
Ferrissima												
Hydracarina												
Hydra												
Hirudinae							1					
Oligochaete	20	17		53	90	12	247	16	34	114	138	
Planaria	1								4	4	3	
Nematoda		2				2	1		1			
Cryptochironomus												
Procladius												
Metriemus												
Total taxa per station		22			23			23			20	

Table 7-1. (Continued)

	99.1			101.4			105.2			108.9		
	A	B	C	A	B	C	A	B	C	A	B	C
Stenonema	26	11	31				52			2	3	
Caenis	8	2	4							5	3	10
Tricorythodes	3	3	11				17			3	2	2
Isonychia			1				1					
Baetis			3				3			1		1
Neureclipsis	29	27	30				16			11	4	8
Hydropsyche							25					
Cheumatopsyche	1		2				13					
Polycentropis	1											
Hydroptila			5				5				1	
Small cased caddis												
Heleidae												
Atherix	2						2					
Hemerodromia			3				1					
Chironomus												
Pseudochironomus	2		3									
Tribelos	11	20	3								6	6
Dicrotendipes	51	80	60				14			20	39	17
Glyptotendipes		1								3	5	4
Polypedilum	23	1	60				93			44	47	24
Micropsectra	113	50	90				79			78	40	42
Rheotanytarsus	7	1	23				33			1	13	
Tanytarsus	37	21	31				21			67	105	38
Cricotopus	32	8	24				37			22	36	7
Psectrocladius	12	4	11							8	14	6
Coryoneura	6	3	9				14			2	3	
Nanocladius	1											
Ablabesmyia	4	18	5				3			6	6	2
Labrundinia										1	1	
Tanypus												
Neohermes												
Acroneuria												
Optioservus			1									
Argia												
Didymops	1		1				1					
Ostracoda	1	3								3	4	10
Hyalella												6
Gammarus												
Physa												1
Gyprinus										1	12	6
Spherium												
Ferrissima		2									1	
Hydracarina												
Hydra		2								5		
Hirudinae												
Oligochaete	29	89	58				4			42	248	184
Planaria	3	2								2	11	45
Nematoda	1						2					
Cryptochironomus												
Procladius												
Metrionemus												
Total taxa per station		32					21				26	

Table 7-1. (Continued)

	112			114.2			119.1			125.5		
	A	B	C	A	B	C	A	B	C	A	B	C
Stenonema	5	5	12	7	7	6	28	23	28	19	19	24
Caenis	2	5	5	4	3	3	8	15	15	4	3	6
Tricorythodes	2			2	6	1	8	14	7	10	10	12
Isonychia										1	1	1
Baetis				2	2	1	1			1	3	
Neureclipsis	40	65	35	3	10	4	18	15	17	19	15	15
Hydropsyche												
Cheumatopsyche							1		1			3
Polycentropis									1			
Hydroptila				4		1						
Small cased caddis					2						2	
Heleidae												
Atherix												
Hemerodromia					1	1						
Chironomus											1	
Pseudochironomus												
Tribelos	1									6	7	5
Dicrotendipes					1					9	61	41
Glyptotendipes	13		16	5	13	4		4	5	1	1	
Polypedilum	75	44	87	94	123	91	15	59	24	23	40	41
Micropsectra	11				2				1	30	123	18
Rheotanytarsus	7	2	1	14	21	7	11	21	25	50	10	31
Tanytarsus	21	11	16	32	61	17	71	35	97	39	34	85
Cricotopus	7			2		17	12	26	7	4	13	7
Psectrocladius	12	3	10	43	34	30	21	109	43	3	3	1
Corynoneura		1		8		4		3	9	3	3	9
Nanocladius	2					2	4	3	5		5	
Ablabesmyia	1			5	2				3	8	8	3
Labrundinia								1	3		2	
Tanypus	7	5	8	11	12	6	6	19	7			
Neohermes					1	1	1					
Acroneuria												
Optioservus												
Argia	1							1				
Didymops		1										
Ostracoda											2	1
Hyalella												
Gammarus	3	2		9	13	24	2	3	1			
Physa												
Gyrinus												
Spherium											2	1
Ferrissima												
Hydracarina	1								2			
Hydra	1											
Hirudinae												2
Oligochaete										13	22	26
Planaria									2		1	
Nematoda	19	8	19	6	11	10	6	46	14			
Cryptochironomus				2	1							
Procladius									1			1
Metriemus												
Total taxa per station		22			25			27			28	

Table 7-1. (Continued)

	133.2			139L			139R			142.7			145.0		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Stenonema	6	3	4	4	16	28	18	13	18	10	24	38	16	25	23
Caenis		2	1	1	7	1									3
Tricorythodes	3			6	20	6	13	18	15	19	28	33	10	9	9
Isonychia				1	1		1			1	1		1		
Baetis	3		1	1	5		1			6	5	4	9	1	4
Neureclipsis	2			7	4	5	1	4	2	12	16	7	1	4	2
Hydropsyche				1											
Cheumatopsyche															
Polycentropis						1	1	1	3						
Hydroptila												1	2		1
Small cased caddis															
Heleidae															
Atherix												1			
Hemerodromia															
Chironomus						3	1	1					1		
Pseudochironomus		1													1
Tribelos	25	14	13	2	4	15	52	42	54	17	9	3	36	13	38
Dicrotendipes	32	41	23	61	154	87	79	28	24	56	51	80	97	108	153
Glyptotendipes		3						1							
Polypedium	13	21	15	26	4	30	32	3	7	26	47	29	30	6	17
Micropsectra	43	17	30	66	72	68	50	19	15	73	76	75	19	18	41
Rheotanytarsus		7	14				6			15	8	8			
Tanytarsus	20	28	15	34	17	19	34	3	16	17	5	35	20	22	18
Cricotopus	5	2	13	9		4	8		1	9	16	9	15	3	3
Psectrocladius						5	2		1	1	4	4	3		2
Corynoneura	2	2	1	2	3	2	1			10	14	13	2	1	1
Nanocladius			1			2						6			1
Ablabesmyia	12	6	4	16	15	23	11	8	5	20	16	15	14	18	26
Labrundinia	2									1		1	1		1
Tanytus	1														
Neohermes										1					
Acroneuria															
Optioservus							1			1					1
Argia				1		1		1							
Didymops															
Ostracoda	42	9	9	3	12	19	11	5	3	7	5	7	6		12
Hyalella				1	2								5	1	4
Gammarus	1											1			
Physa	6	1													
Gyrinus	8	6	6												22
Spherium								1							1
Ferrissima		4	8			1		1		1			1	2	1
Hydracarina	1														
Hydra	1	7													
Hirudinae															
Oligochaete	129	129	172	17	36	33	76		1	87	73	35	36	12	50
Planaria	51	7	4			2	2		2						
Nematoda		1				1	1								
Cryptochironomus	2			2		6	3					3			4
Procladius															
Metriemus															
Total taxa per station		30				27		27		28			28		

8. **Comparison of Laboratory Toxicity Test Data and Receiving Water Biological Impact**

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 effluent 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 sensitivities of the species in the community are 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. 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 in this study).

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

Compound the above described considerations with multiple discharges as well as inputs from tributaries and non-point sources such as agricultural run-off and leachate from landfills and one should logically expect an unpredictable effect on various components of the community. Figure 5-1 shows two clearly different trends between zooplankton and macro-invertebrates, evidence of the above effects.

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 this report and in the other reports (Mount *et al.*, 1984; Mount, Steen and Norberg-King, 1985), it is clear that there is no set response pattern of the community. 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 *Ceriodaphnia* to resemble each other in sensitivity or 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. They cannot be eliminated.

The on site testing period of this study began at extremely high flows as a result of rains in the upper basin. The apparent oxygen demand of the river water and the fungus problem are discussed in Chapter 4. The flows diminished greatly after the testing period (Table 4-11) and a decision was made to re-run the ambient station toxicity tests. Resources prohibited another on site study in which a new sample could be used each day. Instead, a sample was collected and shipped to ERL-D and was used to renew the test solution daily. This approach is totally dependent on one grab sample being representative whereas the usual procedure of using seven different samples for the 7-day exposure period makes any one sample less important.

For comparisons of the toxicity prediction to the community response, only the September data have been used because those samples were collected at a flow much more representative of the flows that

prevailed during most of the period in which the substrates were in the river and when the zooplankton was sampled. Since the toxicity tests were done on one grab sample taken at the same time as the zooplankton samples, conditions should have been more similar for zooplankton than for the macroinvertebrates which were responding to the preceding four weeks of exposure. For acute effects such as spills or short high concentration exposures, the zooplankton and the toxicity tests should be most closely similar. For chronic effects requiring several days to be manifested, the similarity might be much less.

8.1 Effluent Tests

Due to the large flows involved and the number of discharges, dye dilution measurements were not economically possible. Therefore, the study design was based on a comparison of ambient tests to field biological data and the effluent dilution tests were not necessary to the comparison. Effluents were tested to provide general-type information and to locate any unusually high sources of toxicity. None of the effluents had instream waste concentrations (IWC) after mixing that were greater than the acceptable effluent concentration (AEC). Since AEC's were measured using water from just upstream of each effluent, the dilution waters contained all IWC's of upstream effluents and any effluent interaction (e.g., additivity or antagonism) present is incorporated into the measurement. An exception to this generality occurs wherever several effluents were diluted with the same water. In these cases all IWC's of upstream effluents were not in the dilution water and additive effects if present would not be measured. An example would be the 5 effluents diluted with Station 90.4 water.

8.2 Comparison of Ambient Toxicity to Biological Response

Table 8-1 contains a summary of the number of taxa, young per female for the *Ceriodaphnia* and weights for the fathead minnows for the field and toxicity data. The highest value for each data set was used to calculate the percent reduction for all other values in the set. The highest percent toxicity and the largest percent reduction in number of taxa were then used to develop Table 8-2 which gives the percent of correctly predicted responses using various arbitrary levels of impact/toxicity. Because there were few impact/toxicity values above 60 percent, the percent of correctly predicted stations is high when 60-80 or 80-100 percent levels are compared because these are all no-effect comparisons. The 20-40 percent toxicity level gives approximately 60 percent correct predictions for 20-40 and 40-60 percent levels of the field data. None of the toxicity values were signif-

icantly different from each other, and all were less than 40 percent below the highest value suggesting that any toxicity if present was slight. Any one level of percent impairment is not being proposed as the correct percentage at this time. This study is not sufficient to judge which impairment of instream biological response data will correspond to a specified level of laboratory toxicity. Similar comparisons for all eight study sites (see Foreword) need to be completed before making decisions or recommendations.

One should expect a general but not a point-by-point correlation between amount of toxicity and number of taxa lost. This expectation is *not* due to error in measurement of toxicity of taxa or experimental variation, but is expected because of the different relative sensitivity of test and community species. Added on top of this variability are the confounding effects of measurement error. In addition, there is the chance collection of a few individuals of a species that does not usually occur in that location and these numbers bias the number of taxa found. Events such as toxic spills before the study period could have residual effects on the community which would not be measured by the toxicity tests. General water quality conditions and physical effects, nontoxic in nature, such as low DO, high temperature, or direct activities of man (like gravel removal or dredging) also might have affected the community in the period preceding the study but would not affect the toxicity values.

As discussed by Mount et al. (1985), point-by-point statistical comparisons, such as analyses of variance, may not show significant differences even though definite trends are evident. Figure 5-1 is a plot of the number of taxa vs. river kilometers. There is definitely a decrease in the number of taxa of macroinvertebrates from upstream to downstream. The number of zooplankton appear to be lower in the upper river down to river kilometer 88.0. The two groups do not reflect the same trends.

Some amount of the change from upstream to downstream might be attributed to changing stream flow or tributary recruitment areas. Gradient would not be involved because the entire study reach was in three pools formed by navigation dams. Sharp increases in numbers of taxa as shown from RK 88 to 68, all of which is in one pool, would suggest other causes such as water quality.

Figure 8-1 is a plot of the percent toxicity for *Ceriodaphnia* and the percent reduction in zooplankton taxa for each station. The correlation ($r = 0.728$) between these values suggest that the trends in taxa are due to toxicity of the water. There certainly was addition of organic matter from the many dischargers, including POTW's and this could have enriched the water enough to produce the increase in

Table 8-1. Number of Taxa, Number of *Ceriodaphnia* Young per Female and Fathead Minnow Weights with the Associated Percent Reduction Using the Highest Value of Each as Zero Percent at Various Stream Station (RK), Kanawha River

River Kilometer	Number of Zooplankton Taxa	Percent Reduction	Number of Macroinvertebrate Taxa	Percent Impact	Highest Taxa Impact	Mean Number of <i>Ceriodaphnia</i> Young per Female	Percent Toxicity	Wt. of Fathead Minnows	Percent Toxicity	Highest Percent Toxicity
20.1	7	30	24	25	30	28.8	11	.614	5	11
25.7	7	30	21	34	34	24.8	23	.619	5	23
51.8	10	0	20	37	37	31.8	2	.596	8	8
61.3	10	0	15	53	53	32.3	0	.577	11	11
66.0L	9	10	16	50	50	--	--	--	--	--
66R	10	0	19	41	41	31.4	3	.539	17	17
68.4	10	0	20	37	37	29.0	10	.605	7	10
71.1	7	30	24	25	30	--	--	--	--	--
76.1L	7	30	24	25	30	27.4	23	.567	13	23
76.1R	7	30	21	34	34	30.4	6	--	--	6
80.5	7	30	19	41	41	25.0	23	--	--	23
83.5	--	--	--	--	--	--	--	--	--	--
84.5	7	30	21	34	34	25.9	20	.565	13	20
87.4L	5	50	14	56	56	23.6	27	.648	0	27
87.4R	3	70	24	25	70	26.9	17	--	--	17
88.0L	5	50	16	50	50	27.3	15	.637	2	15
88.0R	4	60	21	34	60	24.1	25	--	--	25
90.4	4	60	22	31	60	23.1	28	.534	18	28
92.5L	5	50	23	28	50	27.6	15	.620	4	15
92.5R	5	50	23	28	50	24.8	23	--	--	23
94.1	4	60	20	37	60	26.4	18	.649	0	18
99.1	4	60	32	0	60	24.0	26	--	--	26
101.4	4	60	--	--	60	26.9	17	.616	5	17
105.2	5	50	21	34	50	24.7	24	--	--	24
108.9	8	20	26	19	20	26.8	17	.563	13	25
112.0	7	30	22	31	31	26.8	17	.552	15	17
114.2	5	50	25	22	50	--	--	--	--	--
118.4	5	50	--	--	50	--	--	--	--	--
119.1	--	--	27	16	16	27.6	15	.649	0	15
125.5	7	30	28	12	30	28.0	13	.597	8	13
133.2	6	40	30	6	40	24.8	23	.590	9	23
139L	7	30	27	16	30	24.3	25	.560	14	25
139R	4	60	27	16	60	21.5	33	.571	12	33
142.7	5	50	28	12	50	24.7	24	.608	6	24
145.0	3	70	28	12	70	22.3	31	.581	21	31

Note: -- No data

Figure 8-1. Percent toxicity to *Ceriodaphnia* vs. percent reduction of zooplankton taxa (Source Table 8-1).

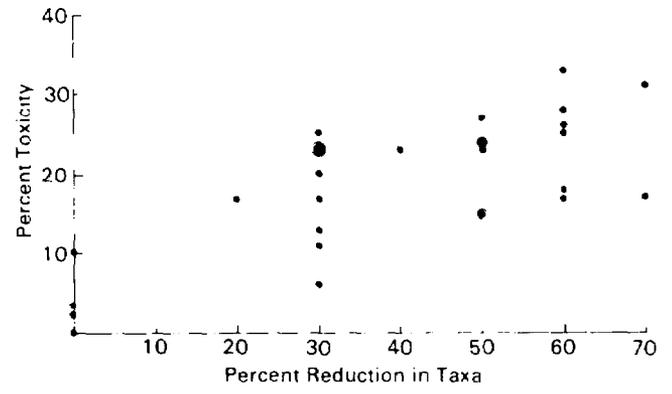


Table 8-2. Percent of Stations Where Reduction in Number of Taxa was Correctly Predicted by Toxicity Tests Using Four Arbitrary Levels of Comparison

Percent Increase in Toxicity	Percent Reduction in Taxa			
	20-40	40-60	60-80	80-100
20-40	57	60	53	45
40-60	3	39	77	100
60-80	3	42	81	100
80-100	3	42	81	100

zooplankton. However, one would expect enrichment to increase density, more than the number of taxa. Evidence for this effect is seen in that the downstream stations had the highest density of zooplankton by several times, but the number of taxa is 30 percent lower between the first and second two.

An examination of Table 8-1 will show that the reduction in taxa in the lower river was greater for the macroinvertebrates and in the upper river, reductions were greater for the zooplankton. The number of macroinvertebrates taxa was greatest at RK 99.1. Upstream of that Station, only two of the 11 values were less than 24, while downstream of RK 99.1 all 20 values were 24 or less which is indicative of the reduced taxa in the lower river.

Figure 8-2 is a plot showing the young per female from the ambient toxicity test and the number of zooplankton taxa plotted against river kilometer. (Recall that the samples for the September ambient toxicity tests and the zooplankton collections were obtained at the same time (Figure 8-2). This plot shows an amazingly similar pattern for both number of taxa and young per female not evident in Figure 8-1. Correlation of percent toxicity of the *Ceriodaphnia* and percent reduction of zooplankton was highly significant ($P \leq 0.005\%$). Since ample food was fed in the ambient toxicity tests to provide for at least 20-25 young per female (the expected number of young per female we obtain in sterile reconstituted water containing no food) nutrient enrichment would not explain the pattern obtained. The pattern would

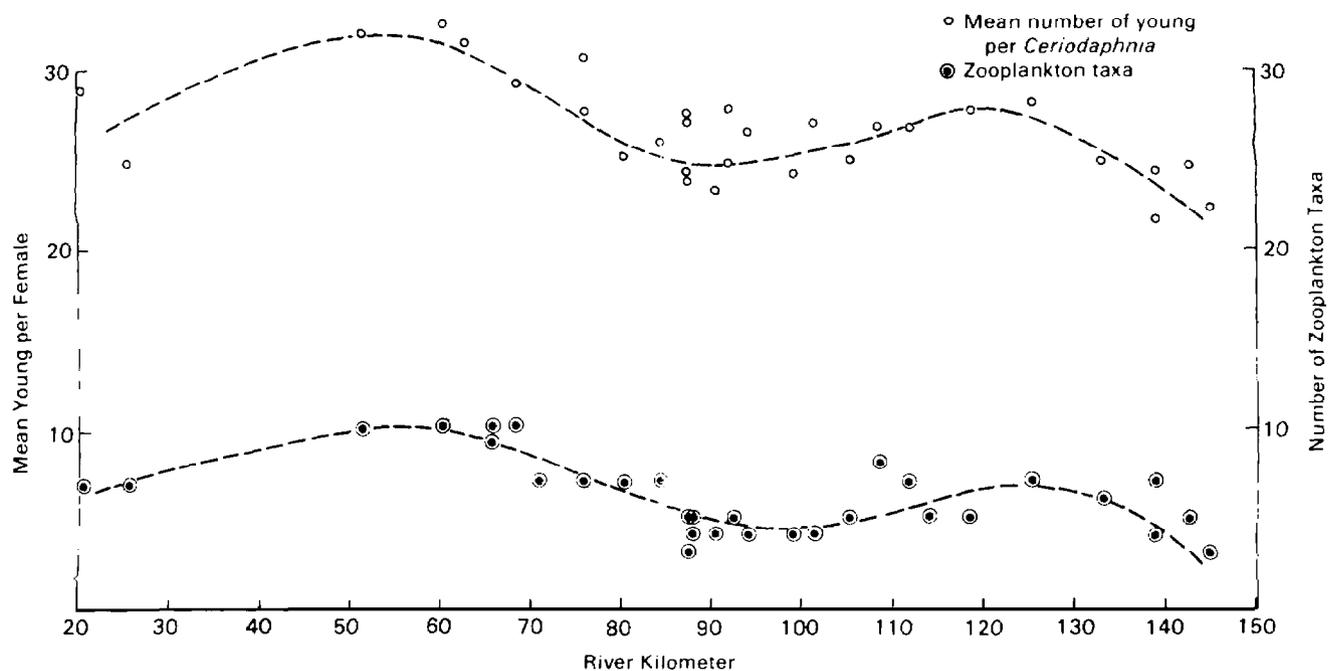
not be up and down but should rather show a continual increase in number of young in the downstream direction. The peak around RK 120 and the depression around RK 90 are not in concert with the nutrient hypothesis. These data provide evidence that the ambient tests are reflecting the effect of water quality on the instream zooplankton populations. And bearing in mind discussion earlier in this section, i.e., that one does not expect a tight correlation between *degree* of toxicity and *amount* of community impact, the obvious correlation shown in Figure 8-2 is also evidence that the ambient tests are reflecting a response of the community to water quality. However, neither test species reflected the response of the macroinvertebrates.

There were many more dischargers present in the study reach that were *not* tested than were tested. None of the effluents tested could account for the ambient toxicity observed. Additivity is not a viable explanation for the difference because the toxicity of the effluents was measured with all upstream effluents present in the dilution water except as noted in section 8-1.

There are several probable reasons for the ambient toxicity observed.

1. Some of the effluents not tested may be more toxic than those tested.
2. Due to the rainfall, the effluent toxicity measured was not representative either because the effluents were not typical or their measured

Figure 8-2. Number of young per female *Ceriodaphnia* and number of zooplankton taxa at various river stations.



toxicity was not typical because the dilution water as a result of the flood condition gave a different response (more suspended solids, BOD, etc.).

3. There are episodic occurrences of toxicity or other unknown sources that were missed in the effluent sampling but which affected the in-stream community and were in some of the September grab samples on which the ambient tests were run.

8.3. Summary

The agreement between the ambient test data and the community response was around 60 percent using 20-40 and 40-60 percent levels for comparison. In other studies in this series, the percent of correctly predicted stations has been generally higher. The correlation between percent toxicity for *Ceriodaphnia* and percent reduction of zooplankton taxa is highly significant ($P \leq 0.005\%$). The remarkable similarity (Figure 8-2) between young per female and number of zooplankton taxa is convincing data that the ambient test measures effects of water quality which are reflected in the community composition. The toxicity tests did not correctly predict the macroinvertebrate response supporting the need for multiple test species and for including various groups in any biological survey to identify impact.

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. 1985. Validity of Effluent and Ambient Toxicity for Predicting Biological Impact on Scippo Creek, Circleville, Ohio. EPA Research Series, EPA-600/3-85-044.
- Mount, D.I., A.E. Steen, and T.J. Norberg-King, Eds. 1985. Validity of Effluent and Ambient Toxicity for Predicting Biological Impact on Five Mile Creek, Birmingham, Alabama. EPA/600/3-85/071.
- 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 Research Series, EPA-600/3-84-084.
- 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.
- Weber, C.I. 1973. Recent Developments in the Measurement 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 Sci. Publ., Ann Arbor, Mich.

Appendix A

Toxicity Test and Analytical Methods

The Kanawha River study was conducted in two parts. One set of tests was conducted 14-21 August 1984. Because the river stage was very high, a second set of ambient tests was done on one set of shipped samples 19-26 September 1984. All tests were performed in a mobile laboratory either on site or on shipped samples at the Environmental Research Laboratory-Duluth, Minnesota.

A.1 On Site Test Methodology

The effluent samples were 24-hour composite samples collected using automatic samplers. The ambient samples were grab samples taken daily for seven days. All samples were put in collapsible polyethylene containers, with a capacity of either 1 or 5 gallons. Composite samples were terminated before 1200 hours on each day. The specific time was different for each effluent. All ambient samples were collected between 0700 and 1400 and were collected close to the artificial substrates.

As the samples were delivered to the mobile lab, they were warmed to 25 °C, and then stacked in an air-conditioned room until used. Effluent dilutions were made using polypropylene-graduated cylinders and polyethylene beakers for mixing. All river water was strained through a fine-mesh screen to remove zooplankton. A 2,000-ml volume of each was made; 200 ml were used for *Ceriodaphnia* tests and the rest for the fathead minnow tests. Initial DO, pH, and conductivity measurements were taken before the sample was split. Dedicated polyethylene containers were used for each concentration for both the *Ceriodaphnia* and the fathead minnow tests. Effluents were diluted with water upstream of each outfall or group of outfalls, and these stations are identified on Table 3-2.

As the ambient samples were collected, they were put in two sets of dedicated polyethylene containers for the *Ceriodaphnia* and fathead minnow tests. Ambient stations were close together, the flow-time between stations was short, and the stations used for dilution water for effluents were scattered among the rest of the stations. Therefore, the initial DO, pH, and conductivity measurements were done only on the dilution water stations and not on the rest of the stations in order to reduce work load. Final DO and pH measurements were taken for all stations. For both

test species, a new sample of effluent or ambient water was used for each daily change.

The *Ceriodaphnia* test followed generally the procedures of Mount and Norberg (1984). Adult *Ceriodaphnia* were transferred to the dilution water two days prior to the initiation of the first tests on site. A young *Ceriodaphnia* (0-6 hour old) was placed in a 1-oz plastic portion cup in 15 ml of test solution. There were ten animals for each treatment. Each day the animal was removed with an eyedropper and placed into a new cup containing new test solution. When young were present, they were counted and discarded. Each set of five effluent concentrations and the dilution water control were randomly assigned to a row on a test board. Each test board held five test organisms per concentration, and each test was split into half-test boards. The ambient station samples were run in the same manner, with six ambient stations randomly arranged in rows on each half test board. In this manner, treatments could be assigned randomly and independently to each half tray. The rotation and shelf assignment of each half tray was randomized each day.

A food suspension was fed daily after each change. The food consisted of three parts: (1) 5 g/L of dry yeast, (2) 5 g/L of Cerophyl®, stirred overnight and filtered through a plankton net, and (3) 5 g/L of trout chow, aerated vigorously for 7 days, settled, and decanted. The yeast suspension and the supernatant from the Cerophyl® and trout chow were mixed in equal parts, and new food was made every 7 days. The mixture, the Cerophyl®, and the yeast components were refrigerated, while the trout chow supernatant was 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 per *Ceriodaphnia* rather than 0.05 ml as was recommended for yeast (Mount and Norberg 1984), because the suspended solids are around 1,800 mg/L, less than half the solids contained in the yeast suspension.

The methods for the fathead minnow tests followed closely those described by Norberg and Mount (1985). The test chambers were 30.5 x 15.2 x 10.2 cm and

*Cerophyl was obtained from Agri-Tech, Kansas City, Missouri. As of January 1985, Cerophyl® was no longer being produced by that manufacturer. Use of trade names does not constitute endorsement.

divided into four compartments; this design allowed four replicates for each concentration. The larval fathead minnows were < 24 hours old and from the ERL-Duluth culture. The fish were assigned to the test compartments by pipetting one or two fish at a time to each replicate test chamber until all replicates had ten fish in each, or forty per concentration. All treatments were re-randomized daily with respect to position on the shelves. Newly hatched brine shrimp were fed to the fish three times a day. The uneaten shrimp were removed daily by siphoning the tanks during test solution renewal. At the same time, the volume in the test chamber was drawn down to 1 cm, after which 2 L of new test solution was added. The laboratory temperature was $25 \pm 1^\circ\text{C}$. A 16-hour light photoperiod was used. Because DO was low at the end of the first 24 hours, test volumes were reduced to 1 L. After 7 days of exposure, the fish were preserved in 4 percent formalin. Prior to weighing, they were rinsed in distilled water. Then each group was oven dried for 18 hours in pre-weighed aluminum pans and weighed on a five-place analytical balance.

A.2 Methods for Shipped Samples

Only ambient samples were tested in the September testing on shipped samples. Five-gallon grab samples were collected on 15 September 1984, cooled using wet ice, and shipped to Duluth and stored at 6°C . Each day, an aliquot was warmed to 25°C , and the new aliquot was exchanged in the *Ceriodaphnia* and fathead minnow tests. Otherwise, the same equipment and procedures were used as described for the onsite tests.

A.3 Statistical Analyses

A.3.1 *Ceriodaphnia dubia*

The statistical analyses were performed using the procedure of Hamilton (1984) as modified by Rogers (1984). 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 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. 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 the overall toxicity effects. Confidence intervals are calculated by the bootstrap procedure. This procedure subsamples the original data set (1,000 times) by means of a computer to obtain a robust estimate of standard error.

A Dunnett's two-tailed t-test is performed with the effluent test data to compare each treatment to the control for significant differences. For the ambient

station data, Tukey's Honestly Significant Difference Test (Sokal and Rohlf, 1981) is used for the ambient toxicity test data to compare stations.

A.3.2 Fathead Minnows

The four groups' mean weights are analyzed statistically with the assumption that the four test-chamber compartments behave as replicates. The method of analysis assumes 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 was then compared to the critical t-statistic for the standard two-tailed Dunnett's test (Steel and Torrie 1960). The survival data were arcsine-transformed prior to the regression analyses to stabilize variances for percent data.

Appendix B

Biological Sampling and Analytical Methods

B.1 Periphyton Survey

The periphytic community was sampled quantitatively using clear acetate strips suspended in the Kanawha River at the same locations as the artificial substrates for the benthic macroinvertebrates (Table 3-2). Triplicate strips were placed in the river at the 33 stations on 14-20 August, 1984 and retrieved on 12-17 September, 1984. 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/m². 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/m². The biomass and chlorophyll *a* data were used to calculate the Autotrophic Index (Weber, 1973), which indicates the relative proportion of heterotrophic and autotrophic components in the periphyton.

B.2 Zooplankton Methods

Zooplankton were collected from thirty-three stations on the Kanawha River in West Virginia on 15, 16, and 17 September, 1984. Samples were collected in triplicate at each station, at 3-foot depths by pumping 200 liters of water through a 153 μm mesh net.

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.

B.3 Macroinvertebrate Methods

Hester-Dendy samplers (round plate, variable spaced, about 0.1 m²) were suspended in the river at 33 locations (Table 3-2). The samplers were set from August 14-20, 1984 and were removed from September 12-17, 1984. The goal was to have the samplers from 2-3 feet from the normal pool surface. Because they were set during high water, the positioning was done by measuring water depth and then calculating the depth from the bottom that should be selected at the existing river stage.

The samplers were retrieved by raising them to just under the surface and then a net was placed beneath them and they were lifted out. The entire sampler and contents was preserved in 10% formalin containing rose bengal stain.

For enumeration, the plates were scraped with a putty knife to remove all material. This material was then washed to remove silt and then strained through a 500μ mesh netting. The organisms were picked from the debris under 8X magnification and placed in 70% alcohol. Identification was to the lowest taxon within the expertise of the analyst.

Appendix C Toxicity Test Data

Table C-1. Water Chemistry Data for Effluent Toxicity Tests. Values are for Both *Ceriodaphnia* and Fathead Minnow Tests and Final Dissolved Oxygen Values are for Daphnids Only

Effluent (RK) ^a	Percent Effluent (v/v)	pH Range	Initial DO (mg/L)		Final DO (mg/L)		Conductivity (umhos)
			Mean	Range	Mean	Range	
POTW 11 (90.4)	100	6.5-7.1	7.4	5.7-8.6	7.4	5.6-7.7	3,075
	30	6.9A ^b	6.9	--	7.0	5.4-8.2	
	10	6.6A ^c	7.2	--	7.3	5.9-7.9	
	3	6.8A ^b	7.4	--	6.7	5.5-7.7	
	1	6.8A ^b	7.3	--	7.3	6.3-7.7	
	Dilution Water (90.4)	6.5-7.0	7.9	7.3-8.8	7.0	6.3-7.6	
POTW 1M (90.4)	100	6.2-6.5	7.2	6.3-7.8	5.6	5.5-5.7	446
	30	6.6A ^b	7.2	--	6.4	5.4-7.0	
	10	6.7A ^c	7.3	--	6.5	5.1-7.5	
	3	6.8A ^b	7.3	--	7.0	5.8-7.8	
	1	6.8A ^b	7.3	--	6.9	6.2-7.8	
	Dilution Water (90.4)	6.5-7.0	7.9	7.4-8.4	6.8	6.3-7.8	
POTW 2 (89.6)	100	6.7-7.0	6.4	2.6-7.9	6.2	5.5-6.8	480
	30	6.7A ^b	7.4	--	7.0	6.2-7.8	
	10	6.5A ^b	7.5	--	6.3	4.9-7.7	
	3	6.5A ^b	7.4	--	7.3	6.5-8.0	
	1	6.5A ^b	7.3	--	7.3	6.4-7.9	
	Dilution Water (90.4)	6.5-7.0	7.9	7.1-8.8	7.2	6.5-7.6	
POTW 3 (66.8)	100	6.6-7.0	6.1	5.7-6.4	6.4	5.7-7.0	545
	30	6.7A ^c	7.0	--	6.3	5.8-7.2	
	10	6.6A ^b	7.0	--	6.4	5.1-7.2	
	3	6.6A ^c	7.1	--	6.6	6.1-7.3	
	1	6.6A ^c	7.0	--	6.4	5.2-7.4	
	Dilution Water (68.4)	6.0A ^b	7.0	--	6.4	4.9-7.0	
A (143.5)	100	5.4-7.8	8.6	7.9-9.2	7.3	6.3-7.9	162
	30	6.7A ^b	8.0	--	7.4	6.3-7.9	
	10	6.6A ^b	7.9	--	7.4	6.3-7.9	
	3	6.5A ^b	7.9	--	7.3	6.2-8.1	
	1	6.5A ^b	7.9	--	7.3	6.4-8.0	
	Dilution Water (145.0)	6.5-6.8	7.5	7.3-7.6	6.8	5.5-7.4	
B (111.0)	100	6.7-7.0	8.2	7.0-8.8	6.9	4.7-8.1	132
	30	6.8A ^b	7.3	--	6.9	4.2-8.1	
	10	6.8A ^b	7.2	--	6.9	3.8-8.0	
	3	6.7A ^c	7.3	--	6.8	3.7-8.1	
	1	6.7A ^c	7.3	--	6.8	4.0-7.9	
	Dilution Water (111.0)	6.8	7.5	7.2-7.9	6.9	5.5-7.9	
C (111.0)	100	6.9-7.0	4.3	1.7-7.3	6.8	--	15,083
	30	6.9A ^b	7.3	--	--	--	
	10	6.9-7.0	7.3	--	7.4	6.4-8.1	
	3	6.9A ^b	7.3	--	7.2	6.3-8.1	
	1	6.8A ^b	7.2	--	6.2	3.2-8.0	
	Dilution Water (114.2)	6.87.0	7.5	7.2-7.9	6.9	4.1-8.0	

Table C-1. (continued)

Effluent	Percent Effluent (v/v)	pH Range	Initial DO (mg/L)		Final DO (mg/L)		Conductivity (umhos)
			Mean	Range	Mean	Range	
D (112.2)	100	7.0-7.2	8.3	7.5-8.9	7.1	5.2-8.2	175
	30	6.9A ^b	7.4	--	6.5	4.0-8.2	
	10	6.9A ^b	7.3	--	6.7	3.7-8.1	
	3	6.8A ^b	7.3	--	6.8	4.7-8.1	
	1	6.8A ^b	7.3	--	6.8	5.3-7.8	
	Dilution Water (114.2)	6.8	7.5	7.2-7.9	7.0	6.4-7.8	
E (88.2)	100	7.0-7.8	7.9	7.5-8.4	6.7	--	1,250
	30	6.8A ^b	7.8	--	8.2	--	
	10	6.5A ^b	7.8	--	8.2	--	
	3	6.5A ^b	7.9	--	7.3	6.5-8.2	
	1	6.6A ^b	7.8	--	7.4	6.4-8.2	
	Dilution Water (90.4)	6.7-7.0	7.4	7.0-7.8	6.9	6.0-8.1	
F (85.8)	100	6.4-6.8	11.8	10.4-14.2	7.6	--	14,083
	30	6.7A ^b	8.8	--	7.8	6.6-8.2	
	10	6.7A ^b	8.1	--	7.3	6.4-8.1	
	3	6.7A ^b	8.0	--	7.3	6.5-8.1	
	1	6.6A ^b	7.6	--	7.2	6.0-8.0	
	Dilution Water (90.4)	6.5-7.0	7.3	7.0-7.6	6.8	6.0-7.9	
G (85.8)	100	6.7-7.1	6.8	4.6-8.0	6.9	6.5-7.3	242
	30	6.7A ^b	7.6	--	6.1	5.1-7.8	
	10	6.6A ^b	7.6	--	6.8	5.7-7.8	
	3	6.7A ^b	7.5	--	7.0	5.5-7.8	
	1	6.6A ^b	7.4	--	7.2	6.2-7.8	
	Dilution Water (86.1)	6.7-6.9	7.1	6.9-7.4	7.2	6.0-8.0	
H (85.8)	100	7.0-7.4	8.2	7.2-9.0	6.7	--	591
	30	6.7A ^b	7.4	--	--	--	
	10	6.6A ^b	7.5	--	7.1	--	
	3	6.5A ^b	7.4	--	6.7	4.5-8.1	
	1	6.6A ^b	7.3	--	7.3	6.4-8.0	
	Dilution Water (86.1)	6.6-6.9	7.1	6.9-7.3	7.1	6.3-7.9	
I (77.4)	100	7.0-7.2	7.1	6.1-7.8	6.9	6.4-7.3	1,017
	30	6.8A ^b	7.5	--	7.3	6.5-7.9	
	10	6.6A ^b	7.4	--	7.1	6.4-8.0	
	3	6.5A ^b	7.2	--	7.2	6.3-8.0	
	1	6.6A ^b	7.2	--	7.2	6.5-7.8	
	Dilution Water (80.5)	6.5-6.6	7.2	7.1-7.2	7.2	6.7-8.1	
J (68.9)	100	6.3-7.0	6.4	4.5-7.1	6.1	5.5-6.7	1,275
	30	6.7A ^b	7.0	--	5.7	5.4-6.3	
	10	6.8A ^b	7.2	--	6.1	4.0-7.2	
	3	6.8A ^b	7.2	--	7.0	6.2-8.0	
	1	6.7A ^b	7.0	--	7.1	5.9-8.0	
	Dilution Water (68.4)	6.8-7.2	7.0	6.9-7.0	7.2	6.5-8.0	
K (67.1)	100	6.9-7.3	7.5	6.6-8.	7.0	--	132
	30	6.8A ^b	7.2	--	--	--	
	10	6.8A ^b	7.1	--	6.7	5.9-7.8	
	3	6.6A ^b	7.2	--	6.4	5.2-7.3	
	1	6.6A ^b	7.1	--	7.3	6.5-8.1	
	Dilution Water (68.4)	6.6-7.2	7.0	7.0	7.3	6.6-8.1	

*RK of the discharger, see Tables 3-1, 3-2.

^bOnly one measurement was made.

Table C-2. Final Dissolved Oxygen Concentrations for Fathead Minnow Larval Growth Tests on Effluents, Charleston, West Virginia, August 1984

Effluent	Percent Effluent (v/v)	DO (mg/L)	
		Mean	Range
POTW 1I (90.4)	100	1.8	1.6-3.8
	30	2.5	1.2-4.6
	10	2.4	1.5-4.2
	3	3.0	2.3-5.2
	1	3.1	2.8-5.2
	Dilution Water (90.4)	3.2	2.6-4.9
POTW 1M (90.4)	100	2.8	0.5-4.5
	30	3.1	1.8-4.5
	10	3.1	1.3-5.2
	3	3.3	1.2-5.4
	1	3.6	1.2-5.9
	Dilution Water (90.4)	3.4	2.0-4.6
POTW 2 (89.6)	100	1.7	0.7-4.5
	30	2.7	1.4-7.6
	10	2.9	1.9-6.2
	3	3.1	2.4-5.1
	1	3.1	2.1-4.6
	Dilution Water (90.4)	3.5	2.8-5.4
A (143.5)	100	3.6	2.8-5.0
	30	3.9	2.7-6.4
	10	3.5	2.9-5.1
	3	3.1	1.6-5.7
	1	3.8	3.1-5.6
	Dilution Water (145.0)	3.4	2.4-5.7

Note: Initial routine chemistry values are in Table C-1.

Table C-3. Initial Water Chemistry Data for Ambient Toxicity Tests with *Ceriodaphnia* and Fathead Minnows on Day 1 of Testing, Charleston, West Virginia, August 1984

Ambient Station (RK)	pH	Initial DO (mg/L)	Conductivity (μmhos)
145.0	6.8	7.9	98
142.7	6.9	8.0	105
139.0R	6.8	8.1	98
139.0L	7.0	8.0	110
133.2	6.9	8.1	102
125.5	6.7	8.3	105
119.1	7.0	8.0	108
114.2	6.8	8.0	87
112.0	6.9	8.0	100
108.9	6.8	8.1	102
105.2	6.9	8.1	95
101.4	6.8	8.1	123
99.1	6.9	7.8	100
94.1	6.8	8.0	100
92.5R	6.9	7.7	95
90.4	6.9	8.0	90
88.0R	6.9	8.1	96
88.0L	6.9	8.0	95
87.4R	6.9	8.0	93
87.4L	6.9	7.8	155
Davis Creek, 85.8	7.0	7.9	900
83.3	7.0	8.1	100
84.5	7.0	8.1	100
84.5	7.0	8.1	168
80.5	6.8	8.0	78
76.1R	6.9	8.0	87
76.1L	6.9	8.2	98
71.1	6.9	8.1	110
68.4	6.9	8.0	121
66.0R	6.7	8.3	100
66.0L	6.9	8.0	108
61.3	6.8	8.1	100
51.8	6.8	8.1	105
25.7	6.8	8.1	96
20.1	6.8	8.3	98

Table C-4. Final Dissolved Oxygen Concentrations for Ambient Toxicity Tests with *Ceriodaphnia* and Fathead Minnow, Charleston, West Virginia, August 1984

Ambient Station (RK)	<i>Ceriodaphnia</i> Final DO (mg/L)		Fathead Minnow Final DO (mg/L)	
	Mean	Range	Mean	Range
145.0	6.6	6.2-7.3	3.8	2.6-5.5
142.7	6.9	6.2-7.9	4.1	1.9-5.5
139.0R	7.2	5.8-7.9	4.1	3.0-5.0
139.0L	7.0	6.2-7.4	3.8	7.5-4.7
133.2	6.9	6.0-7.7	3.8	2.8-4.7
125.5	6.9	6.1-8.0	4.1	3.1-4.9
119.1	7.3	7.1-7.6	3.9	2.2-5.4
114.2	6.4	5.6-7.0	3.8	2.3-6.1
112.0	7.4	6.1-8.1	3.7	2.6-4.7
108.9	6.7	6.0-7.5	4.0	3.5-4.9
105.2	6.9	5.5-7.8	4.3	3.3-5.1
101.4	7.3	5.8-7.9	4.0	3-4.7
99.1	6.1	5.1-7.5	4.6	3.5-5.9
94.1	7.0	6.2-7.4	4.4	3.2-5.6
92.5R	6.7	5.5-7.7	4.5	3.4-5.6
90.4	6.8	6.0-7.5	4.2	2.8-5.2
88.0R	7.2	6.0-7.7	3.9	3.1-5.4
88.0L	6.25	6.2-6.3	4.2	3.1-5.1
87.4R	7.3	6.1-8.1	3.9	2.2-5.0
87.4L	7.0	6.1-8.0	4.4	3.0-5.5
Davis Creek, 85.8	6.9	5.8-7.8	3.9	2.4-4.8
83.3	7.1	6.3-7.6	4.1	2.7-5.4
84.5	6.7	4.6-8.0	4.2	3.2-5.3
80.5	6.9	6.1-7.4	3.9	2.8-4.9
76.1R	7.4	6.1-8.1	4.2	3.0-5.4
76.1L	7.0	6.0-7.8	3.8	2.4-4.9
71.1	6.9	6.1-8.1	4.0	2.7-5.2
68.4	6.8	5.6-7.6	3.9	2.1-5.5
66.0R	6.1	4.8-7.7	4.2	2.9-5.6
66.0L	6.4	5.8-7.1	3.8	2.8-4.7
61.3	6.7	6.2-7.1	4.1	3.1-5.1
51.8	6.9	6.1-7.9	4.2	2.9-5.6
25.7	6.5	5.1-7.4	4.2	2.9-5.3
20.1	6.2	5.7-7.2	3.8	2.4-4.5

Table C-5. Water Chemistry Data for *Ceriodaphnia* and Fathead Minnow Ambient Toxicity Tests, Kanawha River, Charleston, West Virginia, September 1984

Ambient Station (RK)	pH	Conductivity (μ mhos)	Initial DO (mg/L)	Fathead Minnow Final DO (mg/L)		<i>Ceriodaphnia</i> Final DO (mg/L)	
				Mean	Range	Mean	Range
145.0	7.1	115	9.0	6.6	5.8-7.1	7.7	7.6-7.8
142.7	7.0	160	9.4	6.8	5.9-7.7	7.8	7.8-7.9
139.OR	7.0	160	9.3	6.8	5.4-7.5	7.8	7.4-8.1
139.OL	7.0	160	9.2	7.1	6.7-7.5	8.0	7.7-8.2
133.2	6.9	140	9.2	6.8	5.7-7.7	7.9	7.7-8.1
125.5	6.8	150	9.1	6.8	5.7-7.4	7.8	7.8-7.9
119.1	6.8	140	9.0	6.8	5.9-7.5	7.5	6.8-7.8
112.1	6.9	125	9.1	6.7	6.1-7.5	7.9	7.8-7.9
108.9	6.8	140	8.8	6.6	5.1-7.7	7.9	7.8-8.1
105.2	7.0	150	9.0	--	--	7.9	7.8-8.0
101.4	7.0	150	8.9	6.8	6.3-7.6	8.0	7.6-8.3
99.1	7.0	140	9.0	--	--	7.9	7.8-7.9
94.1	7.0	150	8.9	6.6	5.5-7.1	7.7	7.6-7.9
92.5R	7.0	120	8.7	--	--	7.7	6.7-8.5
92.5L	7.0	135	9.0	7.0	6.8-7.2	7.7	7.4-7.9
90.4	7.0	140	9.1	6.8	5.4-7.5	7.6	7.0-7.9
88.OR	7.0	360	8.4	--	--	7.9	7.8-8.1
88.OL	7.2	140	9.0	6.7	4.9-7.3	7.7	6.7-8.5
87.4L	7.0	290	9.2	--	--	7.9	7.7-8.1
87.4L	7.0	250	8.7	6.8	6.0-7.5	7.7	7.4-7.8
84.5	6.9	170	8.9	6.8	5.8-7.4	7.8	7.6-8.0
80.5	--	--	--	--	--	7.6	7.3-7.9
76.1R	7.0	180	8.3	--	--	7.8	6.9-8.5
76.1L	6.8	180	9.0	6.8	5.6-7.3	7.8	7.8-9.9
68.4	6.9	200	9.0	6.8	5.2-7.6	7.8	7.6-7.9
66.0	6.8	210	8.8	6.7	5.9-7.4	7.8	7.6-7.9
61.3	6.8	220	8.7	6.5	4.6-7.4	7.9	7.8-8.1
51.8	6.9	170	8.9	6.7	6.1-7.1	7.8	7.6-8.1
25.7	6.8	160	9.1	6.9	6.3-7.3	7.7	6.6-8.4
20.1	7.0	150	8.9	6.5	5.3-7.5	7.7	7.5-8.1

Appendix D Biological Data

Table D-1. Routine Chemistry Data for August and September for the Stream Stations. Readings Were Taken When Artificial Substrates Were Set and Removed, Kanawha River

Station (RK)	Conductivity (μ mhos)		DO (mg/l)		pH		Temp (°C)	
	Aug.	Sept.	Aug.	Sept.	Aug.	Sept.	Aug.	Sept.
20.1	129	157	7.6	7.4	7.0	7.0	22.6	21.8
25.7	135	153	6.9	7.0	7.3	7.0	22.5	21.9
51.8	130	198	7.1	6.7	7.2	7.4	23.2	22.5
61.3	143	223	7.2	6.8	7.2	7.1	23.6	23.4
66.0L	130	203	7.3	7.0	7.3	7.2	24.3	23.1
66.0R	145	204	7.5	7.2	7.3	7.1	24.8	22.8
68.4	220	197	7.6	6.7	7.4	7.1	24.7	23.3
71.1	133	184	7.9	7.1	7.3	7.2	25.2	22.6
76.1L	134	177	7.8	7.5	7.3	7.3	25.0	22.4
76.1R	134	180	7.9	7.8	7.4	7.3	24.8	22.7
80.5	139	187	7.5	7.4	7.3	7.2	24.7	22.4
84.5	140	167	8.0	7.5	7.2	7.2	24.5	21.7
87.4	146	220	7.6	7.9	7.3	7.4	24.1	22.5
87.4	136	196	7.7	7.9	7.5	7.3	24.5	21.9
88.0R	118	139	8.1	7.7	7.4	7.3	23.8	21.7
88.0L	279	310	7.3	7.8	7.4	7.4	26.0	23.0
90.4	115	148	8.0	7.7	7.3	7.2	22.2	21.6
92.5L	115	150	7.9	7.8	7.4	7.2	22.2	22.3
92.5R	107	101	7.9	7.8	7.3	7.1	21.9	22.2
94.5	116	152	7.8	7.8	7.3	7.2	22.3	22.6
99.1	117	149	7.9	7.7	7.4	7.3	22.4	22.3
101.4	115	154	8.1	7.8	7.4	7.2	22.3	22.4
105.2	111	148	7.9	8.0	7.2	7.1	22.2	22.5
108.9	105	173	8.4	7.2	7.4	7.3	22.3	23.2
112.0	97	169	8.9	7.2	7.4	7.9	24.4	23.1
114.2	94	171	8.3	7.4	7.4	8.0	22.8	23.0
119.1	95	180	8.4	8.7	7.3	7.8	22.7	22.4
125.5	94	180	8.3	8.4	7.2	7.8	22.1	24.0
133.2	97	170	8.3	8.0	7.4	7.7	22.1	21.4
139.0L	96	123	8.3	8.8	7.4	7.3	21.6	21.3
139.0R	96	127	8.4	9.1	7.5	7.8	21.5	21.4
142.7	99	134	8.4	7.8	7.6	7.6	21.7	21.4
145.0	96	110	8.2	7.8	7.6	7.6	21.8	19.9

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