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Validity of Effluent and Ambient Toxicity Tests for Predicting Biological Impact, Skeleton Creek, Enid, Oklahoma

Edited by

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Notice

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 Testing Program are:

1. To investigate the validity of effluent toxicity tests in predicting 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 provide practical case examples of how such testing procedures can be applied to effluents discharged to a receiving water.
4. To field test short-term chronic toxicity tests involving the test organisms, *Ceriodaphnia* 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 the 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. Such limits determined on fresh effluent may not reflect toxicity after aging in the stream and fate processes change effluent composition. This problem is less important since permit limits are normally applied at the edge of the mixing zone where aging has not yet occurred.

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 report presents the site study on Skeleton Creek, Enid, Oklahoma, which was conducted in August 1983. The stream is small and receives discharges from two industries and one publicly owned treatment works.

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 TEST DATA AND RECEIVING WATER BIOLOGICAL IMPACT
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Executive Summary

Skeleton Creek was studied in August 1983 and was the fourth site study. Skeleton Creek is located in an agricultural area in northwestern Oklahoma, near Enid. The creek has a shallow gradient with mostly sand and sandstone bedrock. A small creek, Boggy Creek receives discharges from both an oil refinery and a publicly owned treatment works (POTW) prior to its confluence with Skeleton Creek. A fertilizer processing plant discharge is located on Skeleton Creek just downstream of the confluence of the two streams.

The toxicity of two effluents and ambient stream stations were evaluated. Hydrological and ecological field surveys were also done. A comparison of the relationship between the measured toxicity of the water samples collected from the stream and the health of the aquatic community at the same stream stations is made.

The results of the toxicity tests found the fathead minnow 7-day growth test to be more sensitive to both effluents than the 7-day *Ceriodaphnia* reproduction test. Station 5, below all three discharges, was the station where the toxicity tests, zooplankton and fish were the most affected.

Both the toxicity test data and the ecological survey data show that impact at the stream stations is correlated with the toxicity measured (number of species lost). Correct predictions were made for 87.5 percent of the stations when any equal level of impairment and toxicity was compared.

The results of this study combined with those previous studies published (Mount et al., 1984, and Mount and Norberg, 1985) and ones yet to be published (i.e., Mount et al., 1985) will be used to recommend the best available approach to predict the impacts of discharges on biological communities using effluent and ambient toxicity tests. The data from this study clearly indicate the utility of effluent and ambient toxicity tests for predicting instream effects.

Quality Assurance

Coordination of the study was done by the principal investigator preceding any field work or toxicity testing. A reconnaissance trip was made to the site in the spring of 1983 to obtain the necessary details regarding each discharge and to make a cursory evaluation of the stream. Following that trip, the details were delineated for setting sampling dates and the specific sampling sites; and the specific measurements to be made for each stream station. This study required coordination in setting artificial substrates, removing the substrates, planning the hydrological and ecological surveys, and collection of effluents and water samples for the toxicity tests by two organizations (see list of contributors). 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. Test organisms for the toxicity tests were laboratory raised.

1. Introduction

Future activities in water pollution control will focus, in part, on the control of toxic pollutants that impact water quality. There are two methods used in controlling toxic impact: pollutant-specific controls and "whole effluent toxicity" controls. Because toxicity testing evaluates a living organism's response, it has an advantage over chemical-specific analyses which may not identify all pollutants in a wastewater sample and which cannot detect toxicity interactions. Toxicity information can provide a basis for permit limits based on state water quality standards for toxicity- or technology-based requirements.

The primary purpose of this study is to investigate the relationship between effluent and ambient toxicity, and community response. Toxicity tests have the potential to predict instream impact.

This report is organized into chapters corresponding to the project tasks. Following an overview of the site description and study design, the chapters are arranged into hydrological survey chapters, toxicity test results, and ecological survey results for the study. An integration of the laboratory and field studies are presented in Chapter 8. All the laboratory methods, hydrology methods, ecological survey methods, and supporting data are presented in the appendices.

2. Study Design and Site Description

The study area was on Skeleton Creek, which originates 6.4 km northeast of Enid, Oklahoma. Boggy Creek begins 3 km south of Enid and flows southeast for 12 km before its confluence with Skeleton Creek, which then flows 105 km before its confluence with the Cimarron River. A Refinery and a Publicly Owned Treatment Works (POTW) discharge treated effluent into Boggy Creek. The POTW is an activated sludge plant. The most upstream discharge is the refinery, but the POTW discharge is only 0.2 km downstream of it. Little mixing occurs before the refinery effluent meets the POTW outfall. A Fertilizer manufacturing plant discharges its treated effluent into Skeleton Creek 0.5 km downstream from the confluence with Boggy Creek. The streams maintain a shallow gradient of 1 m/km. During the 1983 field sampling period, the POTW pumped its treated wastewater at night to both the Refinery and Fertilizer Plant for use as process water and the POTW discharged directly into Boggy Creek during the day. The Refinery discharged continuously to Boggy Creek while the Fertilizer Plant discharged intermittently into Skeleton Creek. Actual discharge flow measurements are given in Chapter 3.

Study components include 7-day *Ceriodaphnia** reproductive toxicity tests and 7-day larval growth tests on fathead minnows on ambient samples from the stream stations and various concentrations of the Refinery and Fertilizer Plant effluents. The POTW effluent was not tested because during the study period the plant anticipated that its discharge would all go to the Refinery and Fertilizer Plant. Also, stream flow and discharge volume measurements, quantitative assessment of the planktonic, macroinvertebrate, and fish communities were made. Artificial substrates were set in the stream July 20 and were removed when field sampling, effluent, and water sampling was completed August 9 to 11, 1983. Water samples for the toxicity tests were collected at locations near where the artificial and natural substrate samples were taken. The toxicity tests were conducted August 14 to 21 at the Environmental Research Laboratory-Duluth. Table 2-1 presents the type of sampling done for each stream station.

The study area on Skeleton Creek and Boggy Creek covered a total of 26.6 river kilometers (RK). River kilometers were estimated from county topographical maps using the confluence with the Cimarron River as zero river kilometers. The streams have been described in reports by Wilhm (1965), Baumgardner (1966), and Namminga (1975). Both creeks are shallow prairie streams with shallow tributaries having low summer or intermittent flows. Pool areas predominate with periodic riffles and runs along their lengths. Twelve sampling stations are located along the study area (Figure 2-1) and are described below. The habitats sampled were pools, riffles, and runs for the benthic macroinvertebrates, pools for the fish, and moving water areas for the plankton. The estimated cover, percent riffle, and percent pool for each station is presented in Table C-1.

The station descriptions are as follows.

Station 1A (RK 2.8)—The uppermost point sampled on Boggy Creek, which was 4.6-6.0 m wide at low flow. The riffle was 0.15 m deep and the pool was 0.45 m deep. The sides were lined with riparian vegetation which provided nearly 100 percent cover. Macroinvertebrates were collected from the small riffle areas created by flat rocks. Seining for the fish survey was conducted in the adjoining pool.

Station 1 (RK 1.2)—Upstream from the Refinery discharge on Boggy Creek. The station was used only for setting artificial substrates for sampling macroinvertebrates. After the substrates were set, construction of a beaver dam impounded water and formed a turbid pool, 0.76 m deep. Riparian vegetation on the shore provided 100 percent cover.

Station 2 (RK 107.0)—On Skeleton Creek 2 km upstream of the confluence of Skeleton Creek and Boggy Creek. Riparian vegetation on the shore provided 90 percent cover. The station had a sandy-bottom pool 0.76 m deep and a shallow run less than 0.15 m deep with a bottom substrate composed of sand and gravel.

Station 3 (RK 0.2)—On Boggy Creek below both the POTW and Refinery discharges and just prior of the

*The species of *Ceriodaphnia* used for this study is not known with certainty. The stock cultures were earlier identified as *C. reticulata* but in November 1983, based on taxonomic verification by Dorothy Berner, Ph.D., Temple University, PA, a second species *C. dubia* was also identified in the stock cultures. The exact determination of the species tested is not critical to the results of this study. Therefore, all references to *Ceriodaphnia* are to genus level only.

Table 2-1. Sample Collections Conducted for the Quantitative Biological Assessment and Ambient Toxicity Tests, Skeleton Creek and Boggy Creek, Enid, Oklahoma, August 1983

Collections	Sampling Stations											
	1A	1	2	3	4	5	5A	6	7	8	9	10
Plankton	X		X	X	X	X	X	X	X	X	X	
Macroinvertebrates												
Natural substrate	X		X	X	X	X	X	X	X	X	X	
Artificial substrate		X	X	X	X	X		X	X	X	X	X
Fish	X		X	X	X	X	X	X	X	X	X	
Ambient Toxicity Test												
Fathead minnow	X		X	X	X	X	X	X	X	X	X	
Ceriodaphnia dubia	X		X	X	X	X	X	X	X	X	X	

confluence with Skeleton Creek. The station was composed of a 0.6 m deep pool with a sand bottom and sand overlying rock, and a shallow run less than 0.15 m deep. Riparian vegetation provided about 80 percent cover.

Station 4 (RK 104.8)—Downstream of the confluence of the creeks and upstream of the Fertilizer Plant discharge. The run (0.3 m deep) substrate was composed of irregular rock with a covering of attached filamentous algae and sand. In addition, a black, flocculent material had aggregated in a few areas on the bottom. Riparian vegetation provided no cover.

Station 5 (RK 104.3)—On Skeleton Creek, 0.3 km downstream of the Fertilizer Plant. The water contained large amounts of floating algae and a black, flocculent material. The station was composed of a 0.6 m deep pool and a 0.15 m deep riffle area with a rocky bottom. Riparian vegetation provided 10 percent cover.

Station 5A (RK 103.6)—At Southgate Road crossing of Skeleton Creek. The station was composed of a pool, 0.45 m deep, with a sand bottom and a riffle, 0.25 m deep, with a sand and gravel bottom. Riparian vegetation provided no cover.

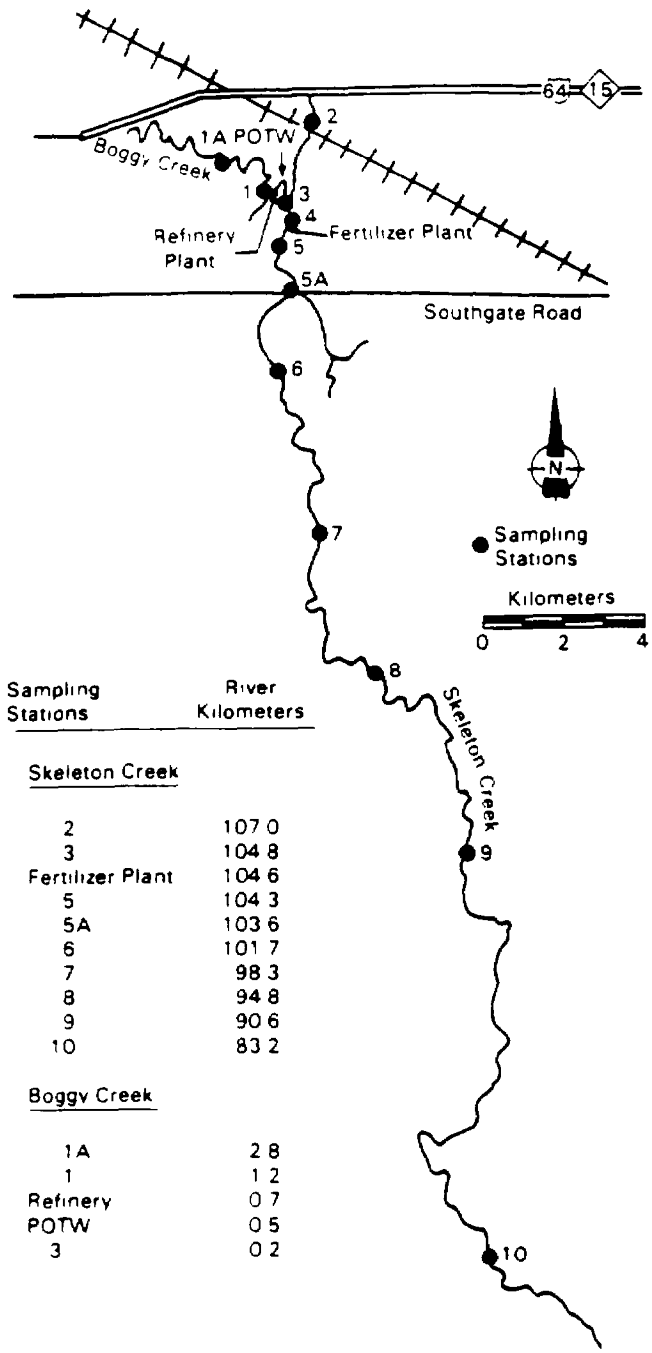
Station 6 (RK 101.7)—On Skeleton Creek, 1.9 km downstream of Station 5A. The station consisted entirely of run habitat approximately 12 m wide and up to 0.25 m deep. Riparian vegetation provided no cover. The substrate was smooth, flat rock covered with some sand or individual rocks.

Station 7 (RK 98.3)—On Skeleton Creek, 3.4 km downstream of Station 6. The station consisted of riffle and pool areas each having a bottom composed of rocks embedded in sand. The riffle was shallow, 0.15 m deep, and the pool was 0.45 m deep. Riparian vegetation provided no cover.

Station 8 (RK 94.8)—On Skeleton Creek, 3.5 km downstream of Station 7. The station consisted of a 0.3 m deep pool with a sand bottom. The riffle was 0.25 m deep and had a sand and gravel bottom. Riparian vegetation provided no cover.

Station 9 (RK 90.6)—On Skeleton Creek, 4.2 km downstream of Station 8. The station was composed of a pool, 0.60 m deep, and a riffle, 0.30 m deep, with a bottom of rocks embedded in sand and clay. The creek banks were red clay and the water was turbid. Riparian vegetation provided about 20 percent cover.

Station 10 (RK 83.2)—The most downstream station 7.4 km farther downstream than Station 9. The station was entirely a pool of approximately 0.76 m depth which appeared to be the result of many wood snags creating a dam. The banks were red clay and the water was turbid. Riparian vegetation provided no cover.



Sampling Stations	River Kilometers
Skeleton Creek	
2	107 0
3	104 8
Fertilizer Plant	104 6
5	104 3
5A	103 6
6	101 7
7	98 3
8	94 8
9	90 6
10	83 2
Boggy Creek	
1A	2 8
1	1 2
Refinery	0 7
POTW	0 5
3	0 2

Figure 2-1 **Map of study site on Skeleton Creek, Enid, Oklahoma.**

3. *Hydrology Survey*

The purpose of the hydrology study of Boggy and Skeleton Creeks was to obtain stream and discharge flow measurements during the study period, and to determine the percent flow contribution from the three dischargers. In addition to these measurements, data was also obtained from a proximate USGS gauging station (downstream from Station 10) and from the operational records of the dischargers. Sampling and analytical methods are presented in Appendix A.

3.1 Discharge Flow Measurements

Stream flows were measured 9-11 August 1983 at the stations shown on Table 3-1. The daily average flows for 8-11 August for the Refinery, the Fertilizer Plant, and the USGS gauging station (Station 07160500 near Lovell, OH) are also given. In addition, the average POTW plant flows for 8-12 August are presented. The Refinery reported a uniform flow of 0.023 m^3/sec. The daily average flow at the POTW varied between 0.066 m^3/sec on 12 August to 0.083 m^3/sec on 9 August. The hourly flows at the POTW deviated from the average values due to the facility's day-night loading cycle. The pumps at the Fertilizer Plant were turned on and off such that the discharge flow was either zero or between 0.072-0.075 m^3/sec (Table 3-2). During the period 8-11 August the pumps were off for 7-10 hours each day. On 10 August, the discharge was on for 22.7 hours

Table 3-2. Effluent Pumping Records and Daily Average Discharge at the Fertilizer Plant, Enid, Oklahoma

Date	Time	Discharge (m^3/sec)	Daily Average Discharge (m^3)
8 Aug	0000 - 1100	0.072	0.050
	1100 - 1755	0.0	
	1755 - 2335	0.072	
	2335 - 2400	0.0	
9 Aug	0000 - 0930	0.0	0.045
	0930 - 2400	0.075	
10 Aug	0000 - 0955	0.075	0.069
	0955 - 1115	0.0	
	1115 - 2400	0.072	
11 Aug	0000 - 0810	0.0	0.049
	0810 - 2030	0.074	

Source: Plant operating records, personal communication

Table 3-1 Measured Flows and Discharges on Skeleton Creek and Boggy Creek, Enid, Oklahoma

Stations	Flow (m^3/sec) 8 Aug	9 Aug	10 Aug	11 Aug	12 Aug
Boggy Creek					
1	..	..	..	0.031	
Refinery*	0.023	0.023	0.023	0.023	
8[2]	..	0.043	..	..	
POTW*	0.074	0.083	0.081	0.067	0.066
3	..	..	0.126	.	
Skeleton Creek					
2	..	..	0.006	.	
4	..	.	0.145	..	
Fertilizer Plant*	0.050	0.045	0.069	0.049	
5	..	..	0.171		
6	..	0.147	..		
7	..	..	..	0.084	
8	..	..	..	0.103	
9	..	0.166	.		
USGS Estimate Flows	0.259	0.227	0.148	0.131	0.126

*Average flow from plant records. During the night, treated wastewater was sent to the Refinery and Fertilizer Plant.
[2]Additional stream measurement taken just downstream of the Refinery

NOTE: The confluence of Boggy Creek and Skeleton Creek is upstream of Station 4

There was a large variation between the flows measured at Stations 5 through 9 and the flows recorded at the USGS station on 9 August (Table 3-1). The measured flows at Station 6 (0.147 m^3 sec) and Station 9 (0 166 m^3 sec) are much less than the 0 227 m^3 sec value reported by the USGS. However, the average USGS flow decreased by 35 percent between 9 and 10 August such that the hourly flows on 9 August must have been decreasing continually Since the Station 6 and 9 flows were measured in the late afternoon, they would be expected to correspond to a lower USGS flow than the reported daily average value On 10 August, the Station 5 flow would be expected to be in better agreement with the USGS flow since the discharge from the Fertilizer Plant stopped for only 1 3 hours. In contrast, on 11 August, the Plant's pump had been on for 2.5 and 3 2 hours before the flows were measured at Stations 7 and 8 which are located 6.3 and 9.8 km, respectively, downstream from the Fertilizer Plant It is possible that the flow increase had not had sufficient time to propagate downstream by the time of the measurement, so that the reported value would be less than the daily USGS values.

3.2 Flow Contribution

Using the measured flow period of 9-11 August, the mean flows were 0 023 m^3 sec for the refinery, 0.077 m^3 sec for the POTW, and 0.054 m^3 sec for the Fertilizer Plant (Table 3-1) The measured upstream flows and the mean discharge flows sum to combined flow at Station 5 of 0 191 m^3 sec The flow of 0.191 m^3 sec exceeds the mean flow at the USGS gauging station for 9-11 August of 0 169 m^3 sec by 13 percent Assuming that water is not being lost from the stream bed, this discrepancy could not result from any combination of over-estimating the upstream flow or the reported discharges or under-estimating the USGS flow The higher flow of 0 19 m^3 sec was used downstream

Table 3-3 Mean Flow and Percent Flow Contribution from Three Discharges for Boggy Creek and Skeleton Creek, Enid Oklahoma, August 1983

Station	Total Flow (m^3 sec)	Percent Flow Contribution: Upstream	Refinery	POTW	Fertilizer Plant
2	0 006	100 0			
1	0 031	100 0			
8[a]	0 054	57 4	42 6		
3	0 131	23 6	17 6	58 8	
4	0 137	27 0	16 8	56 2	
5	0 191	19 4	12 0	40 3	28 3
6	0 191	19 4	12 0	40 3	28 3
7	0 191	19 4	12 0	40 3	28 3
8	0 191	19 4	12 0	40 3	28 3
9	0 191	19 4	12 0	40 3	28 3

[a]Additional stream measurement taken just downstream of the refinery. Total flow value is station 1 plus the Refinery mean flow.

Source Tables 3 1 and 3-2

4. Laboratory Toxicity Tests

Toxicity tests were performed on two effluents and water collected from nine stream stations to measure subchronic effects on growth of larval fathead minnows (*Pimephales promelas*) and chronic effects on reproduction of *Ceriodaphnia*. Descriptions of the toxicity test methods are presented in Appendix B. A wide span of effluent concentrations were used so acute mortality could be measured as well if it existed. The objective of the effluent tests was to measure the minimum concentration of each effluent that would cause acute mortality and chronic effects on the growth of the fathead minnows or reproduction of the *Ceriodaphnia*. The ambient toxicity tests were conducted to measure if toxicity exists either before or after an effluent was discharged to estimate the persistence of toxicity. The effect levels can then be compared to the extant effluent concentrations in Skeleton Creek to predict where the impact on stream population occurs, if any. The validity of these predictions is determined by examining the biologic condition of the stream at the locations where the effluent concentrations occurred as determined by the hydrological survey.

The effluent and ambient samples were collected, cooled, and transported to Duluth for toxicity testing. All tests were run with one composite sample of each effluent or stream station.

4.1 Chemical and Physical Test Conditions

The laboratory temperature was maintained at 25 ± 1°C over the test period. Routine water chemistry measurements for the effluent and ambient tests are given in Table 4-1. Dissolved oxygen (DO) and pH were monitored daily, and the initial pH, DO, conductivity, hardness, and temperature measurements were for both the fathead minnows and *Ceriodaphnia* tests. The pH values were all within 7.3 to 8.6, except for the 100 percent Refinery effluent which was 5.7 to 6.7. The initial DO values were all 7.6 to 8.4 mg/L except for the 100 percent Fertilizer Plant effluent which was 6.0 mg/L. Table 4-1 also gives the mean final DO values for the fathead minnow tests. The Refinery effluent dilution test and the ambient station tests had final DO values ranging from 5.1 to 6.7 mg/l. However, at the 30 and 100 percent Fertilzier Plant concentrations the mean final DO's were 3.1 and 0.5 mg/l. The dilution water and other concentrations of the Fertilizer Plant had DO values ranging from 6.2 to 4.7 mg/l. Other site studies with waters of high BOD levels and DO levels of less than 1 mg/l have also been encountered. In one study (Mount and Norberg-King, in press) the average weights of the fathead minnows were higher than the previous studies. An assessment of this situation has led to the conclusion that dissolved oxygen measurements taken by the dissolved oxygen probe do not accurately reflect the micro-environmental conditions where the fathead minnows are living. The fathead minnows were observed moving towards the surface of the water where in all probability the oxygen concentrations are much higher than that measured by the dissolved oxygen probe. Apparently the behavior of the fish causing them to stay near the surface when the dissolved oxygen levels are low makes the test nearly independent of low DO effects. The highest conductivities were observed in the whole effluents. Hardness ranged form 297 to 725 mg/L $CaCO_3$. In Table 4-2 the final DO and pH values for the *Ceriodaphnia* tests are shown. All values were within acceptable ranges.

4.2 Effluent Toxicity Test Results

Tables 4-3 and 4-4 contain the weight and survival data for the fathead minnow effluent tests. Survival in the Refinery effluent was significantly lower ($P \leq 0.05$) at 30 percent while weights were significantly lower at 10 percent. Therefore, the Acceptable Effluent Concentration (AEC) estimate was 5.5 percent (which is the geometric mean of the No Observable Effect Concentration (NOEC) and Lowest Observable Effect Concentration (LOEC) for the Refinery effluent. Survival in the Fertilizer Plant effluent was significantly lower only at the 100 percent, while the weight data was significant at the 10 percent effluent concentration. The AEC was than 5.5 percent for the Fertilizer Plant.

Table 4-5 contains the *Ceriodaphnia* effluent test data as well as a quality control using laboratory water. The mean number of young per female was significantly lower than the dilution water young production at 30 percent for the Refinery effluent. This gives an AEC of 17.3 percent. The Fertilizer Plant had a significantly lower young production compared to the dilution water at the 30 percent effluent

Table 4-1. Routine Chemistry Data for Effluent and Ambient Tests

Percent Effluent (v/v) or Ambient Sample	Initial pH Range	Dissolved Oxygen: Mean Initial (Range)	Dissolved Oxygen: Mean Final[a] (Range)	Temperature (°C)	Hardness (mg/L)	Conductivity (µmhos/cm)
Refinery						
Dilution Water (1A)	7.9-8.0	8.4 (8.2-8.9)	6.0 (4.6-6.9)	25	297	1 220
1	7.9-8.0	8.4 (8.3-8.7)	6.4 (4.7-7.1)	25	--	1 280
3	7.8-8.0	8.4 (8.3-8.7)	6.1 (5.0-7.1)	25	-	1 340
10	7.7-8.0	8.4 (8.3-8.7)	6.4 (5.4-7.0)	25	--	1 580
30	7.3-8.0	8.3 (7.8-8.7)	6.4 (5.3-7.4)	25	--	2 320
100	5.7-6.7	7.6 (6.7-8.4)	6.7 (6.4-7.0)	25	--	4 680
Fertilizer Plant						
Dilution Water (1A)	7.9-8.0	8.2 (7.7-8.6)	6.2 (5.3-7.2)	25	297	1 280
1	7.9-8.0	8.2 (7.4-8.6)	6.0 (5.6-6.5)	25	-	1 320
3	7.9-8.1	8.2 (7.6-8.7)	5.5 (4.6-6.0)	25	--	1 410
10	7.9-8.0	8.3 (7.6-8.9)	4.7 (3.7-5.6)	25	--	1 800
30	7.9-8.0	8.2 (7.9-8.7)	3.1 (2.6-4.2)	25	--	2 700
100	8.1-8.2	6.0 (-)	0.5 (-)	25	--	5 900
Ambient Station						
2	7.7-8.2	7.9 (7.3-8.4)	7.0 (5.9-8.2)	25	477	1 620
3	7.8-8.1	7.9 (6.8-8.5)	6.0 (5.0-8.0)	25	376	1 980
4	7.9-8.2	7.6 (6.2-8.4)	6.0 (4.5-7.6)	25	380	2 040
5	7.9-8.1	7.7 (6.0-8.5)	5.1 (3.1-6.8)	25	725	3 480
5A	8.1-8.5	8.2 (7.0-8.8)	6.0 (4.3-7.8)	25	466	2 050
6	8.1-8.5	8.2 (7.6-8.6)	6.1 (4.4-7.6)	25	663	2 880
7	7.9-8.2	8.0 (6.2-8.6)	5.4 (3.9-6.9)	25	730	3 500
8	8.2-8.3	8.5 (8.2-8.8)	6.2 (4.9-7.6)	25	700	3 390
9	8.5-8.6	8.5 (8.0-8.8)	6.6 (5.5-8.1)	25	710	3 200

[a]Final dissolved oxygen values are for fathead minnows tests only. Table 4-2 contains *Ceriodaphnia* final chemistry values.

concentration, which gives an AEC of 17.3 percent. The quality control sample young production was in the normal range (Mount and Norberg, 1984).

4.3 Ambient Toxicity Test Results

Tables 4-6 and 4-7 contain the survival and weight data for the fathead minnow ambient tests. Station 5 had significantly lower survival ($P \leq 0.05$), while the weights of Stations 3, 4, 5, 5A, 6, and 8 were significantly lower when compared to Station 9. Table 4-8 presents the results of the survival and mean young production of the *Ceriodaphnia*. Stations 2, 5, 7, and 8 had significantly lower mean number of young per female using the highest value of young

Table 4-2. **Final Dissolved Oxygen and Final pH for *Ceriodaphnia* Effluent and Ambient Toxicity Tests**

Percent Effluent or Ambient Sample	pH	Mean DO (mg/L)	DO Range
Refinery			
Dilution Water (1A)	8.1	7.8	7.3-8.2
1	8.2	7.8	7.4-8.2
3	8.4	7.9	7.4-8.4
10	8.4	7.8	7.4-8.3
30	8.3	7.8	7.5-8.3
100	7.1	7.9	7.6-8.2
Fertilizer Plant			
Dilution Water (1A)	7.4	7.9	7.7-8.3
1	8.3-8.4	7.5	7.2-7.8
3	8.4	7.8	7.2-8.1
10	8.3-8.4	7.2	6.9-7.4
30	8.2-8.3	6.8	6.3-7.1
100	7.9	4.8	4.2-6.2
Ambient Station			
2	8.5	7.9	7.2-8.2
3	8.4	7.9	7.2-8.4
4	8.4	7.9	7.2-8.4
5	8.5	7.9	7.3-8.3
5A	8.4	7.8	7.3-8.3
6	8.4	7.8	7.3-8.3
7	8.3	7.9	7.4-8.4
8	8.4	8.0	7.7-8.3
9	8.4	8.0	7.7-8.3

production (Station 9) for the comparison. Survival was significantly lower only at Station 3.

4.4 Discussion

For the effluent dilution tests the fathead minnows were affected at concentrations lower than the *Ceriodaphnia*. The upstream water (1A) used as the dilution water resulted in good growth of the fathead minnows and high young production of the *Ceriodaphnia*. Station 9, which is the most downstream station, produced the best growth for the fathead minnows and the highest young production for the *Ceriodaphnia*. It appears that all effects of toxicity were removed at the downstream location.

Table 4-3. **Seven-Day Survival of Larval Fathead Minnows in Two Effluents**

		Percent Effluent (v/v)					
Effluent	Replicate	100	30	10	3	1	Dilution Water (1A)
Refinery	A	0	30	80	90	80	100
	B	0	30	90	90	100	90
	C	0	50	90	90	90	90
	D	0	60	100	100	100	90
	Mean	0*	43*	90	93	93	93
Fertilizer Plant	A	0	80	80	80	90	90
	B	0	80	90	90	100	100
	C	0	80	90	100	100	80
	D	0	70	80	90	100	90
	Mean	0*	78	85	90	[illegible]	90

*Significantly lower from the dilution water ($P \leq 0.05$)

Table 4-4. Mean Individual Dry Weight (mg) After Seven Days for Larval Fathead Minnows Exposed to Two Effluents

		Percent Effluent (v/v)					
Effluent	Replicate	100	30	10	3	1	Dilution Water 1A
Refinery	A	0	0.19	0.38	0.56	0.57	0.56
	B	0	0.24	0.40	0.61	0.58	0.64
	C	0	0.32	0.41	0.52	0.61	0.52
	D	0	0.30	0.47	0.56	0.63	0.61
	Weighted Mean[b]	0*	0.276*	0.418*	0.562	0.599	0.608
	SE	..	0.038	0.026	0.026	0.026	0.018
Fertilizer Plant	A	0	0.33	0.46	0.57	0.54	0.58
	B	0	0.24	0.51	0.57	0.58	0.57
	C	0	0.31	0.46	0.66	0.65	0.65
	D	0	0.31	0.56	0.59	0.58	0.75
	Weighted Mean[b]	0*	0.297*	0.497*	0.600	0.589	0.608
	SE	..	0.030	0.029	0.028	0.027	0.019

*Significantly lower from the dilution water (P ≥ 0.05)
[b]Explanation of weighted mean calculation is in Appendix B

Table 4-5 Percent Survival and Young Production of *Ceriodaphnia* in Two Effluents

Sample	Mean Percent Survival	Mean Number of Young per Female	Confidence Intervals
Refinery			
Dilution Water (1A)	100	33.5	27.5-39.5
1	100	34.8	29.2-40.4
3	100	31.5	26.8-36.2
10	100	37.4	33.2-41.7
30	100	23.5*	18.5-28.5
100	0*	0*	.
Fertilizer Plant			
Dilution Water (1A)	100	25.3	18.9-31.7
1	90	25.1	19.3-31.0
3	100	25.0	19.3-30.7
10	90	31.5	24.6-38.5
30	70	11.4*	7.8-15.1
100	0*	0*	
Lake Superior Water[b]	90	18.1	14.1-22.2

*Significantly different from the dilution water for each test (P < 0.05)
[b]Quality Control water sample

Table 4-6. Seven-Day Percent Survival of Larval Fathead Minnows in the Ambient Toxicity Test

	Station Number								
Replicate	2	3	4	5	5A	6	7	8	9
A	100	100	80	10	90	90	100	100	90
B	90	100	90	60	90	90	80	90	90
C	100	90	80	50	100	90	80	100	90
D	100	80	80	60	100	80	80	90	[illegible]
Mean	98	93	83	45*	95	88	85	95	95

*Significantly lower from Station 9 (P < 0.05)

Table 4-7 Mean Individual Dry Weight (mg) After Seven Days for Larval Fathead Minnows in the Ambient Toxicity Test

	Station Number								
Replicate	2	3	4	5	5A	6	7	8	9
A	0 66	0 49	0 46	0 58	0 63	0 63	0 69	0 56	[illegible]
B	0 69	0 43	0 59	0 31	0 60	0 66	0 76	0 68	[illegible]
C	0 63	0 58	0 52	0 28	0 69	0 59	0 69	0 65	0 82
D	0 61	0 48	0 56	0 42	0 75	0 63	0 78	0 72	0 68
Weighted Mean[b]	0 646	0 494[a]	0 525[a]	0 429[a]	0 670[a]	0 627[a]	0 728	0 650[a]	0 762
S D	0 033	0 034	0 036	0 040	0 033	0 035	0 035	0 033	[illegible]

[a]Significantly lower from Station 9 (P ≤ 0 05)

[b]Explanation of weighted mean calculation is in Appendix B

Table 4-8. Percent Survival and Mean Young Production of *Ceriodaphnia* in the Ambient Toxicity Test

Ambient Station	Percent Survival	Mean Number of Young per Female	Confidence Intervals
2	100	11 7[a]	9 9-13 5
3	30[a]	14 0	10 6-17 3
4	90	16 4	11 5 21 3
5	70	8 1[a]	5 8-10 3
5A	80	22 8	18 7-26 8
6	100	19 0	16 0-22 0
7	80	12 8[a]	10 0-15 5
8	60	9 9[a]	5 8-14 1
9	80	24 3	17 9 30 4

[a]Significantly different from Station 9 (P ≤ 0 05)

5. Plankton Community Survey

This survey investigated the plankton community by measuring the occurrence and density of organisms in Skeleton Creek and Boggy Creek. The primary emphasis was to collect zooplankton, but algae were also collected and enumerated. The number of species and individuals are used to determine alterations in composition and/or density. The sampling and analytical methods are presented in Appendix C. Samples were not collected at Stations 1 and 10.

5.1 Community Structure

Algae were the dominant planktonic organisms at every station on Skeleton Creek and Boggy Creek (Table 5-1). The number of algae were lowest at Stations 1A and 2, but the numbers increased at Stations 3 and 4 by 8 and 7.5 times, respectively. The highest algal densities were found at Stations 5, 6, and 8 where there were over 8,000 organisms/liter. Solitary diatoms composed the great majority of the algal population.

The numbers of crustaceans and rotifers collected were low. Often the only crustaceans found were nauplii. The highest density of crustaceans was 2.44 organisms/liter at Station 7. At Station 5, no crustaceans were collected. The highest density of rotifers was also at Section 7 (30.65 organisms/liter). Non-loricate forms composed the majority of the rotifers and the remainder were from the Family Branchionidae.

5.2 Evaluation of the Plankton Community

Rotifers were the most abundant zooplankton group in Skeleton Creek and Boggy Creek. In general, densities of rotifers were low at the upstream stations, but consistently increased downstream to a

Table 5-1 **Mean Density (number/liter) of Planktonic Organisms Collected in Skeleton Creek and Boggy Creek, Enid, Oklahoma, August 1983**

Taxa	Sampling Station 1A	2	3	4	5	5A	6	7	8	9
Crustaceans										
Cladocera	··	··	0 56	0 07	··	··	··	··		
Copepoda	··	··	0 06	0 06	··	··	··	0 26		0 04
Nauplii	0 15	0 24	1 45	0 31	··	0 05	0 34	2 18	0 55	
Total crustaceans	0 15	0 24	2 07	0 44	··	0 05	0 34	2 44	0 55	0 04
Rotifers										
Branchionus spp	0 04	··	0 74	0 26	0 99	1 33	3 26	7 22	0 55	[illegible]
Small Branchionidae	0 14	0 48	1 88	0 71	1 21	2 12	2 00	5 87	1 54	0 88
Non-loricate forms	··	··	2 51	4 21	5 61	7 12	9 33	17 56	1 65	0 82
Total rotifers	0 18	0 48	5 13	5 18	7 81	10 57	14 59	30 65	3 74	2 40
Algae										
Padiastrum	0 05	0 12	0 63	0 68	1 2[illegible]	1 14	7 16	7 28	6 50	7 28
Desmids	0 72	1 67	0 48	0 63	0 62	0 02	0 11	0 49		0 96
Solitary diatoms	112 1	149 0	908 2	1 116 5	9 435 9	2 382 5	2,238 3	5 573 6	12 727 5	3 309 6
Total algae	112 87	150 79	909 31	1 117 81	9 437 73	2 383 66	2 245 57	5 581 37	12 734 0	3 317 84
Others										
Chironomidae	0 19	0 24	0 48	0 32	0 22	1 36	0 85	0 61	0 33	0 04
Trichoptera	··	··	··	··	··	··	··	··		0 04
Heleidae	0 04	··	··	··	··	··	··	··		
Nematoda	0 05	0 12	0 12	··	0 22	0 07	0 28	0 39	0 22	
Total others	0 28	0 36	0 60	0 32	0 44	1 43	1 13	1 00	0 55	0 08
Total Zooplankton Taxa	3	2	5	5	3	4	4	4	4	4

Note: ·· indicates organisms were not found

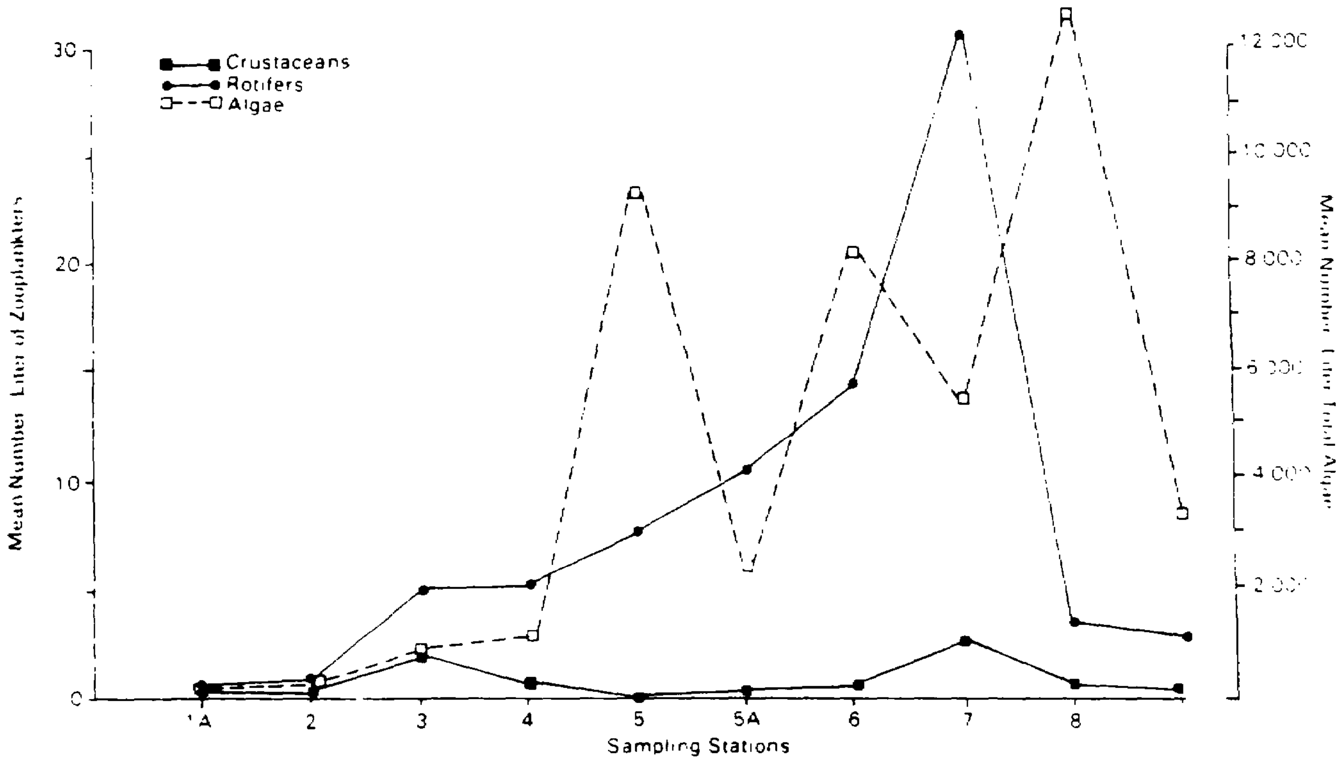


Figure 5-1 Densities of crustaceans, rotifers, and algae at Skeleton Creek and Boggy Creek, Enid, Oklahoma, August 1983

maximum at Station 7 and densities decreased below Station 7 (Figure 5-1). Results of a one-way analysis of variance (ANOVA) indicated that the difference in densities between stations was highly significant ($P = 0.0001$). Tukey's test (Sokal and Rohlf 1981) results indicated that Station 6 and 7 were significantly different ($P < 0.05$) from all other stations.

Despite the low densities of crustaceans, there were significant differences ($P = 0.0001$) between stations from results of a one-way ANOVA. Tukey's test results indicated that Stations 3 and 7 were significantly different ($P < 0.05$) from the other stations. Crustacean densities were highest at Station 3 and 7 while less than 0.6 organisms/L at other stations (Figure 5-1).

6. *Macroinvertebrate Community Survey*

The survey investigated the macroinvertebrate community of Boggy Creek and Skeleton Creek. Samples were collected from natural and artificial substrates. The macroinvertebrate community is considered to be a good indicator of changes in water quality due to their limited mobility. The degree of community stability can be ascertained by measuring species composition and dominance. An alteration in community structure, species composition, or biomass beyond normal variations would be regarded as an adverse effect. In addition, the increased abundance of nuisance insect larvae or other benthic species would be regarded as an adverse effect.

Although both natural and artifical substrates were used to quantify the macroinvertebrate communities, not all stations were sampled by both methods (Table 2-1 and Appendix C). A description of the sampling and analytical methods is presented in Appendix C. Additional data are included in Appendix D.

6.1 Community Composition

The macroinvertebrate communities of Boggy Creek and Skeleton Creek were composed of 55 taxa. The number of taxa at each station varied from 13 to 28. Major taxa were identified as those which contributed a minimum of 5 percent of the total number of organisms from at least one station. The changes in abundance and percent composition of these major taxa are presented for the two substrate types.

6.1.1 Natural Substrates

Two taxa were more abundant than the other major taxa: a chironomid—*Dicrotendipes* sp. and a coleopteran—*Berosus* sp. *Dicrotendipes* composed over 30 percent of the benthic density at Stations 2, 3, and 5, and *Berosus* constituted over 30 percent of the benthos at Stations 5A, 6, and 7 (Table 6-1). There are another fifteen taxa which contributed ≥5 percent of the populations for at least one station. These taxa were in six taxonomic groups: Diptera (Chironomidae), Ephemeroptera, Odonata, Trichoptera, Gastropoda, and Oligochaeta.

The macroinvertebrate population in Skeleton Creek and Boggy Creek is primrily composed of insects. Fifteen of the seventeen major taxa are insects. The Chironomidae family (midges) had the most taxa (9). Two taxa each were from the Ephemeroptera (mayflies) and Trichoptera (caddisflies) families. One major taxon each were identified from the Coleoptera (beetles), Odonata (dragonflies, damselflies) and Physidae (pouch snails) families, and Oligochaeta.

6.1.2 Artifical Substrates

The same two taxa were found to be most abundant using the artifical substrates as with the natural substrates: a chironomid—*Dicrotendipes* sp. and a coleopteran—*Berosus* sp. (Table 6-2). *Dicrotendipes* sp. composed greater than 30 percent of the macroinvertebrates population at five stations. In contrast, *Berosus* sp. composed approximately 50 percent at Station 6, 27 percent at Station 7, and less than 6 percent at the other stations. There are another fourteen major taxa which contributed ≥5 percent of the populations for at least one station. These taxa were in six taxonomic groups: Diptera (Chironomidae), Ephemeroptera, Odonata, Trichoptera, Amphipoda, and Gastropoda.

The macroinvertebrate population, collected using artificial substrates, in Skeleton and Boggy Creek is primarily composed of insects. Fourteen of the sixteen major taxa are insects. The Chironomidae family (midges) had the greatest number of major taxa (eight). Similar to the results for natural substrates, two taxa each were from the Ephemeroptera (mayflies) and Trichoptera (caddisflies) families and one each from the Coleoptera (beetles), Odonasta (dragonflies, damselflies), Physidae (pouch snails) and Talitridae (scuds) families.

6.1.3 Comparison Between Substrate Types

The taxa collected from the natural and artificial substrates for dominant taxa were very similar. The most abundant major taxa were the same for the two substrates. A difference between the two substrates occurred in the non-insect taxa. Physidae and Oligochaeta were the non-insect major taxa collected from the natural substrates while Physidae and Talitridae were the non-insect major taxa collected from the artifical substrates.

Table 6-1   **Mean Percent Composition of Major Macroinvertebrate Taxa Collected from Natural Substrates in Skeleton Creek and Boggy Creek, Enid, Oklahoma, August 1983**

Taxa	Sampling Station 1A	2	3	4	5	5A	6	7	8	9
Diptera										
Dicrotendipes sp	25.0	40.0	34.9	18.3	36.5	16.1	14.5	10.8	5.1	[illegible]
Polypedilum sp	10.9	5.8	1.3	0.5	0.9	0.4	0.7	0.2	31.2	18.5
Ablabesmyia sp	4.5	0.5	0.8	2.6	5.8	2.9	3.7	11.7	8.2	[illegible]
Chironomus sp	1.0		36.5	17.0	16.4	9.2	0.2	0.5		
Tanypus sp	0.3	0.9	2.4	20.0	0.5	1.3	--	0.2		[illegible]
Tanytarsus sp	6.4	3.0	7.8	12.8	1.2	1.2	0.5	1.9	16.8	1.4
Pseudochironomus sp	0.5	13.7	--		--	--	0.2	0.2	0.5	
Cricotopus sp	1.0	5.8	0.5	--	10.1	4.6	11.2	15.9	8.2	[illegible]
Chironomidae pupae	2.3	--	4.6	0.8	18.9	7.9	6.8	4.1	8.1	1.8
Total Chironomidae	54.5	72.6	89.5	73.1	90.9	43.6	38.7	45.5	78.7	23.2
Ephemeroptera										
Caenis sp	17.8	0.9	1		0.7	1	0.5	1.8		
Baetis sp	0.8				--		--	1.6	0.5	[illegible]
Coleoptera										
Berosus sp	6.4	3.9	0.8	15.2	2.9	30.1	40.0	42.8	6.3	1.2
Odonata										
Argia sp	6.4	--		--		0.1	1.3			
Trichoptera										
Cheumatopsyche sp	--	--	--		0.1		0.2	1.3	1.4	43.7
Hydropsyche sp	--	--				--			0.3	10.5
Gastropoda										
Physidae	2.1	6.7	0.3	6.9	2.3	0.3	2.2	5.8	0.9	0.3
Oligochaeta (unidentified)	3.3	12.5	7.8	0.5	0.8	21.1	7.9	7.1	9.0	[illegible]

Source: Table D-1

Note: -- indicates not collected

6.2 Station Comparisons

6.2.1 Natural Substrates

The greatest number of organisms collected from natural substrates was at Station 5 and Chironomidae taxa comprised 90 percent of these (Tables D-1 and 6-1). Collections were greater than 2,100 organisms/m² except at Stations 1A, 2, and 3 where collections were less than 1,500 organisms/m².

There are noticeable differences in the abundance of many of the major taxa between stations. There were also differences in the abundance patterns between taxa. The mean density of *Dicrotendipes* sp. varied by over two orders of magnitude, from a maximum at Station 5 of 1,568/m² to only 7/m² at Station 9 (Figure 6-1). Mean densities of *Dicrotendipes* sp. at the other stations were 120-620/m². Results of a two-way ANOVA indicated that these differences in numbers between stations were highly significant (P = 0.001), and results of Tukey's test indicated that Station 9 was significantly different ($P \leq 0.05$) than all the other stations, except Station 3. The mean density of *Berosus* sp. varied by two orders of magnitude from a low of 11/m² at Station 3 to a maximum at Station 5A of 1,159/m² (Table D-1, Figure 6-2). Highly significant differences (P = 0.0001) were found in numbers of *Berosus* sp. between stations [illegible] ANOVA and Tukey's test results indicated that Station 3 was different (P [illegible] 0.05) than Stations 6 and 7 [illegible] *Berosus* composed at least 40 percent of the community.

Examination of the abundance trends for the two most abundant macroinvertebrate taxa collected from natural substrates indicated that densities of *Dicrotendipes* sp. peaked distinctly at Station 5, while densities of *Berosus* sp. peaked immediately downstream at Station 5A (Figures 6-1 and 6-2). Other major taxa also had maximum densities at Stations 5 or 5A: *Chironomus* sp., *Cricotopus* sp., Chironomidae pupae, and unidentified oligochaetes. The contribution of the Chironomidae to the composition at each station was overwhelming at Stations 3 and 5, where that family composed 90 percent of the taxa. In contrast, *Polypedilum* sp., was found in greatest abundance at Stations 8 and 9 where other chironomids were least abundant. *Cheumatopsyche* sp. was also most abundant at Station 9.

There were nonsignificant differences between stations of the number of chironomid taxa. [illegible] the total number of taxa, ANOVA results [illegible] that there were very significant differences (P [illegible]

Table 6-2 **Mean Percent Composition of Major Macroinvertebrate Taxa Collected from Artificial Substrates in Skeleton Creek and Boggy Creek, Enid, Oklahoma, August 1983**

	Sampling Station									
Taxa	1	2	3	4	5	6	7	8	9	10
Diptera										
Dicrotendipes sp	58.0	63.7	45.0	53.3	31.3	7.2	26.0	0.6	2.9	3.8
Polypedilum sp	0.6	0.7	0.7	0.2	0.3	0.2	0.1	40.2	37.4	25.3
Ablabesmyia sp	14.1	6.7	33.0	13.1	15.1	8.6	4.1	4.9	4.3	19.2
Chironomus sp	5.5	0.4	3.6	1.0	14.6	5.4	10.3	0.1	0.1	
Psectrocladius sp	--		5.3	2.4	0.5	1.4	2.9	0.8	0.8	0.4
Tanypus sp	0.7	1.5	0.1	2.2	10.5	1.4	2.5		0.1	
Tanytarsus sp	4.6	2.4	0.1	0.1	0.1	0.5	0.4	21.8	4.8	[illegible]
Chironomidae pupae	4.4	9.5	4.4	2.2	11.7	2.5	0.9	5.5	3.1	14.7
Total Chironomidae	89.5	86.7	94.2	76.2	93.5	28.8	48.3	74.1	55.6	71.6
Ephemeroptera										
Caenis sp	7.0	1.9	0.2	0.8	0.3	0.4	1.0			0.4
Baetis sp	0.4	1.3	0.3	1.9	1.5	8.5	16.0	1.3	2.7	
Coleoptera										
Berosus sp		2.0	0.2	6.2	3.1	52.3	27.0	4.3		
Odonata										
Argia sp	2.1	6.3	3.0	5.5	0.5	5.4	4.5	0.8	2.8	17.5
Trichoptera										
Cheumatopsyche sp	--	-	--					13.6	3.1	
Hydropsyche sp						0.2		3.7	31.1	9.4
Amphipoda										
Talitridae	0.9		0.7	6.6	0.7	1.2	2.5	0.1		
Gastropoda										
Physidae		6.1	0.2	12.7	0.3	0.9	0.2	0.2		

Source: Table D-2

Note: -- indicates not collected

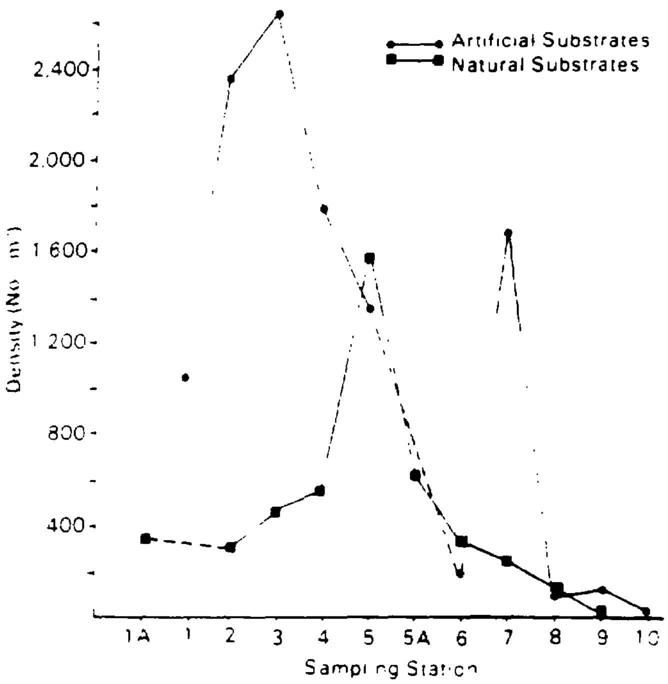


Figure 6-1 **Mean densities of *Dicrotendipes* sp. collected from Skeleton Creek and Boggy Creek, Enid, Oklahoma, August 1983**

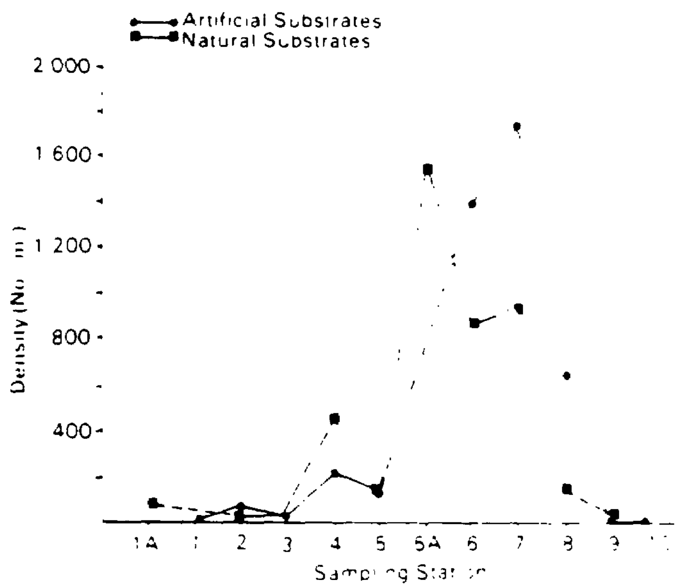


Figure 6-2 **Mean densities of *Berosus* sp. collected from Skeleton Creek and Boggy Creek, Enid, Oklahoma, August 1983**

between stations and Tukey's test did indicate that Stations 1A and 5 were significantly different (P 0.05) from Station 3. The lowest number of taxa were found at Stations 2, 3, and 8 with 13-14 taxa (Table D-1). The greatest number of taxa occurred at Stations 1A, 5, and 6 with 24-25 taxa.

6.2.2 Artifical Substrates

The greatest number of organisms collected by the artificial substrates was at Station 8 and of these 75 percent were from the Chironomidae family (Tables D-2 and 6-2). The mean number of organisms collected at Station 8 (14,951) is almost two and one-half times greater than at next highest values at Stations 3 and 7.

Similar to the data collected from natural substrates, there are differences in abundance between stations and in patterns of abundance for the major taxa. For one of the two most abundant major taxa, *Dicrotendipes* sp., peak mean densities were observed at Stations 3 and 7 with variations of up to two orders of magnitude (Figure 6-1). These station differences were highly significant ($P = 0.0006$) as shown by ANOVA results on the number of *Dicrotendipes* sp. and the Tukey's test results indicated that Station 3 was significantly different ($P \leq 0.05$) than Stations 8, 9, and 10. Mean densities of *Berosus* sp. varied by approximately two orders of magnitude with a maximum density at Station 7 of 1,755 organisms m^2 (Figure 6-2). Results of an ANOVA indicated that, similar to the case with natural substrates, the differences in *Berosus* sp. abundance between stations was highly significant ($P = 0.0001$). In addition, Stations 6, 7, and 8 were different ($P \leq 0.05$), due to the high abundance from all other stations. Peak mean densities also occurred at Station 7 for *Baetis* sp. (Ephemeroptera) and *Argia* sp. (Odonata), and at Station 8 for *Polypedilum* sp. and *Tanytarsus* sp. (Chironomidae).

Examination of the total number of macroinvertebrate taxa by ANOVA indicated that there were highly significant differences ($P = 0.0001$) between stations. Tukey's test results indicated that Station 8 was significantly different ($P \leq 0.05$) from Station 10. The number of chironomid taxa varied between stations, and these differences were significant ($P = 0.045$) according to ANOVA results, and Tukey's test results did not show significant differences. This finding is consistent with the results from the natural substrates—that there were no differences in the number of chironomid taxa between stations.

6.2.3 Gear Comparison

While the same major taxa were collected by collecting gears, the numbers and locations of taxa were not the same. In addition, the total number of organisms collected was generally higher using the artificial substrates (Table 6-3). However, these differences are expected due to the nature of the two substrates. The artificial substrates are composed of smooth homogenous surfaces which were suspended in the water column for 20 days and are relatively immobile during that period. The natural substrate of Skeleton Creek is principally bedrock with overlying shifting sand. The shifting sand offers some degree of instability to benthic fauna.

The Chironomidae family was the most abundant of any of the macroinvertebrate groups collected using either the natural or the artificial substrates. The number of chironomid taxa was similar for all stations for both substrates. The Chironomidae (midges) composed the largest proportion of their community, up to 90 percent at Station 5, with few exceptions. From the natural substrates, a trichopteran (caddisfly) composed over 40 percent of the community at Station 9 and from the artificial substrates, a coleopteran (beetle) composed over 50 percent of the community at Station 6.

There were two taxa which were noticeably more abundant in Skeleton and Boggy Creek using either substrate. The most abundant of the major taxa *Dicrotendipes* sp. (a chironomid) showed highly significant differences in numbers between stations for both substrates. The second most abundant taxon *Berosus* sp. (a coleopteran) also showed highly significant differences in numbers between stations for both substrates.

The number of chironomid taxa did not change significantly between stations. However, the abundance and composition for the other major taxa varied between the two types of substrates, and the total number of taxa did vary significantly between stations. For the natural substrates, the number of taxa collected at Station 3 was significantly lower than the number collected at Stations 1A and 5. The number of taxa collected by artificial substrates at Station 8 was significantly greater than Station 10.

6.3 Evaluation of the Macroinvertebrate Community

The macroinvertebrate community of Skeleton Creek and Boggy Creek was dominated by insects. The most abundant taxonomic group was the Chironomidae family (midges). Other major taxa were from the Coleoptera (beetles), Ephemeroptrae (mayflies), Trichoptera (caddisflies), and Odonata (dragonflies, damselflies) families.

The community composition changed between stations. Statistically signficant differences between stations were found using the total number of taxa collected, although the stations which were different

Table 6-3. Total Taxa Collected by Artificial and Natural Substrates and the Combined Macroinvertebrate Taxa at Each Sampling Station

	Sampling Station											
	1A	1	2	3	4	5	5A	6	7	8	9	10
Natural Substrate	25		14	13	16	23	16	24	18	13	19	
Artificial Substrate	--	13	21	20	21	20	--	23	25	28	18	13
Combined Substrate Total[a]	--	--	25	22	25	29	--	32	28	30	25	

[a]Total number of unique taxa in either natural or artificial substrate sample; numbers were tallied using Tables D-1 and D-2

NOTE -- Means no sample was available; see Chapter 2 for clarification

varied with substrate type. In addition, changes in the number of taxa for the most abundant group, the chironomids, were nonsignificant between stations.

7. *Fish Community Survey*

This study investigated the fish community in Skeleton Creek and Boggy Creek. Species abundance and composition were used as measures of community stability. A description of the sampling and analytical methods is in Appendix C. A list of fish species and families are given in Table D-7.

7.1 Community Structure

The fish community at Skeleton and Boggy Creek was composed of 11 taxa (Table 7-1). These taxa represented five families of fish. Three species were present at all but one station: red shiner (*Notropis lutrensis*), sand shiner (*Notropis stramineus*) and mosquitofish (*Gambusia affinis*) although the latter was not abundant. The most abundant taxa were the red and sand shiners and the early juvenile cyprinids (minnows). Most of the taxa collected in Skeleton Creek and Boggy Creek were from the Cyprinidae (minnows) or Centrarchidae (sunfish) families.

The number of fish taxa collected varied between 3-7 per station except at Station 5 where none were caught. The largest number of fish taxa were collected at Stations 2, 3 and 9 and the least at Station 7 and 8. The greatest numbers of fish were caught at Stations 3 and 6. The proportion of run habitat at Station 3 was 50 percent and approximately 100 percent at Station 6. At Station 3, red shiners composed over 35 percent of the catch (Table 7-1). In contrast, at Station 6, red shiners composed over 75 percent and sand shiners composed over 20 percent of the catch. Total number of other fish species caught were quite low, under 20 fish per station, with the exception of the mosquitofish.

7.2 Evaluation of the Fish Community

Another fish survey of Boggy and Skeleton Creek had been conducted in 1982 (JRB Associates, 1983). In that survey, four stream collection stations were used in locations similar to Stations 1, 3, 5, and 9 used in this study. The number of fish taxa varied between 2 and 6 in that earlier study (JRB Associates, 1983). Of the six species they collected, the most abundant was the red shiner. Lower catches and numbers of taxa at the two intermediate sites were regarded by JRB Associates (1983) as indicative of degradation.

Results of this 1983 Skeleton Creek survey revealed higher catches of fish and more numbers of taxa than previously (JRB Associates, 1983). Results of X[2] test on the number of taxa indicated no significant difference between stations, using either Station 1A (upstream) or Station 2 (maximum) as the expected value.

Table 7-1 Number of Fish Collected by Seine from Skeleton and Boggy Creeks, Enid, Oklahoma, August 1983

Taxa	1A	2	3	4	5	5A	6	7	8	9
					Sampling Station					
Notropis lutrensis	79	18	500	47		15	835	98	176	294
Notropsis stramineus	24	7	2	874		22	232	55	349	[illegible]
Notropis umbratilis			20	1						
Pimephales promelas	3	1		1		1	12			[illegible]
Phenacobis mirabilis										3
Notemigonus crysoleucas		5								
Lepomis megalotis	3	5	2							
Lepomis humilus			4							
Lepomis cyanellus										[illegible]
Ictalurus melas		5								
Gambusia affinis		24	[illegible]	6		3	11	53	27	[illegible]
Early juvenile Notropis	398	71	800	30		1				[illegible]
Early juvenile Carpoides							1	2		
Total number of fish	507	136	1,329	959	0	42	1,091	208	552	[illegible]
Total number of taxa[a]	4	7	6	5	0	4	4	3	3	[illegible]

[a]X[2] test results indicate nonsignificant differences between stations, using either Station 1A or Station 2 as [illegible]

The number of species is similar between stations which indicates that the community structure is unchanged between stations. However, there were fluctuations in the number of fish collected. Most notable was the paucity of the fish collected at Station 5A, where very few of the abundant red shiner, sand shiner, and young juvenile cyprinids were caught.

8. *Comparison Between Laboratory Toxicity Tests and Instream Biological Response*

8.0 Background

The comparison between toxicity measured in the laboratory on a few species and the impact occurring in the stream on whole communities must compensate for a very limited database from which to predict. The sensitivity of the test species relative to that of species in the community is almost never known and certainly not in these toxicity tests. Therefore, when toxicity is found, there is no method to predict whether many species in the community, or just a few, will be adversely affected at similar concentrations, since the sensitivity of the species in the community is not known. For example, at a given waste concentration, if the test species has a toxic response and if the test species is very sensitive, then only those species in the community of equal or greater sensitivity would be adversely affected. 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 mean sensitivity of organisms in the community if the test species is chosen without knowledge of its sensitivity (as was the case here).

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

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

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

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

The same is true of the test species. Sometimes the *Ceriodaphnia* respond like the periphyton and other times like the fish community. 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 or zooplankton,
2. Fathead minnow toxicity to always resemble toxicity to fish,
3. Fathead minnows and other fish to display the same relative sensitivity to different effluents.

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

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

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

Several measures of community structure are based on number of species, e.g., diversity and community loss index. Since diversity measures are little affected by changes in the number of species (or taxa) that are in very low densities in the community, diversity is an insensitive measure for some perturbations which can be measured by toxicity tests. The community loss index is based only on the presence or absence of specific species relative to a reference station and would be useful except that habitat differences between stations heavily affect 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 indictor 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. Identification of taxa to different levels can reduce the sensitivity of species richness. Even though species richness has numerous sources of error as a representative measure of community health, it remains the best measure for *comparison with* toxicological data. Species sensitivity will respond in the most direct way to toxic response of the community with the least interference.

8.1 Prediction of Instream Community Impacts Based on Effluent Dilution Test Results

The calculated Acceptable Effluent Concentrations (AEC) for each test species and effluent tested are presented in Table 8-1, as well as the Instream Waste Concentration (IWC) for each effluent downstream of the discharge. The AEC is based on the most sensitive endpoint of the most sensitive species. The Refinery IWC was about three times higher than the AEC while the IWC of the Fertilizer Plant was about five times the AEC. Based on these results, there should be noticeable ambient toxicity at the stations below each discharge and adverse effects on the instream biological community since some species would be

Table 8-1. Comparison Between the Acceptable Effluent Concentration (AEC) and the Instream Waste Concentration (IWC) for Effluents Tested

	AEC (Percent)		IWC (Percent)	
Effluent	Fathead Minnow[a]	Ceriodaphnia[b]	Station 3	Station 5
Refinery	5.5	17.3	17.6	12.0
Fertilizer Plant	5.5	17.3		28.3

[a]Calculated from data in Table 4-4
[b]Calculated from data in Table 4-5
Data from Table 3-3

expected to be as sensitive as the most sensitive test species.

For Station 3 below the Refinery, the IWC was estimated at 17.6 percent, which was much higher than the AEC. Therefore, toxicity instream at Station 3 was predicted, and ambient toxicity was increased at Station 3 (Table 8-2). At Station 4, Skeleton Creek and Boggy Creek have joined and a slight decrease in ambient toxicity was expected and was observed. Since the IWC of the Fertilizer Plant was five times the AEC at Station 5, ambient toxicity was predicted. The results of the ambient toxicity tests at Station 5 corroborated the prediction of the effluent dilution test by showing increased toxicity at Station 5. The prediction of impact at Station 5 could also have been made using the IWC of the Refinery (Table 8-1).

8.2 Comparison of Ambient Toxicity Test Results and Field Data

In order to make a prediction of impact from single species data, the station with the least toxicity or the most numbers of taxa was considered the least impacted and used as zero percent impact for comparative purposes. The percent impact at all other stations was then calculated from that value and each measurement (fathead minnow toxicity, daphnid toxicity, and reduced species richness) could have used a different reference station as zero percent impact (Table 8-2). The data for the number of benthic macroinvertebrate taxa from both the artificial and natural substrates were combined in order to obtain a total number of taxa found at each station where both kinds of samples were collected. The comparisons on Table 8-2 include Stations 2 through 9 only as Station 1A was sampled for macroinvertebrates on natural substrates only since Station 1 had become impounded by a beaver dam after the artificial substrates were set. This made the comparisons of the natural and artificial substrates impossible as the locations and the conditions the invertebrates were exposed to were quite different. Also, since Station 5A was added during the August field sampling no artificial substrate sample was collected and therefore 5A is eliminated from the overall comparison too. The zooplankton data are of limited value as few crustaceans and rotifers were collected. The trends of the percent increase in toxicity as predicted by combining the ambient toxicity test data are compared to the percent reduction in the number of taxa for the various biological field components in Table 8-2.

Table 8-2. Percent Increase in Toxicity and Percent Reduction in Number of Taxa for the Instream Biological Community

Station	*Ceriodaphnia* Young Production	Fathead Minnow Weight	Zooplankton Taxa	Combined Macroinvertebrate Taxa[b]	Fish Taxa
2	52	15	60	22	0
3	42	35	0	31	14
4	32	31	0	22	29
5	66	44	40	9	100
6	22	18	20	0	43
7	47	4	20	12	57
8	59	15	20	6	57
9	0	0	20	22	14

[a]Percent values were obtained by using the highest value for each measurement as the basis for zero percent impact.
[b]This is the total number of unique taxa found in either the artificial or natural substrates and totaled for the comparison. [illegible]
Sources: Tables 4-6, 4-7, 5-1, 6-3 and 7-1

Table 8-3 Percent of Correct Predictions Using Four Levels of Defined Impact

Combined Toxicity Data (Percent)	Combined Biological Field Data Percent			
	20-100	40-100	60-100	80-100
20-100	87.5	75.0	37.5	25.0
40-100	62.5	75.0	67.5	50.0
60-100	12.5	50.0	100	100
80-100	0	37.5	75.0	87.5

Source Table 8-3

Table 8-3 was constructed in the following manner. If both the toxicity data and all biological field data values were below 20 percent, a correct prediction was registered. If one or more toxicity value and one or more taxa values were over 20 percent, a correct prediction was registered. This was done for all stations and the correct prediction placed in the upper left cell of the table. The same procedure was used for each cell only changing the percentage to the appropriate value for that cell. The 20 percent incremental categories are arbitrarily selected.

The largest percentages of correct predictions were obtained, in general, when comparable percentages were compared, i.e., the highest values lie along a diagonal from upper left to lower right. This pattern is evidence that the degree of toxicity is related to the degree of taxa reduction. To verify this trend quantitatively, the degree of toxicity and reduction of taxa was evaluated by a correlation analysis. The correlation of the combined toxicity data (the greatest toxicity of either the fathead minnows and the *Ceriodaphnia*) and the reduction of the biological field data (fish, zooplankton, and invertebrates) was significant ($P \leq 0.01$). Figure 7-1 plots the greatest percent toxicity at each station with the greatest reduction in the field data that was subjected to the correlation analysis.

One level of percent reduction or increase in toxicity is not being proposed as the best percentage at this time. Each study that has been done will compare which reduction of the instream biological response data best corresponds to a specified level of laboratory toxicity. Comparisons for all sites studied need to be completed before any decisions and recommendation on the best percentage are made.

8.3 Summary

Ambient toxicity was measured at both stations where effluent tests predicted toxicity. There was a highly significant correlation between number of taxa and degree of toxicity.

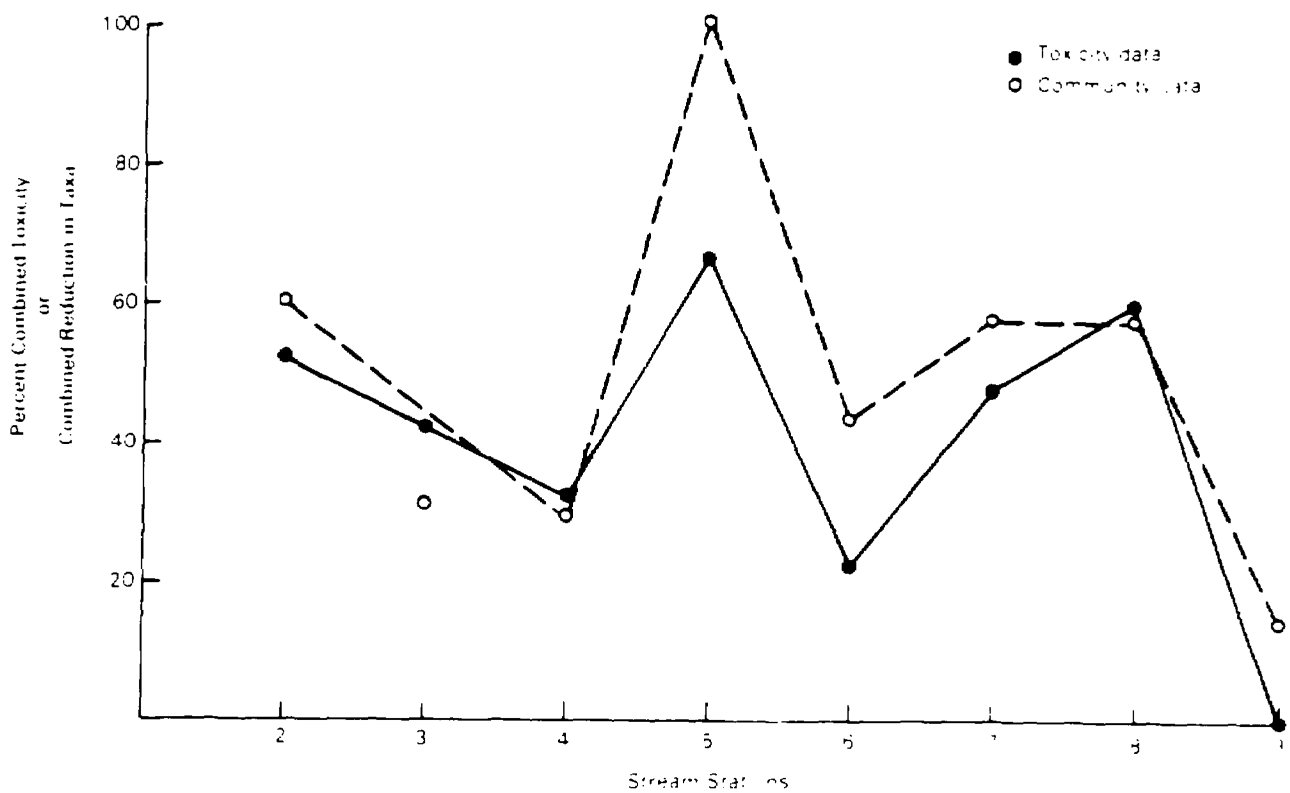


Figure 8-1 A comparison of percent toxicity and percent reduction of the taxa (Source. Table 8 3)

References

Baumgardner, R K 1966 Oxygen Balance in a Stream Receiving Domestic and Oil Refinery Effluents. Ph.D Thesis, Oklahoma State University. 70 pp

Hamilton, M A 1984 Statistical Analysis of the Seven-Day *Ceriodaphnia reticulata* Reproductivity Toxicity Test. EPA Contract J3905NASX-1 16 January. 48 pp.

JRB Associates 1983. Demonstration of the Site-Specific Criteria Modification Process Boggy and Skeleton Creeks, Enid, Oklahoma Draft Report prepared for U S EPA, Criteria of Standards Division, Washington, D C , EPA Contract 68-01-6388.

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. and T J Norberg-King. In press Validity of Effluent and Ambient Toxicity Testing for Predicting Biological Impact on the Kanawha River, Charleston, West Virginia. EPA/600 Research Series.

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/600/8-85/015.

Mount, D I., N. A Thomas, T J. Norberg, M T Barbour, T H Roush, and W. F. Brandes 1984 Effluent and Ambient Toxicity Testing and Instream Community Response on the Ottawa River, Lima. Ohio EPA Research Series, EPA 600/3-84/084

Namminga, H E 1975. Heavy Metals in Water, Sediments, and Chironomids in a Stream Receiving Domestic and Oil Refinery Effluents. Ph.D. Thesis, Oklahoma State University 108 pp.

Norberg, T. J and D I Mount 1985 A New Fathead Minnow (*Pimephales promelas*) Subchronic Toxicity Test. Environ. Toxicol Chem 4(5).

Robins, C R , R M Bailey, C E Bond, J R. Brooker, E A Lachner, R N Lea, and W B Scott, eds 1980 A List of Common and Scientific Names of Fishes from the United States and Canada American Fisheries Society Special Publication No 12, Fourth Edition Committee on Names of Fishes, Bethesda, Maryland 174 pp.

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

Wilhm, J L. 1965. Species Diversity of Benthic Macroinvertebrates in a Stream Receiving Domestic and Oil Refinery Effluents. Ph D Thesis, Oklahoma State University 42 pp

Appendix A
Hydrological Sampling and Analytical Methods

A.1 Flow Measurements

Stream flows were measured from 9-11 August using a Teledyne Gurley Pygmy flowmeter Measurements were made once at Stations 1 through 9, including an additional measurement downstream of the Refinery At each station, measurements were made at intervals of 0 3 to 0 6 m depending on the width of the transect such that a minimum of 10 velocity measurements were made

The water depth was recorded with each measurement Following standard hydrological methods for shallow streams (< 0 75 m), velocity measurements were made at depths of 60 percent of the water column

A.2 Flow Contribution Calculations

The mean contribution, in percent of the total flow, was calculated using the measured stream velocities, plant operating records and USGS gauging station data The upstream flow values form Stations 1 and 2 for Boggy Creek and Skeleton Creek, respectively, were used

Appendix B
Toxicity Test and Analytical Methods

B.1 Sampling and Sample Preparation

A 24-hour composite sample of Refinery effluent was collected 10-11 August 1983, as well as composite samples of the stream stations. Automatic ISCO samplers were set to collect an aliquot every 15 minutes and composite samples were collected in 5-gal polyethylene containers. The Fertilizer Plant effluent was a partial composite and a partial grab sample from the holding ponds on 10 August. The samples were cooled to approximately 10°C and transported to ERL-Duluth where they were stored until use at 8°C. Testing began 14 August 1983. Test solutions were renewed daily. Each day 2 L were removed and warmed to 25°C. The effluent and the dilution water were warmed separately, and dissolved oxygen levels checked for supersaturation. Ambient stations were also warmed to 25°C over a propane heater and aerated until saturation was 100 percent.

The effluents were diluted with river water (Station 1A) that was collected upstream of the Refinery. Dilutions were made using polypropylene or polyethylene beakers and glass graduated cylinders. Two liters of each concentration were made and 0.200 L were used for the *Ceriodaphnia* tests and the rest for the fathead minnow tests. After the 2 L were prepared, the dissovled oxygen (DO), pH, hardness, and conductivity were measured. The DO and pH meters were calibrated daily prior to readings. At the time of renewal, the DO was measured in one compartment in each fathead minnow test chamber (see Section B.3) and in at least one cup of the *Ceriodaphnia* test in each exposure. DO was measured daily early in the morning after the lights were on to evaluate any effects of diurnal DO cycles. DO values in the 100 and 30 percent of the Fertilizer Plant effluent were low, but otherwise no effects due to DO levels were noticed. A series of effluent concentrations of 100, 30, 10, 3, and 1 percent were used in the effluent dilution tests. For the ambient toxicity tests, the samples were run without dilution.

B.2 *Ceriodaphnia* Test Method

Adult *Ceriodaphnia* from the ERL-Duluth culture were used as brood stock, and the adults were not acclimated in the dilution water prior to testing. The tests were started with less than 6-hour-old *Ceriodaphnia*. Glass beakers, 30-ml which contained 15 ml of test solution, were used. Test solutions were renewed daily and young, if present, were counted and discarded. The animals were fed 0.05 ml of a yeast food every day, for a concentration of 250 μg yeast. Temperatures were maintained at 25 ± 1°C by means of a constant temperature cabinet. The test procedure was that of Mount & Norberg, 1984.

B.3 Fathead Minnow Test Method

The methods used followed closely those described by Norberg and Mount (1985). The test chambers were 30.5 cm x 15.2 cm x 10.2 cm high and are divided into four compartments; this design allowed four replicates for each concentration. The larval fathead minnows were less than 24 hours old post hatch and were from the ERL-Duluth culture. The fish were assigned to the test compartments by pipetting 1 or 2 fish at a time to each replicate test chamber across all concentrations until all replicates had ten fish in each or forty per concentration. 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 ± 1°C. A 16-hour light photoperiod was used.

After seven 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 preweighed aluminum weigh pans and weighed on a five-place analytical balance.

B.4 Quantitative Analyses

B.4.1 Ceriodaphnia

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

B.4.2 Fathead Minnows

The four groups' mean weights are statistically analyzed 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 is then compared to the critical t-statistic for the standard two-tailed Dunnett's test (Steele and Torrie 1960) The survival data are arcsine-transformed prior to the regression analyses to stabilize variances for percent data to show significant differences, however, actual survival values of the replicates are given in Tables 4-3 and 4-6

Appendix C
Biological Sampling and Analytical Methods

Estimated pool and riffle proportions and percent cover information are provided in Table C-1. Table 2-1 provides information on which stations were sampled for each survey.

C.1 Plankton Survey

Plankton were collected from ten stations on Skeleton Creek and Boggy Creek near Enid, Oklahoma, on 8-11 August 1983. Duplicate samples were collected at each station using a Wisconsin-type plankton net with a 80-μm mesh. The net was held stationary in the water for two minutes (only one minute at Station 9). The samples were transferred to bottles pre-charged with formalin. The volume filtered was calculated the time required for a float to travel a 3-m distance and the net diameter, assuming 100 percent filtering efficiency.

The samples were thoroughly mixed and an aliquot removed. Two subsamples from each replicate sample were analyzed using a Sedgewick-Rafter counting chamber. Identifications were made using a compound microscope at 100X magnification. All organisms in the chamber were enumerated and identified to a convenient taxon, except the solitary diatoms. For diatoms, one short-dimension optical strip was enumerated. Abundance was standardized to number per liter for density comparisons.

The crustacean and rotifer densities were analyzed by Analysis of Variance (ANOVA). One-way ANOVAs were performed to determine differences between stations. Tukey's Honestly Significant Difference (HSD) tests were conducted to determine which stations were different when a significant difference was detected using the ANOVAs.

C.2 Macroinvertebrate Survey

C.2.1 Sample Collection

C.2.1.1 Natural Substrates

Natural substrates at ten stations were sampled on Skeleton Creek and Boggy Creek from 8-11 August 1983. A 1-ft^2 Hess-style sampler was used with a 800 x 900-um mesh net. Triplicate samples were collected in riffle areas or similar areas and then preserved in 10 percent formalin.

Table C-1. Station Description Information and Estimated Proportions of Riffle and Pool for Skeleton Creek and Boggy Creek, Enid, Oklahoma

Station	Estimated Percent Cover	Percent Riffle	Riffle Width (m)	Riffle Length (m)	Percent Pool	Pool Width (m)	Pool Length[a] (m)	Estimated area (m^2) for Fish Seine
1A	100	16	4.5	6.1	84	6.1	30	210
1	100	0	--[c]	--[c]	100	--[c]	--[c]	
2	90	40[b]	0.9	6.1	60	3.6	9.1	106
3	80	50[b]	6.1	9.1	50	9.1	18.2	191
4	0	100[b]	9.1	30.5	0	--	-	214
5	10	40	4.6	6.1	60	9.1	15.2	106
5A	0	23	3.0	9.1	77	9.1	30.5	214
6	0	100[b]	12.2	30.5	0	--		210
7	0	27	6.1	9.1	73	12.2	24.4	171
8	0	16	6.1	6.1	84	13.7	30.5	214
9	20	27	12.2	9.1	73	12.2	24.4	171
10	0	0	--[c]	--[c]	100	10.7	--[c]	

[a]Estimated sampled length for the fish survey; actual length may be longer.
[b]Run habitat only.
[c]These stations sampled with artificial substrates only.

C.2.1.2 Artificial Substrates

Quadruplicate Hester-Dendy multiplate artificial substrates were suspended in the water column at each of the ten stream stations on 20 July 1983 and removed on 9 August resulting in a 20-day colonization period. Substrates were collected using a small-mesh net. Each substrate was disassembled and scraped and the collections were preserved in 10 percent formalin. The Hester-Dendy substrates have an effective surface area of 0.093 m^2.

C.2.2 Sample Analysis

The samples were washed in tap water and flooded with a sugar solution to separate debris and organisms. The floating organisms were removed and placed in 70 percent ethanol. The debris was examined to detect non-floating or entangled organisms using a dissecting microscope at 8X magnification. Organisms were enumerated and identified to genus or lowest reasonable taxa. Abundance was standardized to number per square meter for density comparisons.

ANOVAs were conducted on the counts of major taxa to determine differences between stations. The two major taxa were *Dicrotendipes* sp. and *Berosus* sp. Tukey's HSD tests were conducted when significance was detected using ANOVA, to determine which stations were different. In addition, ANOVAs were conducted on the number of Chironomidae taxa and the total number of macroinvertebrate taxa to discern differences between stations. Tukey's HSD was used when the ANOVAs showed significant differences to identify which of the stations were different.

C.3 Fish Survey

Fish seining was done at ten stations on Skeleton Creek and Boggy Creek on 8-11 August 1983. At most 30.5 m of the stream was seined at each station using a woven net 1.2 m x 9.1 m, with a 0.5-cm mesh. Collections were preserved in 10 percent formalin. Fish were enumerated and identified to species or lowest practicable taxon.

The number of fish taxa per station were examined using a X^2 test. This test was performed with Station 1A as the expected value, and again with Station 2 as the expected value.

Appendix D
Biological Data

Table D-1. Mean Density (No./m²) of Benthic Macroinvertebrates Collected from Natural Substrates in Skeleton Creek and Boggy Creek, Enid, Oklahoma, August 1983

Taxa	Sampling Station									
	1A	2	3	4	5	5A	6	7	8	9
Ephemeroptera										
Caenis sp	251	7	14	--	29	39	11	39	--	-
Tricorythodes sp	7	4	--	--	11	79	86	--	--	86
Baetis sp	11	--	--	--	--	--	--	36	11	197
Stenonema sp	--	--	--	--	--	--	--	--		7
Choroterpes sp	--	--	--	--	--	--	--	--	-	29
Total Ephemeroptera	269	11	14	0	40	118	97	75	11	319
Trichoptera										
Hydroptilidae	29	--	--	--	--	--	4	--	--	--
Cheumatopsyche sp	14	--	--	--	4	--	4	29	104	1 137
Hydropsyche sp	--	--	-	--	--	--	--	--	7	273
Hydropsychidae pupae	--	--	--	--	--	--	--	--	--	22
Total Trichoptera	43	0	0	0	4	0	8	29	111	1 432
Coleoptera										
Laccophilus sp	--	--	--	--	4	--	--	-	-	
Peltodytes sp	--	--	--	4	--	--	--	-	-	
Berosus sp	90	29	11	459	126	1.159	861	947	151	32
Stenelmis sp	14	--	--	--	7	--	4	--		18
Dubiraphia sp	--	--	--	--	4	--	--	-	-	
Total Coleoptera	104	29	11	463	141	1 159	865	947	151	50
Odonata										
Dromogomphus sp	--	--	--	--	4	--	--	--	-	
Gomphus sp	--	--	--	--	--	--	--	-	--	11
Plathemis sp.	--	--	--	22	--	--	--	--	--	
Libellula sp	--	--	--	4	--	--	--	--	-	
Argia sp	90	--	--	--	--	4	29	--	--	
Total Odonata	90	0	0	26	4	4	29	0	0	11
Megaloptera										
Corydalis sp	--	--	--	--	--	--	-	-	--	22
Neohermes sp	--	--	--	--	--	--	--	4	-	
Total Megaloptera	0	0	0	0	0	0	0	4	0	22
Diptera										
Chironomus sp	14	--	488	513	703	355	4	11	--	
Dicrotendipes sp	352	298	466	553	1 568	621	312	240	122	7
Polypedilum sp	154	43	18	14	39	14	14	4	746	481
Cryptochironomus sp	--	22	7	4	4	--	--	--	11	4
Pseudochironomus sp	7	102	--	-	--	--	4	4	11	
Kiefferulus sp	--	--	--	--	25	29	14	--	--	
Tanytarsus sp	90	22	104	387	50	47	11	43	402	36
Micropsectra sp	25	--	--	-	--	--	--	--	--	-
Cricotopus sp	14	43	7	--	434	176	240	352	197	4
Psectrocladius sp	--	--	--	--	4	--	7	-		
Ablabesmyia sp	65	4	11	79	248	111	79	258	196	25
Pentaneura sp	11	--	--	--	--	--	--	-		
Tanypus sp	4	7	32	606	22	50	-	4	--	
Procladius sp	--	--	--	29	--	--	-	-		
Chironomidae pupae	32	--	61	25	811	305	147	90	194	47

Table D-1 (Continued)

Taxa	Sampling Station 1A	2	3	4	5	5A	6	7	8	9
Palpomyia sp	--	--	-	--	--	-	-	4		
Probezzia sp	11	14	7	65	4	4	-	4		
Simuliidae	4	--	--	-	--	-			--	
Diptera pupae[a]	-	7	--	-	-	-	--	--		
Total Diptera	783	562	1,201	2 275	3,912	1 712	832	1 014	1 879	604
Hemiptera										
Belastoma sp	--	-	-	-	--	--	4			
Corixidae	--	--	--	--	61	32	68	4	-	
Total Hemiptera	0	0	0	0	61	32	72	4	0	0
Others										
Gastropoda										
Physidae	29	50	4	208	97	11	47	129	22	7
Ancylidae	32	--	--	--	--	--	4	-		
Pelecypoda										
Sphaeriidae	4	--	--	-	--	-	18	-		
Amphipoda										
Talitridae	7	--	--	39	4	--	4	-		
Oligochaeta (unidentified)	47	93	104	14	36	814	169	11	215	158
Annelida										
Hirudinea	--	-	--	-	-	-	7			
Total number of taxa[b]	25	14	13	16	23	16	24	18	13	19
Total number of individuals/m²	1 408	745	1,334	3 025	4 299	3 850	2 152	2 213	2 389	2 603

[a]Unidentified, non-Chironomidae pupae

[b]Does not include pupae

Note: Values are rounded to nearest integer

Table D-2. Mean Density (No./m²) of Macroinvertebrates Collected from Artificial Substrates in Skeleton Creek and Boggy Creek, Enid, Oklahoma, August 1983

Taxa	Sampling Stations 1	2	3	4	5	6[a]	7	8	9	10
Ephemeroptera										
Caenis sp	126	70	11	27	11	11	65			3
Tricorythodes sp	--	--	--		--			19	75	3
Baetis sp	8	54	16	65	65	226	1 043	188	126	
Stenonema sp	--	8	3					5	16	3
Choroterpes sp	--	-		-	-			8		
Total Ephemeroptera	134	132	30	92	76	237	1 108	220	217	9
Trichoptera										
Chimarra sp			-					13		
Hydroptilidae								3		
Cheumatopsyche sp	-	--	--					2 027	145	
Hydropsyche sp						4		554	1 473	[illegible]
Hydropsychidae pupae		-	-			-		70	86	3
Total Trichoptera	0	0	0	0	0	4	0	2 667	1 704	[illegible]
Coleoptera										
Tropisternus sp			-			7	8			
Laccophilus sp	-					7				
Berosus sp		73	13	207	135	1 387	1 755	654		
Stenelmis sp	--	5	8	11	-		5	108	46	
Total Coleoptera	0	78	21	218	135	1 401	1 768	762	46	[illegible]
Odonata										
Libellula sp		3		3						
Argia sp	38	234	177	183	22	143	230	[illegible]	[illegible]	[illegible]
Hetaerina sp							--			
Ischnura sp		40		43	3	22	5			
Total Odonata	38	277	177	227	25	165	[illegible]	[illegible]	[illegible]	[illegible]

Table D-2. (Continued)

Taxa	Sampling Stations 1	2	3	4	5	6[a]	7	8	9	10
Megaloptera										
Chauliodes sp	--	--	--	--	--	--	--	13	--	
Neohermes sp	--	--	59	24	3	7	8	32	--	
Total Megaloptera	0	0	59	24	3	7	8	45	0	0
Diptera										
Chironomis sp	99	13	212	35	631	143	667	5	3	--
Dicrotendipes sp	1.038	2.368	2.640	1.790	1.352	190	1 691	83	126	30
Polypedilum sp	11	27	43	5	5	4	8	6.024	1 769	202
Cryptochironomus sp	--	--	3	--	--	7	11	5	--	
Pseudochironomus sp	13	8	--	--	--	--	38	--	--	--
Kiefferulus sp	--	3	5	--	357	--	--	--	48	30
Tanytarsus sp	83	89	3	3	5	14	27	3.255	226	8
Tribelus sp.	--	--	--	--	--	--	--	--	--	24
Cricotopus sp.	--	--	99	11	56	36	19	5	48	3
Psectrocladius sp.	--	--	314	81	22	36	186	124	40	3
Ablabesmyia sp	253	250	1.938	441	653	229	267	728	204	153
Pentaneura sp	8	16	11	22	3	--	--	30	--	
Corynoneura sp.	--	--	--	--	--	--	--	--	13	
Tanypus sp	13	54	5	75	454	36	164	--	3	--
Procladius sp.	--	8	--	19	--	--	--	--	--	
Chironomidae pupae	78	353	260	75	505	65	56	817	148	118
Palpomia sp	--	27	--	--	--	--	3	3	--	--
Probezzia sp	5	8	--	--	3	--	--	3	--	--
Tabannus sp	--	--	--	--	--	--	3	--	--	--
Atherix sp.	--	--	--	--	--	--	--	3	--	--
Hemerodromia sp	--	--	--	--	--	--	--	3	--	--
Total Diptera	1.601	3.224	5.533	2.557	4.046	764	3.140	11.088	2 628	571
Hemiptera										
Belastoma sp.	--	--	--	--	--	4	--	--	--	--
Corixidae	--	--	--	--	--	7	3	--	3	
Total Hemiptera	0	0	0	0	0	11	3	0	3	0
Others										
Gastropoda										
Physidae	--	226	11	427	13	25	13	32	--	--
Amphipoda										
Talitridae	16	--	40	223	32	32	161	19	--	--
Oligochaeta	--	--	--	8	--	--	--	--	--	
Annelidae										
Hirudinea	--	--	--	--	5	--	8	--		--
Total Number of Taxa[b]	13	21	20	21	20	23	25	28	18	13
Total Number of Individuals/m^2	1.789	3.717	5.871	3.356	4.325	2.650	6.505	14 951	4 730	798

[a]Station 6 had only three replicates

NOTE Values are rounded to nearest integer

Table D-3. Analysis of Variance and Tukey's Studentized Range Test Results for Zooplankton, Skeleton Creek, August 1983

Crustaceans

Dependent Variable ln Count

Source	dF	Sum of Squares	Mean Square	F Value	PR > F
Station	9	27 67	3 07	16 93	0 0001
Error	30	5 44	0 18		
Corrected total	39	33 12			

Tukey's Studentized Range Test

Station	7	3	8	4	6	2	1A	9	5A	5
Mean	2 44	2 06	0 55	0 44	0 34	0 24	0 15	0 05	0 05	0 0

Table D-3 (Continued)

Rotifers

Dependent Variable ln Count

Source	dF	Sum of Squares	Mean Square	F Value	PR > F
Station	9	2985.23	331.69	197.82	0.0001
Error	30	50.30	1.68		
Corrected total	30	3035.53			

Tukey's Studentized Range Test

Station	7	6	5A	5	4	3	8	9	2	1A
Mean	30.64	14.59	10.57	7.80	5.18	5.13	3.74	2.39	0.47	0.18

Table D-4. Analysis of Variance and Tukey's Studentized Range Test Results for the Two Most Abundant Macroinvertebrate Taxa from the Natural Substrates, Skeleton Creek, August 1983

Dicrotendipes sp

Dependent Variable Count

Source	dF	Sum of Squares	Mean Square	F Value	PR > F
Station	9	33.96	3.77	5.12	0.0001
Error	20	14.73	0.74		
Corrected total	29	48.69			

Tukey's Studentized Range Test

Station	5	5A	3	6	4	2	1A	7	8	9
Mean	4.78	4.00	3.61	3.39	3.15	3.04	3.03	3.03	2.50	0.46

Berosus sp

Dependent Variable Count

Source	dF	Sum of Squares	Mean Square	F Value	PR > F
Station	9	51.30	5.70	8.03	0.0001
Error	20	14.19	0.71		
Corrected total	29	65.49			

Tukey's Studentized Range Test

Station	7	6	5A	4	5	8	1A	9	2	[illegible]
Mean	4.40	4.38	4.12	3.31	2.52	2.49	1.94	1.36	1.23	[illegible]

Table D-5. Analysis of Variance and Tukey's Studentized Range Test Results for the Two Most Abundant Macroinvertebrate Taxa from the Artificial Substrates, Skeleton Creek, August 1983

Dicrotendipes sp

Dependent Variable Count

Source	dF	Sum of Squares	Mean Square	F Value	PR > F
Station	9	297 239 27	33 026 58	4 77	0 0006
Error	29	200.670 17	6.916 67		
Corrected total	38	497.909 44			

Tukey's Studentized Range Test

Station	3	2	4	7	5	1	6	9	8	10
Mean	245 50	220 25	166 50	157 25	125 75	96 50	17 67	11 75	7 75	2 75

Berosus sp

Dependent Variable Count

Source	dF	Sum of Squares	Mean Square	F Value	PR > F
Station	9	120.450 58	13.383 40	64 26	0 0001
Error	29	6.039 42	208 25		
Corrected total	38	126.490 00			

Tukey's Studentized Range Test

Station	7	6	8	4	5	2	3	1	9	10
Mean	163 25	129 33	60 75	19 25	12 50	6 75	1 25	0 0	0 0	0 0

Table D-6. Analysis of Variance and Tukey's Studentized Range Test Results for Numbers of Macroinvertebrate Taxa, Skeleton Creek, August 1983

Natural Substrate Data

Total Number of Taxa

Dependent Variable Count

Source	dF	Sum of Squares	Mean Square	F Value	PR > F
Station	9	174 87	19 44	3 74	0 0067
Error	20	104 00	5 20		
Corrected total	29	278 97			

Tukey's Studentized Range Test

Station	1A	5	6	9	5A	7	4	8	2	3
Mean	17 00	15 67	15 00	14 33	13 33	12 67	12 00	11 00	10 67	8 67

Total Number of Chironomidae Taxa

Dependent Variable Count

Source	dF	Sum of Squares	Mean Square	F Value	PR > F
Station	20	28 83	3 20	1 48	0 2224
Error	9	43 43	2 17		
Corrected total	29	72 17			

Tukey's Studentized Range Test was not performed since the ANOVA results were nonsignificant

Table D-6 (Continued)

Artificial Substrates

Total Number of Taxa

Dependent Variable Count

Source	dF	Sum of Squares	Mean Square	F Value	PR F
Station	9	380 33	42 26	10 44	0 0001
Error	29	117 42	4 04		
Corrected total	38	497 75			

Tukey s Studentized Range Test

Station	8	7	4	6	2	9	3	5	1	10
Mean	18 75	17 25	16 75	16 67	15 50	14 75	14 00	13 50	9 75	8 50

Total Number ofChironomidae Taxa

Dependent Variable Count

Source	dF	Sum of Squares	Mean Square	F Value	PR F
Station	9	31 06	3 44	2 27	0 0453
Error	29	43 92	1 51		
Corrected total	38	74 92			

Tukey's Studentized Range Test

Station	7	4	5	6	9	3	2	8	1	10
Mean	8 75	8 25	7 75	7 67	7 50	7 25	6 75	6 50	5 25	5 75

Table D-7. List of Fish Species and Families Collected from Skeleton Creek and Boggy Creek Near Enid, Oklahoma[1]

Family	Scientific Name	Common Name
Cyprinidae (minnows)	*Notropis lutrensis*	Red shiner
	Notropis stramineus	Sand shiner
	Notropis umbratilis	Redfin shiner
	Pimephales promelas	Fathead minnow
	Phenacobis mirabilis	Suckermouth minnow
	Notemigonus crysoleucas	Golden shiner
	Notropis spp	Early juvenile cyprinids
Centrarchidae (sunfish)	*Lepomis megalotis*	Longear sunfish
	Lepomis cyanellus	Green sunfish
	Lepomis humilis	Orangespotted sunfish
Ictaluridae (catfish)	*Ictalurus melas*	Black bullhead
Poeciliidae (livebearers)	*Gambusia affinis*	Mosquitofish
Catastomidae (suckers)	*Carpiodes* spp	Early juvenile catastomids

[1]Names follow Robins et al. (1980)

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