Aquatic Macroinvertebrate Water Quality Assessment
Boggy Branch of Brushy Creek
Atmore, Alabama

Environmental Indicators Section
Field Operations Division
Alabama Department of Environmental Management

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Boggy Branch at Atmore, Alabama

Introduction

The Boggy Branch of Brushy Creek at Atmore, Alabama is utilized by the City of Atmore (NPDES permit # AL0049557) and by Masland Carpets (NPDES permit # AL0021997) as a receiving stream for their treated wastewater effluents. Staff members of the Environmental Indicators Section, Field Operations Division of the Alabama Department of Environmental Management (ADEM), at the request of the Municipal Branch of the Water Division of ADEM, conducted an aquatic macroinvertebrate water quality assessment in an effort to assess the effects of the wastewaters on the aquatic macroinvertebrate community of Boggy Branch. An assessment was performed on December 10-11, 1996, approximately two weeks after short-term chronic toxicity tests of both effluents demonstrated that the Atmore wastewater treatment plant (WWTP) and Masland Carpets were not meeting their toxicity requirements (See Appendix A). A more comprehensive assessment was performed on October 1-2, 1997 to evaluate the stream conditions during a low flow season of the year.

Sampling Locations and Methodology

The following sampling locations were chosen for Boggy Branch (see Figure 1) based on effluent discharge locations. In addition, an established ecoregional reference stream with similar stream characteristics and habitat types was sampled to further assess the conditions of the stream.

Station	Description
BOB-1 (control)	Boggy Branch of Brushy Creek at unnamed Escambia County Road. T1N, R5E, S25, SE¼, NE¼. Latitude: 31° 01' 03.7" N Longitude: 087° 30' 48.8" W
BOB-2	Boggy Branch of Brushy Creek near eastern point of Atmore WWTP and downstream of Masland Carpets effluent discharge. T1N, R5E, S25, SE¼, NE¼. Latitude: 31° 01' 01.4" N Longitude: 087° 30' 53.3" W
BOB-3	Boggy Branch of Brushy Creek approximately 100 yards downstream of Atmore WWTP effluent discharge. T1N, R5E, S25, SE¼, NE¼. Latitude: 31° 00' 58.3" N Longitude: 087° 30' 56.0" W
HLB-1 (ecoregional reference)	Halls Creek in Baldwin County. Latitude: 31° 03' 10.4" N Longitude: 087° 50' 12.6" W

Macroinvertebrate samples were collected using the intensive Multihabitat Bioassessment method (MB-I) described in the ADEM Standard Operating Procedures (SOP) and Quality Control Assurance (QCA) Manual, Volume 2 (1996). Habitat quality was assessed using the

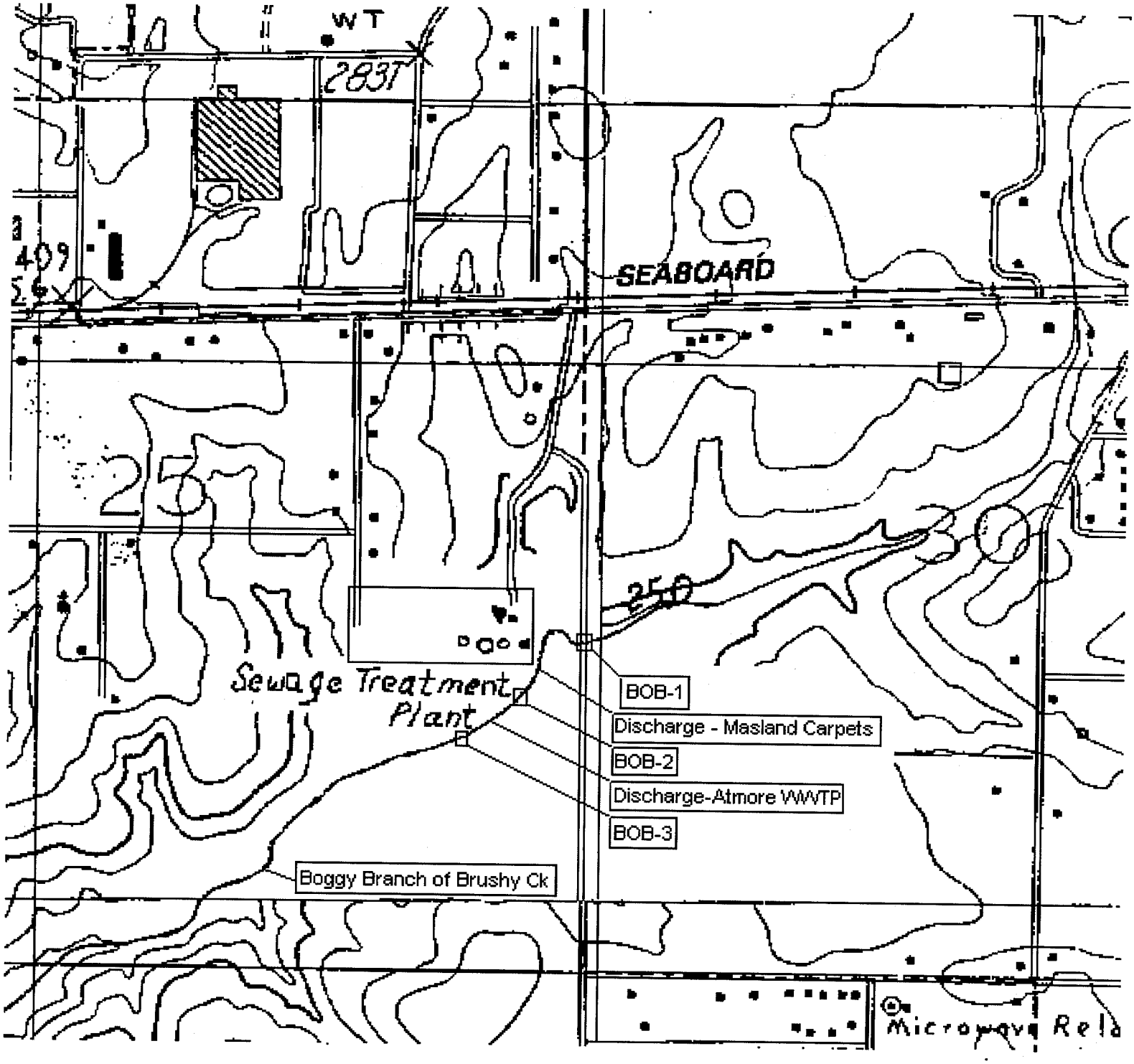

Figure 1
Sampling Locations
Boggy Branch of Brushy Creek

modified Barbour & Stribling (1996) habitat assessment form. All macroinvertebrate sample assessments were calculated using the Biological Condition Scoring Criteria (BCSC) (EPA 1989). Table 2 provides a simplified interpretation of the biological metrics used to evaluate this stream.

Instream water samples collected for field parameters and chemical analyses were grab collections using the methodology outlined in Volume 1 of the ADEM SOP and QCA Manual (1994).

Samples collected from the WWTP discharge for toxicity testing were 24-hour composite samples taken at the permitted sampling point. The toxicity test was conducted as specified in NPDES permit numbers AL0021997 and AL0049557 and per methodology outlined in ADEM SOP and QCA Manual , Volume 4 (1994).

Sample handling techniques, physical data collection and chain-of-custody procedures utilized during this assessment were as described in the ADEM Standard Operating Procedures and Quality Control Assurance Manual, Volumes 1 (1994), 2 (1996) and 4 (1994). Chain-of-custody was maintained by locking the samples in a departmental vehicle when not in sight of a Field Operations employee.

Discussion and Results

A. Physical

Boggy Branch of Brushy Creek is a first order stream over the length of the study reach. It drains primarily urban and suburban developments and lies within the Southern Pine Plains and Hills sub-ecoregion (65f). Boggy Branch has an estimated 50%-80% shaded canopy with trees as the dominant riparian vegetation on moderately stable banks. The stream is approximately eight feet wide with a predominantly sandy bottom and varies in depth from approximately 2 feet in run areas to 3 feet in pool areas. As indicated by the permitted in-stream waste concentration of 100% (see Appendix A), the flow of this stream is comprised largely of the combined effluent discharges of the Atmore WWTP and Masland Carpets. Multiple habitats suitable for colonization by aquatic macroinvertebrates are present and habitat assessments at all sampling locations are similar (Table 3). Boggy Branch of Brushy Creek lies within the Perdido River drainage basin.

The ecoregional reference site HLB-1 was similar to the study stations in stream characteristics and habitat types. The habitat quality (Table 3) of the three study locations was within eighty-eight percent of the ecoregional reference chosen.

B. Chemical

The Water Use Classification for Boggy Branch of Brushy Creek is Fish and Wildlife (F&W). The F&W classification specifies the waters to be suitable for fishing, propagation of fish, aquatic life, and wildlife, and any other usage except for swimming, and water contact sports or as a source of water supply for drinking or food processing purposes (Rules and Regulations: Water Quality Criteria and Use Classifications, Water Division-Water Quality Program, ADEM, Ch.335-6-10).

As shown in Figure 2 *(dissolved oxygen)*, data collected from Boggy Branch during December 1996 indicated that the waters at all stations appeared to be meeting the dissolved oxygen standard for the F&W classification (5.0 mg/L). However, samples collected during October 1997 indicated that BOB-2 fell below the 5.0 mg/L standard. BOB-1 and BOB-3 were documented as being above the standard by 0.3 mg/L and 0.1 mg/L, respectively.

Stream pH data (Figure 2*(pH)*) from both assessments indicated that the addition of the two effluents was altering the pH more than one standard unit as compared to the control BOB-1. The F&W classification for pH states that, "Sewage, industrial wastes, or other wastes shall not cause the pH to deviate more than one unit from the normal or natural pH, nor be less than 6.0, nor greater than 8.5."

Similarly, specific conductivity and turbidity (Figure *2(conductivity)*, *(turbidity)*) showed increases above background below the two effluents. The F&W classification for turbidity states that, "There shall be no turbidity of other than natural origin that will cause substantial visible contrast with the natural appearance of waters or interfere with any beneficial uses which they serve. Furthermore, in no case shall turbidity exceed 50 Nephelometric units above background. Background will be interpreted as the natural condition of the receiving waters without the influence of man-made or man-induced causes."

Boggy Branch of Brushy Creek does not appear to be meeting its Water Use Classification of Fish and Wildlife. It should be noted that for pH, conductivity and turbidity, increases above background were noted immediately downstream of the effluent discharge of Masland Carpets. While an increase above background was also noted below the Atmore WWTP effluent discharge, pH, conductivity and turbidity concentrations were appreciably lower than at BOB-2.

C. Aquatic Macroinvertebrate Assessment

Table 2 provides a simplified interpretation of the biological metrics used to evaluate this stream and should be referred to during the following discussion.

As demonstrated in Figures 3 through 7 and Tables 3 through 4, aquatic macroinvertebrates collected from Boggy Branch during both assessments showed an adverse impact from the addition of the effluent from the Masland Carpets discharge. Compared to the control station BOB-1, BOB-2 experienced a reduction in the number of taxa groups present. The dominant taxa group was the Family Chironomidae (Figures 4 through 7, Table 4). When analyzed according to the biological condition scoring criteria developed by EPA (Plafkin 1989), BOB-2 when compared to BOB-1 (Table 3) was evaluated as moderately impaired.

BOB-3, when compared to the control station BOB-1, showed a slight improvement in both the numbers of organisms and types of organisms present (Figures 4 through 7, Table 4). This is most probably the result of an increased stream flow due to the addition of the effluent of the Atmore WWTP. When analyzed according to the biological condition scoring criteria (Table 3), BOB-3 when compared to BOB-1 was evaluated as nonimpaired to slightly impaired.

At the time of this study, Field Operations personnel collected aquatic macroinvertebrate data from an established ecoregional reference station (HLB-1) with similar stream characteristics, habitat types, and within the same ecoregion as Boggy Branch. Figures 4-7, and

Tables 3 and 4 indicate that, as compared to a reference station, Boggy Branch is moderately impaired at BOB-1 and BOB-2, but only slightly impaired at BOB-3.

Conclusions

The results of this study indicate the water quality of the Boggy Branch of Brushy Creek below Masland Carpets but above the Atmore WWTP to be moderately impaired. Degradation to the macroinvertebrate community below the discharge was evidenced by decreased taxa richness and increased community tolerance at BOB-2. Although nutrient concentrations increased below the discharge (Table 1), there was no associated increase in total number of organisms collected. These results are indicative of an invertebrate community negatively impacted by toxic wastes (Welsh 1992). The results of the short-term chronic toxicity tests indicated a toxic effect present in the effluent (Appendix A & B). In addition, changes in DO, BOD, TSS, and turbidity indicate organic enrichment. Associated water samples suggest that increased concentrations of conductivity, and/or chloride may be causing the impairment.

Water quality data collected below the Atmore WWTP indicates Boggy Branch to be slightly impaired but improving due to increased flow. Degradation to the macroinvertebrate community below the discharge was evidenced by decreased taxa richness at BOB-3. Although nutrient concentrations remained elevated below the discharge (Table 1), there was no associated increase in total number of organisms collected, again, indicative of an invertebrate community negatively impacted by toxic wastes. The results of the short-term chronic tests toxicity tests indicated a toxic effect present in the effluent (Appendix A & B). In addition, changes in DO, BOD, TSS, and turbidity indicate organic enrichment. Associated water samples suggest that increased concentrations of zinc, and/or chloride may be causing the impairment.

Figure 2

Field Parameters

Boggy Branch of Brushy Creek at Atmore, AL.

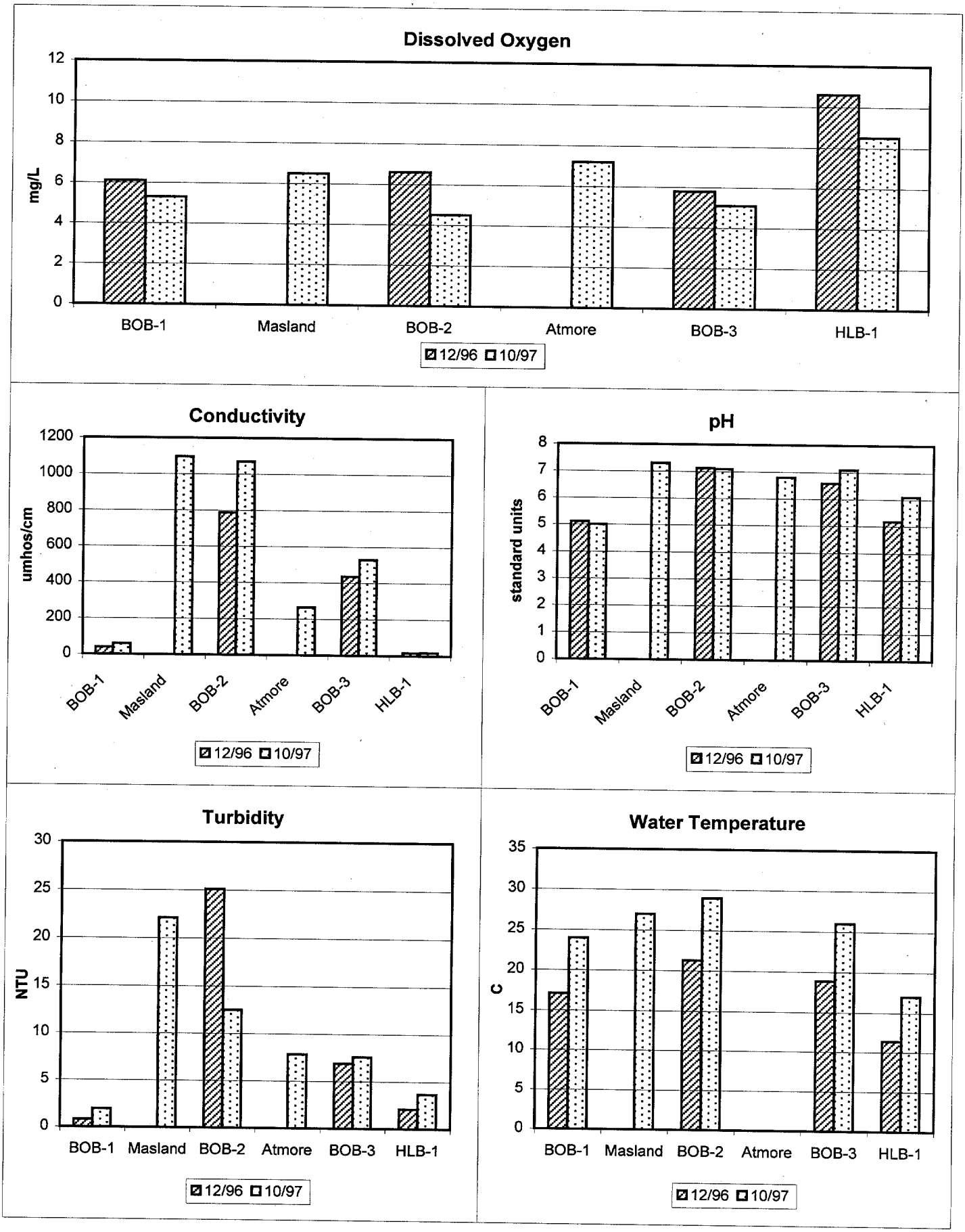

Figure 3

Individual Metrics

Boggy Branch of Brushy Ck, Atmore, AL.

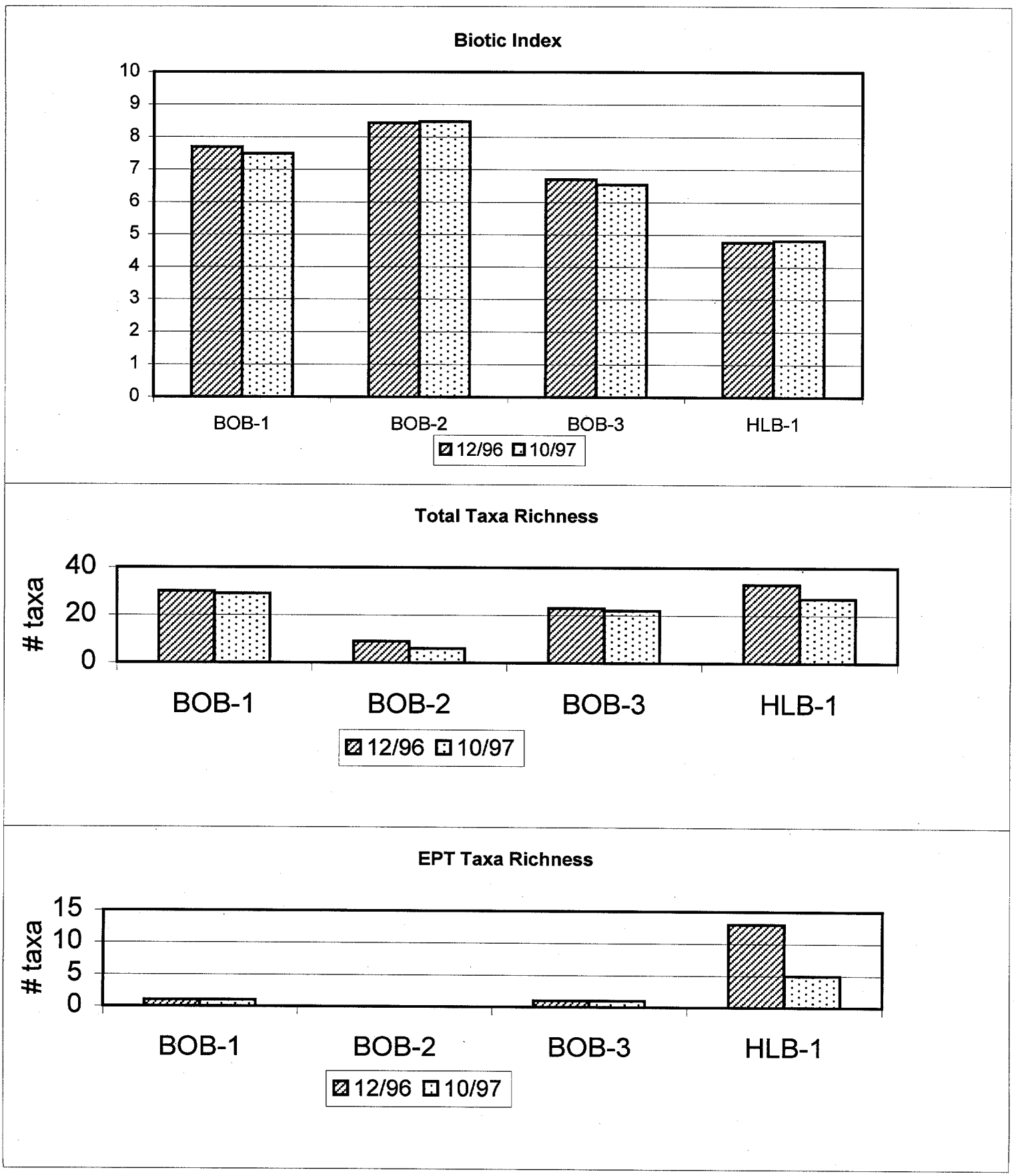

Figure 4

% Composition of Taxa Groups
Boggy Branch of Brushy Creek, Atmore, AL.
December 10-11, 1996

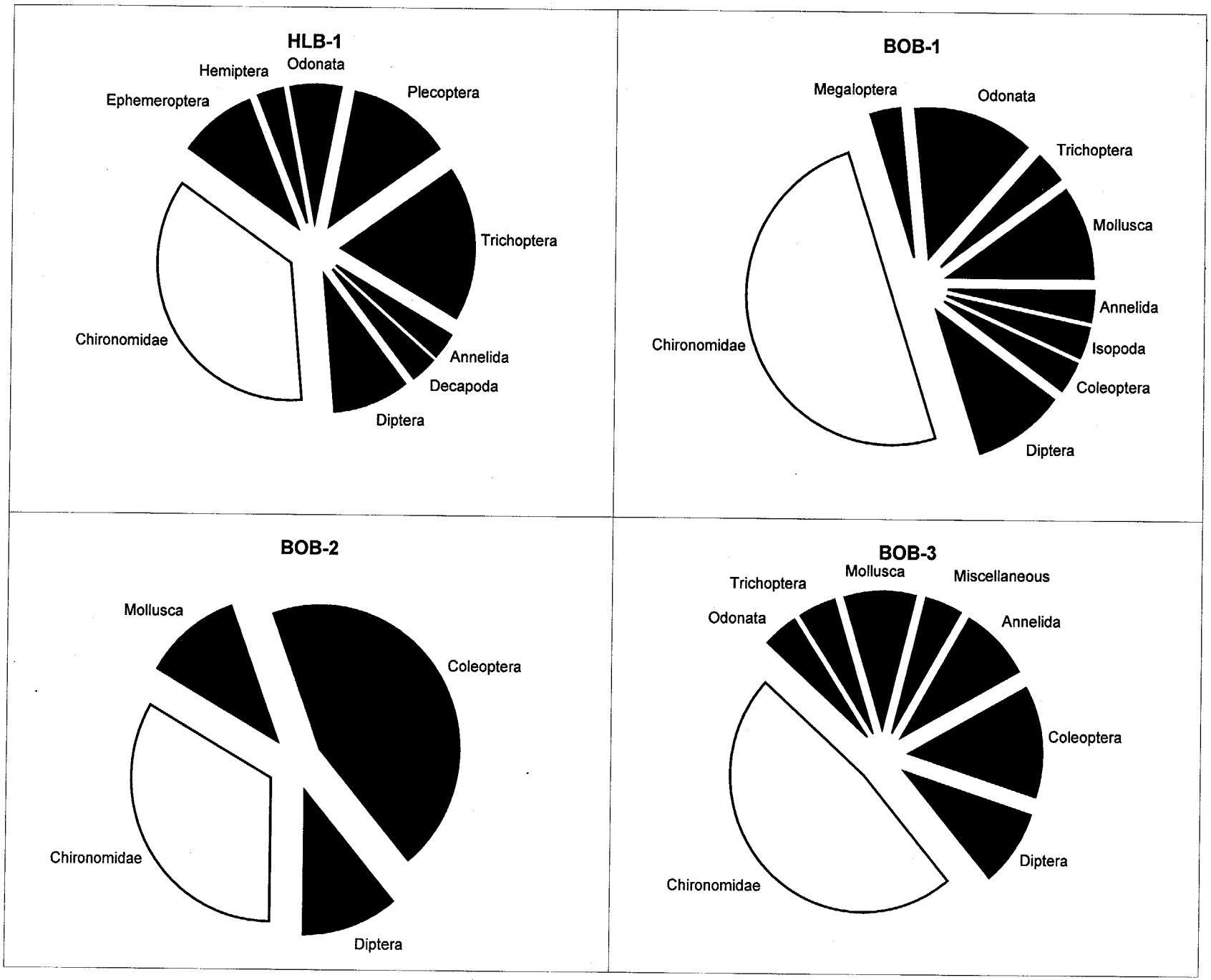

Figure 5

% Composition of Taxa Groups
Boggy Branch of Brushy Creek, Atmore, AL.
November 1-2, 1997

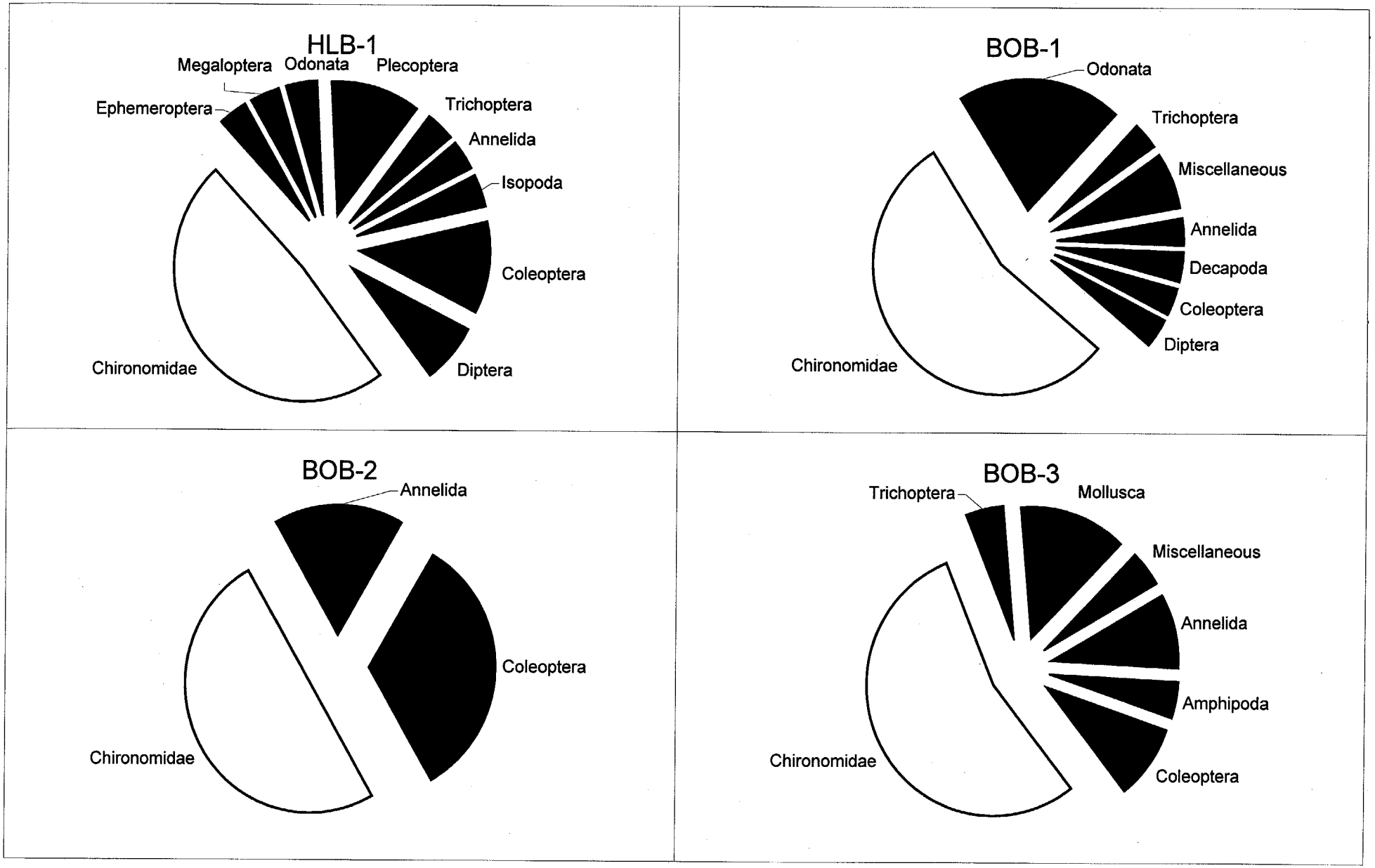

Figure 6

% Composition of Organisms
Boggy Branch of Brushy Creek, Atmore, AL.
December 10-11, 1996

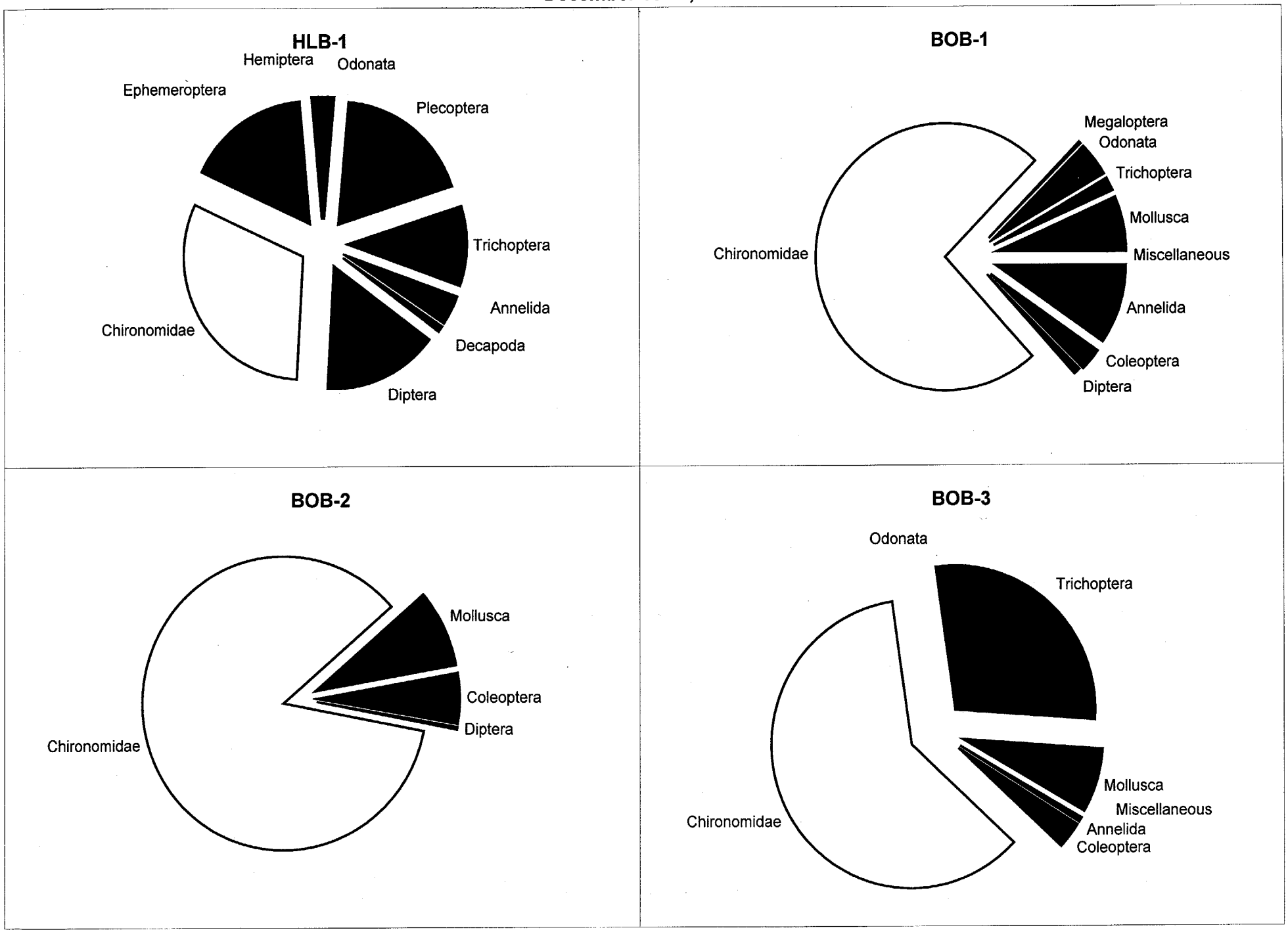

Figure 7

% Composition of Organisms
Boggy Branch of Brushy Creek, Atmore, AL.
November 1-2, 1997

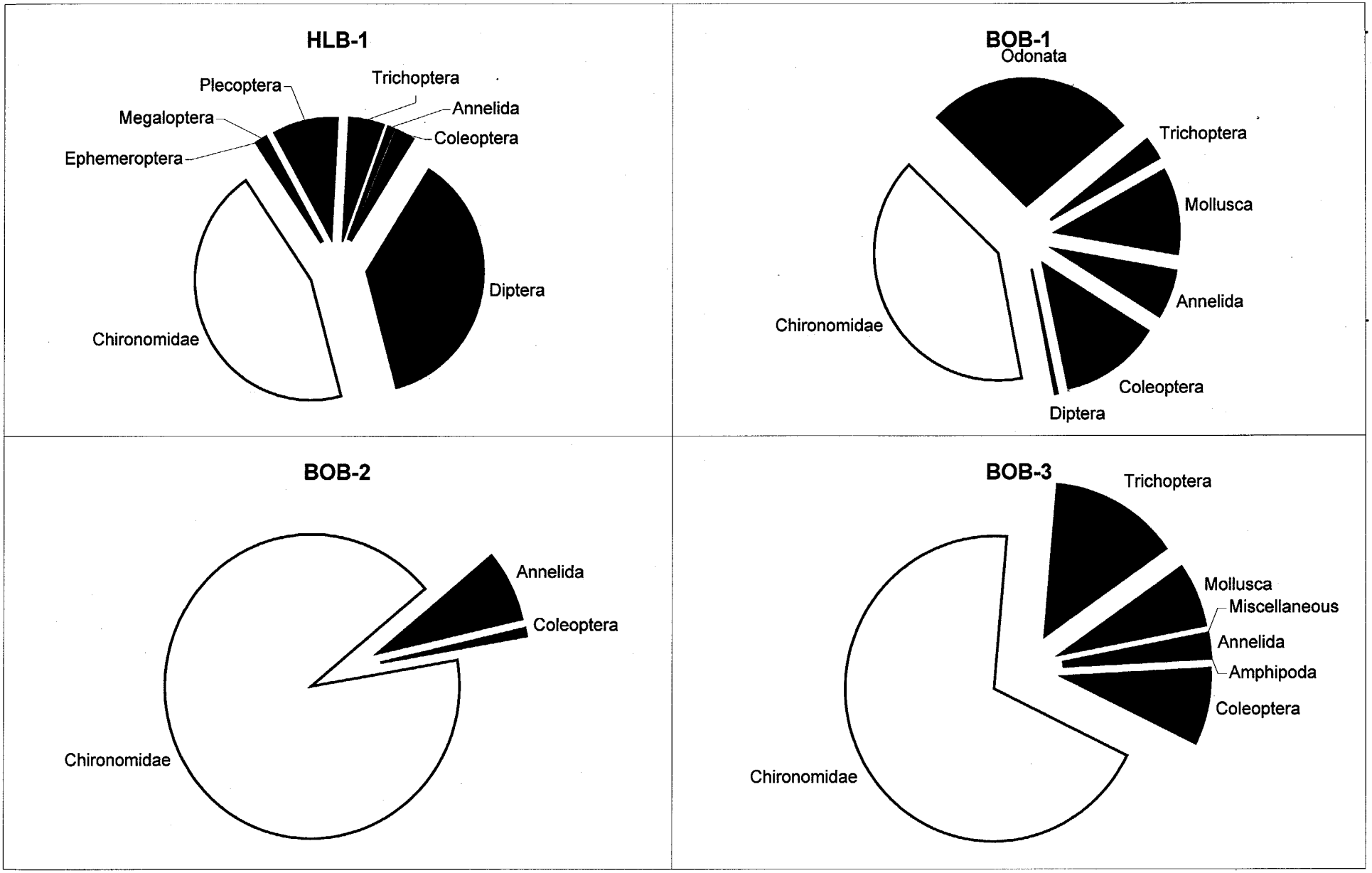

TABLE 1
Boggy Branch @ Atmore WQDS
December 10-11,1996 October 1-2, 1997

Station	Date	Time	Temp-H_2O	Temp-Air	Flow	SpCond	DO	pH	Weather	Turb	BOD_5	COD	Alkalinity	TDS	TSS	TON
	MMDDYY	HHMM	°C	°C	cfs	µmho/cm	mg/l	Units		NTUs	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Storet Code			00010	00020	00060	00095	00300	00400	47501	82079	00310	00335	00410	00515	00530	00605
BOB-1	12/11/96	0931	17.1	21		39	6.1	5.11	1 (Clear)	0.8						
BOB-2	12/11/96	0753	21.3	19		788	6.6	7.13	1 (Clear)	25.1						
BOB-3	12/11/96	0849	18.9	19		436	5.8	6.6	1 (Clear)	6.9						
HLB-1	12/10/96	1215	11.4	21		16	10.6	5.18	1 (Clear)	2.1						
BOB-1	10/01/97	1525	24	30	0.65	59.6	5.3	5	1 (Clear)	1.95	0.8	3.42	8	53	1	0.20K
BOB-2	10/01/97	1400	29	29	0.31	1070	4.5	7.1	1 (Clear)	12.5	7.4	83.7	56	717	21	0.36
BOB-3	10/01/97	1225	26	29	3.44	529	5.1	7.1	1 (Clear)	7.6	2.4	34.1	32	381	6	0.14
Masland Carpets - eff	10/02/97	1115	27	25	0.79	1096	6.5	7.3	1 (Clear)	22.1	13.6	25.8	74	76	23	1.42
Atmore WWTP - eff	10/02/97	1140	40*	42*	1.31	265	7.2	6.8	1 (Clear)	7.83	1.5	18.9	19	346	8	1.08
HLB-1	10/02/97	0735	17	9	16.65	18.3	8.5	6.1	1 (Clear)	3.67	0.5	8.89	4	35	1K	0.2K
DUP-1 (HLB-1)	10/02/97	0745	17.5	9		20.4	8.5	6.1	1 (Clear)	3.71	0.7	8.89	5	39	1	0.2K

Station	Date	Time	NH_3-N	NO_3+NO_2	TKN	PO_4-P	Cyanide	Hardness	Ca	Mg	Chloride	As	Cd	Cr^{+6}	Cr^T	Cu
	MMDDYY	HHMM	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Storet Code			00610	00620	00625	00665	00720	00900	00916	00927	00940	01002	01027	01032	01034	01042
BOB-1	10/01/97	1525	0.015K	0.59	0.150	0.05		9.7	2.4	0.893	10.3					
BOB-2	10/01/97	1400	0.43	30.58	0.79	3.28		47.9	16	1.927	92.6					
BOB-3	10/01/97	1225	0.13	17.74	0.27	1.82		33.4	10.6	1.674	64.6					
Maslin Carpets - eff	10/02/97	1115	2.08	28.85	3.5	3.16		45.4	14.5	2.243	92.8					
Atmore WWTP - eff	10/02/97	1140	0.015K	16.05	1.08	2.49	0.004K	44.5	13.5	2.629	74.4	10K	3K	20K	15K	20K
HLB-1	10/02/97	0735	0.015K	0.08	0.150	0.004K		2.7	0.5	0.358	5					
DUP-1 (HLB-1)	10/02/97	0745	0.015K	0.09	0.150	0.03		3.4	0.5	0.534	5					

Station	Date	Time	Pb	Ni	Ag	Zn	Fecal Coliform	Ethion	Malathion	Parathion	Diazinon	Methyl Parathion	Phosdrin	Hg
	MMDDYY	HHMM	µg/L	µg/L	µg/L	µg/L	org/100 mL	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Storet Code			01051	01067	01077	01092	31613	39398	39530	39540	39570	39600	39610	71900
BOB-1	10/01/97	1525					120							
BOB-2	10/01/97	1400					140							
BOB-3	10/01/97	1225					390							
Maslin Carpets - eff	10/02/97	1115					160							
Atmore WWTP - eff	10/02/97	1140	2K	9K	15K	61	190	10K	30K	15K	10K	12K	50K	0.5K
HLB-1	10/02/97	0735					140							
DUP-1 (HLB-1)	10/02/97	0745					83							

* - equipment malfunction (?)

Table 2

BIOMETRIC INTERPRETATION

METRIC	RANGE	INTERPRETATION
Habitat Assessment	170-220 118-169 60-117 0-59	Optimal Sub-optimal Marginal Poor
Total Taxa Richness EPT Taxa Richness		Generally Increases with Increasing Water Quality
Biotic Index		Generally Increases With Decreasing Water Quality
% Contribution of Functional Feeding Types %Shredders %Scrapers %Predators %Collector Gatherers %Collector Filterers %Macrophyte Piercers %Others		Percentages and Composition Should be similar to background station for similar stream sizes and habitat composition

BIOLOGICAL CONDITION SCORING CRITERIA

% Comparison to Reference Score	Biological Condition Category	Attributes
>81%	**Nonimpaired**	Comparable to best situation within ecoregion. Balanced trophic structure Optimum community structure for stream size and habitat
82-52%	**Slightly impaired**	Community structure less than expected Composition lower than expected due to loss of intolerant spp % contribution of tolerant forms increases
52-19%	**Moderately impaired**	Fewer species due to loss of most intolerant forms Reduction in EPT index
<19%	**Severely impaired**	Few species present

Table 3

	BOB-1 (Control)		BOB-2		BOB-3		HLB-1 (Reference)	
	Dec-96	Oct-97	Dec-96	Oct-97	Dec-96	Oct-97	Dec-96	Oct-97
Habitat Assessment	142	165	122	147	154	160	138	154
Habitat Quality (% Comparability to Reference)	103%	107%	88%	95%	112%	104%		
Taxa Richness	30	29	9	6	23	22	33	27
Biotic Index	7.69	7.49	8.43	8.47	6.7	6.54	4.76	4.81
EPT/EPT+Chiro	0.02	0.06	0	0	0.32	0.17	0.59	0.24
% Contib. Dom Taxa	33.3	12.63	62	65	28	45	12	36.91
EPT Index	1	1	0	0	1	1	13	5
Shredders/Total	0.08	0.08	0.38	0.5	0.28	0.55	0.24	0.28
Community Loss Index								
Compared to Control			3.00	4.33	0.60	0.63		
Compared to Reference	0.70	0.77	3.55	4.80	0.95	1.00		
Biological Condition								
Compared to Control			**Moderately Impaired**	**Moderately Impaired**	**Nonimpaired**	**Slightly Impaired**		
Compared to Reference	**Moderately Impaired**	**Slightly Impaired**	**Moderately Impaired**	**Moderately Impaired**	**Slightly Impaired**	**Slightly Impaired**		

Table 4

% Composition of Taxa Groups

% of Taxa in Each Group

	BOB-1 (Control)		BOB-2		BOB-3		HLB-1 (Ref)	
	Dec-96	Oct-97	Dec-96	Oct-97	Dec-96	Oct-97	Dec-96	Oct-97
Percent Annelida	3.33	3.45		16.67	8.7	9.09	3.03	3.7
Percent Amphipoda						4.55		
Percent Isopoda	3.33							3.7
Percent Decapoda		3.45					3.03	
Percent Coleoptera	3.33	3.45	44.44	33.33	13.04	9.09		11.11
Percent Diptera	10	3.45	11.11		8.7		9.09	7.41
Percent Chironomidae	50	55.17	33.33	50	47.83	54.55	36.36	48.15
Percent Ephemeroptera							9.09	3.7
Percent Hemiptera							3.03	
Percent Megaloptera	3.33							3.7
Percent Odonata	13.33	20.69			4.35		6.06	3.7
Percent Plecoptera							12.12	11.11
Percent Trichoptera	3.33	3.45			4.35	4.55	18.18	3.7
Percent Mollusca	10	6.9	11.11		8.7	13.64		
Percent Other					4.35	4.55		
	100	100	100	100	100	100	100	100

% of Organisms in Each Group

	BOB-1 (Control)		BOB-2		BOB-3		HLB-1 (Ref)	
	Dec-96	Oct-97	Dec-96	Oct-97	Dec-96	Oct-97	Dec-96	Oct-97
Percent Annelida	9.65	6.1		7.56	0.64	1.62	3.88	0.79
Percent Amphipoda						0.25		
Percent Isopoda	0.18							0.16
Percent Decapoda		0.29					0.97	
Percent Coleoptera	2.57	12.63	5.47	0.89	2.93	8.13		2.37
Percent Diptera	0.8	0.44	0.48		0.15		15.53	37.07
Percent Chironomidae	74.14	40.06	85.37	91.55	60.69	69.08	31.07	44.64
Percent Ephemeroptera							16.5	0.32
Percent Hemiptera							0.97	
Percent Megaloptera	0.09							0.95
Percent Odonata	4.25	27			0.12		1.94	0.16
Percent Plecoptera							18.45	8.83
Percent Trichoptera	1.59	2.76			28.31	13.98	10.68	4.73
Percent Mollusca	6.64	10.74	8.68		7.03	6.69		
Percent Other	0.09				0.12	0.25		
	100	100	100	100	100	100	100	100

Appendix A

ALABAMA DEPARTMENT OF ENVIRONMENTAL MANAGEMENT
FIELD OPERATIONS DIVISION
ECOLOGICAL STUDIES SECTION
TOXICS UNIT

TOXICITY TEST REPORT

1. GENERAL

NPDES PERMIT NO.: 0049557 DSN: 001 COUNTY: Escambia
Facility Name: Atmore WWTP
Receiving Water: Boggy Br. Design Flow: ---
Total 24-Hour Flow: (1) 0.744 MGD (2) 0.678 MGD (3) 0.673 MGD
Test Type: Short-term Chronic Screening
Test Id. #: 961119-01

Test Organism	Date/Time Started YYMMDD HHMM	Date/Time Ended YYMMDD HHMM	Control Validity (Acceptable/Unacceptable)
Ceriodaphnia dubia	961119 1440	961126 1400	Acceptable
Pimephales promelas	961119 1430	961126 1335	Acceptable

2A. SUMMARY OF RESULTS FOR SCREENING TEST

Test Org.	Effluent Conc.	Test Number (1) Surv	(1) Repro	(1) Grow	(2) Surv	(2) Repro	(2) Grow	(3) Surv	(3) Repro	(3) Grow	(4) Surv	(4) Repro	(4) Grow
C. d.	100%	PASS	FAIL	N/A	-----	-----	-----	-----	-----	-----	-----	-----	-----
P. p.	100%	PASS	N/A	PASS	-----	-----	-----	-----	-----	-----	-----	-----	-----

3. LABORATORY ANALYSES OF UNDILUTED SAMPLES(S)

Sample Id.	pH su	Alkalinity mg/L as CaCO3	Hardness mg/L as CaCO3	Conductivity umhos/cm @ °C	TRC mg/L
961119-01	6.3	20	39	379 at 25.3	0.02
961121-01	6.6	23	38	366 at 25.6	0.02
961123-01	6.7	19	41	370 at 25.0	-----

4. SAMPLE COLLECTION:

Were split samples collected?: samples were collected during a scheduled CSI
Were samples collected as specified in NPDES Permit (Location and/or Type)? yes

Sample Id.	Sample(s) Collected YYMMDD HHMM to YYMMDD HHMM	Arrival Temp (°C)	Used in Test(s) YYMMDD to YYMMDD
961119-01	961118 --- to 961119 ---	2.5	961119 to 961120
961121-01	961120 0830 to 961121 0830	4	961121 to 961122
961123-01	961122 0930 to 961123 0930	4	961123 to 961125

PERMITTEE: Atmore WWTP NPDES #: 0049557 DSN: 001 TEST Id #: 961119-01

5.CONTROL/DILUTION WATER

Carboy #	Preperation YYMMDD	Begin Use YYMMDD	Initial Water Chemistries			
			pH (su)	Alkalinity (mg/L)	Hardness (mg/L)	Conductivity @ °C (umhos/cm)
C-5	961115	961119	8.1	63	78	157 at 25.3
C-3	961119	961121	7.9	63	78	151 at 25.1
C-2	961119	961122	7.9	62	78	148 at 25.1
C-1	961120	961123	7.9	62	76	147 at 25.4

6. TOXICITY TEST INFORMATION

Test Organism	Organism Age	Organism Source	Org./Test Vessel	Replicates/Conc.
C.d.	<8h	ADEM In-house cultures	1	10
P.p.	<8h	ADEM In-house cultures	10	4

Test Organism	Temperature Range (°C)	D.O. Range (mg/L)	pH Range (su)	Light Intensity Average (ft-c)
C.d.	24.0 - 26.0	7.9 - 10.0	6.3 - 7.8	81
P.p.	24.0 - 26.0	3.8 - 10.0	6.3 - 7.1	81

7. FEEDING: Fed Daily

Brine Shrimp Fed 0.15 mL Suspension of Newly Hatched Larvae 2 Times Daily.
YCT Fed 0.15 mL Suspension Containing 1800 mg/L TSS Daily.
Algae Fed 0.15 mL Suspension Containing 3.6×10^7 Algal Cells/mL Daily.

8. REFERENCE TOXICANT TESTS

TOXICANT - Sodium Chloride (NaCl)

Test Organism	Test Date YYMMDD	Results LC50 (mg/L)	95% Confidence Interval (mg/L)
C.d.	961113	1489.36	1249.01/1775.97
P.p.	961113	7323.58	7157.28/7493.75

9. TEST CONDITION VARIABILITY

A. Deviations From Standard Test Conditions: The composite collection times for the first sample were not recorded on the custody form. The initial D.O. levels were slightly above 100% saturation. The temperature was slightly below the acceptable level on one occasion in the C. dubia test. These deviations did not appear to adversely affect the test results.

B. Test Solution Manipulations or Test Modifications

- ☐ Dechlorination
- ☐ Aeration during the test
- ☐ Aeration prior to test initiation or sample renewal
- ☐ Filtration
- ☐ pH adjustment
- ☒ NO sample modifications

PERMITTEE: Atmore WWTP NPDES #: 0049557 DSN: 001 TEST Id #: 961119-01

11. CHRONIC SCREENING TOXICITY TESTS RESULTS

TEST ORGANISM: Ceriodaphnia dubia

Test Validity:
Is survival in the CONTROL ≥ 80%? Yes
Are Average Neonates/Surviving Female in the CONTROL ≥ 15.0? Yes
Did 60% of the CONTROL Females Produce Their Third Brood? Yes

MORTALITY

CHRONIC TOXICITY INDICATED? PASS

Solution Concentration (%)	% Survival at 7 days
Control (0%)	100
100	100

STATISTICAL ANALYSES (Using proportion surviving):	COMMENTS:
☒ No Statistical Analysis Necessary	

REPRODUCTION

CHRONIC TOXICITY INDICATED? FAIL

Solution Concentration (%)	Reproduction (Average # young /female)
Control (0%)	22.1
100	16.5

STATISTICAL ANALYSES (Using number of neonates):

Shapiro Wilk's Test (Normality)
Test Statistic: 0.946 Critical Value: 0.868 (Parametric)
Normally Distributed
☒ Yes (if test stat is > critical value) GOTO VARIANCE F-TEST
☐ No (if test stat is < critical value) GOTO WILCOXON RANK SUM TEST

F-TEST
F Statistic: 1.653 Critical F: 6.54
Variance
☒ Equal (if f stat is < critical f) GOTO T-TEST
☐ Unequal (if f stat is > critical f) GOTO MODIFIED T-TEST

T-TEST
t Statistic: 2.991 Critical t value: 1.73
Significant Difference
☒ YES (if t stat is > critical t) FAIL
☐ NO (if t stat is < critical t) PASS

COMMENTS:

PERMITTEE: Atmore WWTP NPDES #: 0049557 DSN: 001 TEST Id #: 961119-01

11. CHRONIC SCREENING TOXICITY TESTS RESULTS

TEST ORGANISM: Pimephales promelas

Test Validity: Is survival in the CONTROL ≥ 80%? Yes
Is mean dry weight of surviving CONTROL fish ≥ 0.25mg? Yes

MORTALITY

CHRONIC TOXICITY INDICATED? PASS

Solution Concentration (%)	% Survival at 7 days
Control (0%)	97.5
100	90

STATISTICAL ANALYSES (Using Survival data as proportion surviving that is arc sine transformed):

Shapiro Wilk's Test (Normality)
Test Statistic: 0.848 Critical Value: 0.749 (Parametric)
Normally Distributed
☒ Yes (if test stat is > critical value) GOTO VARIANCE F-TEST
☐ No (if test stat is < critical value) GOTO WILCOXON RANK SUM TEST

F-TEST
F Statistic: 4.666 Critical F: 47.50
Variance
☒ Equal (if f stat is < critical f) GOTO T-TEST
☐ Unequal (if f stat is > critical f) GOTO MODIFIED T-TEST

T-TEST
t Statistic: 1.152 Critical t value: 1.94
Significant Difference
☐ YES (if t stat is > critical t) FAIL
☒ NO (if t stat is < critical t) PASS

COMMENTS:

GROWTH

CHRONIC TOXICITY INDICATED? PASS

Solution Concentration (%)	Mean dry weight (mg)
Control (0%)	0.755
100	0.916

STATISTICAL ANALYSES (Using mean dry weights):

☒ No Statistical Analysis Necessary

COMMENTS:

Signature: Janet Olen Date: 961202

ALABAMA DEPARTMENT OF ENVIRONMENTAL MANAGEMENT
FIELD OPERATIONS DIVISION
ECOLOGICAL STUDIES SECTION
TOXICS UNIT

TOXICITY TEST REPORT

1. GENERAL

NPDES PERMIT NO.: 0021997 DSN: 001 COUNTY: Escambia
Facility Name: Masland Carpets
Receiving Water: Boggy Br.
Total 24-Hour Flow: (1) 0.44 MGD (2) 0.48 MGD (3) 0.35 MGD Design Flow: ---
Test Type: Short-term Chronic Screening
Test Id. #: 961119-02

Test Organism	Date/Time Started YYMMDD HHMM	Date/Time Ended YYMMDD HHMM	Control Validity (Acceptable/Unacceptable)
Ceriodaphnia dubia	961119 1440	961126 1430	Acceptable
Pimephales promelas	961119 1430	961126 1355	Acceptable

2A. SUMMARY OF RESULTS FOR SCREENING TEST

Test Org.	Effluent Conc.	Test Number (1) Surv	(1) Repro	(1) Grow	(2) Surv	(2) Repro	(2) Grow	(3) Surv	(3) Repro	(3) Grow	(4) Surv	(4) Repro	(4) Grow
C. d.	100%	FAIL	N/A	N/A	-----	-----	-----	-----	-----	-----	-----	-----	-----
P. p.	100%	PASS	N/A	FAIL	-----	-----	-----	-----	-----	-----	-----	-----	-----

3. LABORATORY ANALYSES OF UNDILUTED SAMPLES(S)

Sample Id.	pH su	Alkalinity mg/L as CaCO3	Hardness mg/L as CaCO3	Conductivity umhos/cm @ °C	TRC mg/L
961119-02	7.4	76	186	810 at 26.7	-----
961121-02	7.4	63	168	707 at 25.9	-----
961123-02	7.5	65	140	647 at 25.0	-----

4. SAMPLE COLLECTION:

Were split samples collected?: samples were collected during a scheduled CSI
Were samples collected as specified in NPDES Permit (Location and/or Type)? yes

Sample Id.	Sample(s) Collected YYMMDD HHMM to YYMMDD HHMM	Arrival Temp (°C)	Used in Test(s) YYMMDD to YYMMDD
961119-02	961118 --- to 961119 ---	2.5	961119 to 961120
961121-02	961120 0915 to 961121 0915	4	961121 to 961122
961123-02	961122 0915 to 961123 0915	4	961123 to 961125

PERMITTEE: Masland Carpets NPDES #: 0021997 DSN: 001 TEST Id #: 961119-02

5.CONTROL/DILUTION WATER

Carboy #	Preperation YYMMDD	Begin Use YYMMDD	Initial Water Chemistries			
			pH (su)	Alkalinity (mg/L)	Hardness (mg/L)	Conductivity @ °C (umhos/cm)
C-5	961115	961119	8.1	63	78	157 at 25.3
C-3	961119	961121	7.9	63	78	151 at 25.1
C-2	961119	961122	7.9	62	78	148 at 25.1
C-1	961120	961123	7.9	62	76	147 at 25.4

6. TOXICITY TEST INFORMATION

Test Organism	Organism Age	Organism Source	Org./Test Vessel	Replicates/Conc.
C.d.	<8h	ADEM In-house cultures	1	10
P.p.	<8h	ADEM In-house cultures	10	4

Test Organism	Temperature Range (°C)	D.O. Range (mg/L)	pH Range (su)	Light Intensity Average (ft-c)
C.d.	24.0 - 26.0	7.9 - 9.8	7.3 - 8.3	81
P.p.	24.0 - 26.0	4.0 - 9.8	7.2 - 7.5	81

7. FEEDING: Fed Daily

Brine Shrimp Fed 0.15 mL Suspension of Newly Hatched Larvae 2 Times Daily.
YCT Fed 0.15 mL Suspension Containing 1800 mg/L TSS Daily.
Algae Fed 0.15 mL Suspension Containing 3.6×10^7 Algal Cells/mL Daily.

8. REFERENCE TOXICANT TESTS

TOXICANT - Sodium Chloride (NaCl)

Test Organism	Test Date YYMMDD	Results LC50 (mg/L)	95% Confidence Interval (mg/L)
C.d.	961113	1489.36	1249.01/1775.97
P.p.	961113	7323.58	7157.28/7493.75

9. TEST CONDITION VARIABILITY

A. Deviations From Standard Test Conditions: The composite collection times for the first sample were not recorded on the custody form. The initial D.O. levels were slightly above 100% saturation. These deviations did not appear to adversely affect the test results.

B. Test Solution Manipulations or Test Modifications

- ☐ Dechlorination
- ☐ Aeration during the test
- ☐ Aeration prior to test initiation or sample renewal
- ☐ Filtration
- ☐ pH adjustment
- ☒ NO sample modifications

PERMITTEE: Masland Carpets NPDES #: 0021997 DSN: 001 TEST Id #: 961119-02

11. CHRONIC SCREENING TOXICITY TESTS RESULTS

TEST ORGANISM: Ceriodaphnia dubia

Test Validity:
Is survival in the CONTROL ≥ 80%? Yes
Are Average Neonates/Surviving Female in the CONTROL ≥ 15.0? Yes
Did 60% of the CONTROL Females Produce Their Third Brood? Yes

MORTALITY

CHRONIC TOXICITY INDICATED? FAIL

Solution Concentration (%)	% Survival at 7 days
Control (0%)	100
100	10

STATISTICAL ANALYSES (Using proportion surviving):

Fishers Exact Test

A = 10 B = 10
a = 10 b = 1
critical value = 6

Is b ≤ critical value?
☒ YES There is a significnat difference (FAIL)
☐ NO There is not a significant difference (PASS)

COMMENTS:

REPRODUCTION

CHRONIC TOXICITY INDICATED? N/A

Solution Concentration (%)	Reproduction (Average # young /female)
Control (0%)	20.8
100	1.2

PERMITTEE: Masland Carpets NPDES #: 0021997 DSN: 001 TEST Id #: 961119-02

11. CHRONIC SCREENING TOXICITY TESTS RESULTS

TEST ORGANISM: Pimephales promelas

Test Validity: Is survival in the CONTROL ≥ 80%? Yes
Is mean dry weight of surviving CONTROL fish ≥ 0.25mg? Yes

MORTALITY

CHRONIC TOXICITY INDICATED? PASS

Solution Concentration (%)	% Survival at 7 days
Control (0%)	97.5
100	97.5

STATISTICAL ANALYSES (Using Survival data as proportion surviving that is arc sine transformed):

☒ No Statistical Analysis Necessary

COMMENTS:

GROWTH

CHRONIC TOXICITY INDICATED? FAIL

Solution Concentration (%)	Mean dry weight (mg)
Control (0%)	0.762
100	0.631

STATISTICAL ANALYSES (Using mean dry weights):

Shapiro Wilk's Test (Normality)
Test Statistic: 0.924 Critical Value: 0.749 (Parametric)
Normally Distributed
☒ Yes (if test stat is > critical value) GOTO VARIANCE F-TEST
☐ No (if test stat is < critical value) GOTO WILCOXON RANK SUM TEST

F-TEST
F Statistic: 3.160 Critical F: 47.5
Variance
☒ Equal (if f stat is < critical f) GOTO T-TEST
☐ Unequal (if f stat is > critical f) GOTO MODIFIED T-TEST

T-TEST
t Statistic: 2.467 Critical t value: 1.94
Significant Difference
☒ YES (if t stat is > critical t) FAIL
☐ NO (if t stat is < critical t) PASS

COMMENTS:

Signature: Janet Dean Date: 9/6/202

Appendix B

ALABAMA DEPARTMENT OF ENVIRONMENTAL MANAGEMENT
FIELD OPERATIONS DIVISION
ENVIRONMENTAL INDICATORS SECTION
BIOASSAY UNIT

TOXICITY TEST REPORT

1. GENERAL

NPDES PERMIT NO.: 0021997 DSN: 001 COUNTY: Escambia
Facility Name: Masland Carpets, Atmore
Receiving Water: Boggy Branch
Total 24-Hour Flow: (1) 0.71 MGD (2) 0.63 MGD (3) 0.17 MGD
Test Type: Short-term Chronic Screening
Test Id. #: 971002-02

Test Organism	Date/Time Started YYMMDD HHMM	Date/Time Ended YYMMDD HHMM	Control Validity (Acceptable/Unacceptable)
Ceriodaphnia dubia	971003 1420	971010 1345	Acceptable
Pimephales promelas	971002 1515	971009 1320	Acceptable

2A. SUMMARY OF RESULTS FOR SCREENING TEST

Test Org.	Effluent Conc.	Test Number (1) Surv	(1) Repro	(1) Grow	(2) Surv	(2) Repro	(2) Grow	(3) Surv	(3) Repro	(3) Grow	(4) Surv	(4) Repro	(4) Grow
C. d.	100%	PASS	FAIL	N/A	-----	-----	-----	-----	-----	-----	-----	-----	-----
P. p.	100%	PASS	N/A	PASS	-----	-----	-----	-----	-----	-----	-----	-----	-----

3. LABORATORY ANALYSES OF UNDILUTED SAMPLES(S)

Sample Id.	pH su	Alkalinity mg/L as CaCO3	Hardness mg/L as CaCO3	Conductivity umhos/cm @ °C	TRC mg/L
971002-02	7.6	81	47	1029 at 24.2	0.05
971004-02	7.7	82	43	1005 at 23.1	-----
971006-02	7.7	70	45	1053 at 25.6	-----

4. SAMPLE COLLECTION:

Were split samples collected?: No
Were samples collected as specified in NPDES Permit (Location and/or Type)? Yes

Pimephales promelas Test

Sample Id.	Sample(s) Collected YYMMDD HHMM to YYMMDD HHMM	Arrival Temp (°C)	Used in Test(s) YYMMDD to YYMMDD
971002-02	971001 1130 to 971002 1115	2	971002 to 971003
971004-02	971003 0830 to 971004 0815	2	971004 to 971005
971006-02	971005 0830 to 971006 0815	2.5	971006 to 971008

Ceriodaphnia dubia Test

Sample Id.	Sample(s) Collected YYMMDD HHMM to YYMMDD HHMM	Arrival Temp (°C)	Used in Test(s) YYMMDD to YYMMDD
971002-02	971001 1130 to 971002 1115	2	971003 to 971004
971004-02	971003 0830 to 971004 0815	2	971005 to 971006
971006-02	971005 0830 to 971006 0815	2.5	971007 to 971009

PERMITTEE: Masland Carpets NPDES #: 0021997 DSN: 001 TEST Id #: 971002-02

5.CONTROL/DILUTION WATER

Carboy #	Preparation YYMMDD	Begin Use YYMMDD	Initial Water Chemistries			
			pH (su)	Alkalinity (mg/L)	Hardness (mg/L)	Conductivity @ °C (umhos/cm)
C-2	971001	971002	8.1	67	77	144 at 22.9
C-1	971002	971007	8.2	66	77	144 at 23.6

6. TOXICITY TEST INFORMATION

Test Organism	Organism Age	Organism Source	Org./Test Vessel	Replicates/Conc.
C.d.	<8 hrs.	ADEM In-house cultures	1	10
P.p.	<24 hrs.	ADEM In-house cultures	15	4

Test Organism	Temperature Range (°C)	D.O. Range (mg/L)	pH Range (su)	Light Intensity Average (ft-c)
C.d.	*23.8 - 25.7	7.6 - 9.9	6.9 - 8.3	69
P.p.	24.1 - 25.8	4.2 - 8.4	7.3 - 7.7	69

*below recommended temperature range

7. FEEDING: Fed Irregular*

Brine Shrimp Fed 0.15 mL Suspension of Newly Hatched Larvae 2 Times Daily.
YCT Fed 0.15 mL Suspension Containing 1800 mg/L TSS Daily.
Algae Fed 0.15 mL Suspension Containing 3.5×10^7 Algal Cells/mL Daily.
**Pimephales promelas* test accidentally fed a.m. brine shrimp on 971009.

8. REFERENCE TOXICANT TESTS

TOXICANT - Sodium Chloride (NaCl)

Test Organism	Test Date YYMMDD	Results LC50 (mg/L)	95% Confidence Interval (mg/L)
C.d.	970909	1672.36	1486.25/1881.77
P.p.	970902	6541.73	6200.48/6901.76

9. TEST CONDITION VARIABILITY

Deviations From Standard Test Conditions: The *Pimephales promelas* test was fed a.m. brine shrimp on 10/9/97. The temperature in the *Ceriodaphnia dubia* test was below the recommended temperature on one occasion. These deviations did not appear to adversely affect test results.

B. Test Solution Manipulations or Test Modifications

- ☐ Dechlorination
- ☐ Aeration during the test
- ☐ Aeration prior to test initiation or sample renewal
- ☐ Filtration
- ☐ pH adjustment
- ☒ NO sample modifications

PERMITTEE: Masland Carpets NPDES #: 0021997 DSN: 001 TEST Id #: 971002-02

11. CHRONIC SCREENING TOXICITY TESTS RESULTS **TEST ORGANISM: Ceriodaphnia dubia**

Test Validity:
Is survival in the CONTROL ≥ 80%? Yes
Are Average Neonates/Surviving Female in the CONTROL ≥ 15.0? Yes
Did 60% of the CONTROL Females Produce Their Third Brood? Yes

MORTALITY **CHRONIC TOXICITY INDICATED? PASS**

Solution Concentration (%)	% Survival at 7 days
Control (0%)	100%
100%	100%

STATISTICAL ANALYSES (Using proportion surviving):	COMMENTS:
☒ No Statistical Analysis Necessary	

REPRODUCTION **CHRONIC TOXICITY INDICATED? FAIL**

Solution Concentration (%)	Reproduction (Average # young /female)
Control (0%)	23.0
100%	17.0

STATISTICAL ANALYSES (Using number of neonates):

☐ No Statistical Analysis Necessary

Shapiro Wilk's Test (Normality)
Test Statistic: 0.885 Critical Value: 0.868 (Parametric)
Normally Distributed ☒ Yes (if test stat is > critical value) GOTO VARIANCE F-TEST
☐ No (if test stat is < critical value) GOTO WILCOXON RANK SUM TEST

F-TEST
F Statistic: 3.724 Critical F: 6.54
Variance ☒ Equal (if f stat is < critical f) GOTO T-TEST
☐ Unequal (if f stat is > critical f) GOTO MODIFIED T-TEST

T-TEST
t Statistic: 3.439 Critical t value: 1.73
Significant Difference ☒ YES (if t stat is > critical t) FAIL
☐ NO (if t stat is < critical t) PASS

COMMENTS:

PERMITTEE: Masland Carpets NPDES #: 0021997 DSN: 001 TEST Id #: 971002-02

11. CHRONIC SCREENING TOXICITY TESTS RESULTS

TEST ORGANISM: Pimephales promelas

Test Validity: Is survival in the CONTROL ≥ 80%? Yes
Is mean dry weight of surviving CONTROL fish ≥ 0.25mg? Yes

MORTALITY

CHRONIC TOXICITY INDICATED? PASS

Solution Concentration (%)	% Survival at 7 days
Control (0%)	95%
100%	96.7%

STATISTICAL ANALYSES (Using Survival data as proportion surviving that is arc sine transformed):

☒ No Statistical Analysis Necessary

COMMENTS:

GROWTH

CHRONIC TOXICITY INDICATED? PASS

Solution Concentration (%)	Mean dry weight (mg)
Control (0%)	0.7596
100%	0.7395

STATISTICAL ANALYSES (Using mean dry weights):

☐ No Statistical Analysis Necessary

Shapiro Wilk's Test (Normality)
Test Statistic: 0.934 Critical Value: 0.749 (Parametric)
Normally Distributed ☒ Yes (if test stat is > critical value) GOTO VARIANCE F-TEST
☐ No (if test stat is < critical value) GOTO WILCOXON RANK SUM TEST

F-TEST
F Statistic: 3.623 Critical F: 47.50
Variance ☒ Equal (if f stat is < critical f) GOTO T-TEST
☐ Unequal (if f stat is > critical f) GOTO MODIFIED T-TEST

T-TEST
t Statistic: 0.682 Critical t value: 1.94
Significant Difference ☐ YES (if t stat is > critical t) FAIL
☒ NO (if t stat is < critical t) PASS

COMMENTS:

Signature: Audra B. Huff Date: 10/20/97

ALABAMA DEPARTMENT OF ENVIRONMENTAL MANAGEMENT
FIELD OPERATIONS DIVISION
ENVIRONMENTAL INDICATORS SECTION
BIOASSAY UNIT

TOXICITY TEST REPORT

1. GENERAL

NPDES PERMIT NO.: 0049557 DSN: 001 COUNTY: Escambia
Facility Name: Atmore WWTP
Receiving Water: Boggy Branch
Total 24-Hour Flow: (1) 1.572 MGD (2) 0.737 MGD (3) 0.785 MGD
Test Type: Short-term Chronic Screening
Test Id. #: 971002-03

Test Organism	Date/Time Started YYMMDD HHMM	Date/Time Ended YYMMDD HHMM	Control Validity (Acceptable/Unacceptable)
Ceriodaphnia dubia	971003 1420	971010 1335	Acceptable
Pimephales promelas	971002 1510	971009 1335	Acceptable

2A. SUMMARY OF RESULTS FOR SCREENING TEST

Test Org.	Effluent Conc.	Test Number (1) Surv	(1) Repro	(1) Grow	(2) Surv	(2) Repro	(2) Grow	(3) Surv	(3) Repro	(3) Grow	(4) Surv	(4) Repro	(4) Grow
C. d.	100%	PASS	PASS	N/A	-----	-----	-----	-----	-----	-----	-----	-----	-----
P. p.	100%	PASS	N/A	PASS	-----	-----	-----	-----	-----	-----	-----	-----	-----

3. LABORATORY ANALYSES OF UNDILUTED SAMPLES(S)

Sample Id.	pH su	Alkalinity mg/L as CaCO3	Hardness mg/L as CaCO3	Conductivity umhos/cm @ °C	TRC mg/L
971002-03	6.9	19	48	416 at 24.0	0.
971004-03	6.8	13	53	408 at 22.5	-----
971006-03	6.8	11	52	436 at 25.1	-----

4. SAMPLE COLLECTION:

Were split samples collected?: No
Were samples collected as specified in NPDES Permit (Location and/or Type)? Yes

Pimephales promelas Test

Sample Id.	Sample(s) Collected YYMMDD HHMM to YYMMDD HHMM	Arrival Temp (°C)	Used in Test(s) YYMMDD to YYMMDD
971002-03	971001 1202 to 971002 1147	2	971002 to 971003
971004-03	971003 0830 to 971004 0815	2	971004 to 971005
971006-03	971005 0830 to 971006 0815	2.5	971006 to 971008

Ceriodaphnia dubia Test

Sample Id.	Sample(s) Collected YYMMDD HHMM to YYMMDD HHMM	Arrival Temp (°C)	Used in Test(s) YYMMDD to YYMMDD
971002-03	971001 1202 to 971002 1147	2	971003 to 971004
971004-03	971003 0830 to 971004 0815	2	971005 to 971006
971006-03	971005 0830 to 971006 0815	2.5	971007 to 971009

PERMITTEE: Atmore WWTP NPDES #: 0049557 DSN: 001 TEST Id #: 971002-03

5.CONTROL/DILUTION WATER

Carboy #	Preparation YYMMDD	Begin Use YYMMDD	Initial Water Chemistries			
			pH (su)	Alkalinity (mg/L)	Hardness (mg/L)	Conductivity @ °C (umhos/cm)
C-2	971001	971002	8.1	67	77	144 at 22.9
C-1	971002	971007	8.2	66	77	144 at 23.6

6. TOXICITY TEST INFORMATION

Test Organism	Organism Age	Organism Source	Org./Test Vessel	Replicates/Conc.
C.d.	<8 hrs.	ADEM In-house cultures	1	10
P.p.	<24 hrs.	ADEM In-house cultures	15	4

Test Organism	Temperature Range (°C)	D.O. Range (mg/L)	pH Range (su)	Light Intensity Average (ft-c)
C.d.	23.3 - 26.0	7.4 - 10.6	6.3 - 7.7	69
P.p.	24.3 - 25.9	3.7 - 8.4	6.3 - 6.9	69

7. FEEDING: Fed Irregular

Brine Shrimp Fed 0.15 mL Suspension of Newly Hatched Larvae 2 Times Daily.
YCT Fed 0.15 mL Suspension Containing 1800 mg/L TSS Daily.
Algae Fed 0.15 mL Suspension Containing 3.5×10^7 Algal Cells/mL Daily.

8. REFERENCE TOXICANT TESTS

TOXICANT - Sodium Chloride (NaCl)

Test Organism	Test Date YYMMDD	Results LC50 (mg/L)	95% Confidence Interval (mg/L)
C.d.	970909	1672.36	1486.25/1881.77
P.p.	970902	6541.73	6200.48/6901.76

9. TEST CONDITION VARIABILITY

A. Deviations From Standard Test Conditions: The *Pimephales promelas* test was fed a.m. brine shrimp on 10/9/97. The *Ceriodaphnia dubia* test temperature was below the recommended range on one occasion. These deviations did not seem to adversely affect test results.

B. Test Solution Manipulations or Test Modifications

- ☐ Dechlorination
- ☐ Aeration during the test
- ☐ Aeration prior to test initiation or sample renewal
- ☐ Filtration
- ☐ pH adjustment
- ☒ NO sample modifications

PERMITTEE: Atmore WWTP NPDES #: 0049557 DSN: 001 TEST Id #: 971002-03

11. CHRONIC SCREENING TOXICITY TESTS RESULTS **TEST ORGANISM: Ceriodaphnia dubia**

Test Validity:
Is survival in the CONTROL ≥ 80%? Yes
Are Average Neonates/Surviving Female in the CONTROL ≥ 15.0? Yes
Did 60% of the CONTROL Females Produce Their Third Brood? Yes

MORTALITY **CHRONIC TOXICITY INDICATED? PASS**

Solution Concentration (%)	% Survival at 7 days
Control (0%)	90%
100%	100%

STATISTICAL ANALYSES (Using proportion surviving):	COMMENTS:
☒ No Statistical Analysis Necessary	

REPRODUCTION **CHRONIC TOXICITY INDICATED? PASS**

Solution Concentration (%)	Reproduction (Average # young /female)
Control (0%)	22.4
100%	22.7

STATISTICAL ANALYSES (Using number of neonates):	COMMENTS:
☒ No Statistical Analysis Necessary	

PERMITTEE: Atmore WWTP NPDES #: 0049557 DSN: 001 TEST Id #: 971002-03

11. CHRONIC SCREENING TOXICITY TESTS RESULTS **TEST ORGANISM: Pimephales promelas**

Test Validity: Is survival in the CONTROL ≥ 80%? Yes
Is mean dry weight of surviving CONTROL fish ≥ 0.25mg? Yes

MORTALITY **CHRONIC TOXICITY INDICATED? PASS**

Solution Concentration (%)	% Survival at 7 days
Control (0%)	96.7%
100%	95%

STATISTICAL ANALYSES (Using Survival data as proportion surviving that is arc sine transformed):

☐ No Statistical Analysis Necessary

Shapiro Wilk's Test (Normality)
Test Statistic: 0.747 Critical Value: 0.749 (Parametric)
Normally Distributed ☐ Yes (if test stat is > critical value) GOTO VARIANCE F-TEST
☒ No (if test stat is < critical value) GOTO WILCOXON RANK SUM TEST

WILCOXON RANK SUM TEST or MODIFIED T-TEST
Sample Rank Sum: 19.0 # of reps 4 Critical Rank Sum: 11.0
Significant Difference ☐ YES (if sample rank sum is < critical rank sum) FAIL
☒ NO (if sample rank sum is > critical critical rank sum) PASS

COMMENTS:

GROWTH **CHRONIC TOXICITY INDICATED? PASS**

Solution Concentration (%)	Mean dry weight (mg)
Control (0%)	0.7679
100%	0.8010

STATISTICAL ANALYSES (Using mean dry weights):

☒ No Statistical Analysis Necessary

COMMENTS:

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