

**State of New Jersey
JAMES McGREEVEY
GOVERNOR**

**SEDIMENT TOXICITY TEST
USING THE AMPHIPOD
Hyalella azteca
Watershed Management Area 18 and 19
(Pennsauken Creek and Rancocas Creek)**



**New Jersey Department of Environmental Protection
BRADLEY CAMPBELL
COMMISSIONER**

January 2002



New Jersey Department of Environmental Protection
Division of Watershed Management
P.O. Box 427, Trenton, NJ 08625-0427

WATER MONITORING MANAGEMENT

James Mumman, Administrator

January 2002

**SEDIMENT TOXICITY TEST
USING THE AMPHIPOD
Hyalella azteca
Watershed Management Area 18 and 19
(Pennsauken Creek and Rancocas Creek)**

**Bureau of Freshwater and Biological Monitoring
Biomonitoring Section**

Assay Number(s): 01H002a, 01H002b, 01H002c

Report Prepared By:
Stacey Hoeltje

Analysts:
Stacey Hoeltje
Anna Marie Signor

Samplers:
Victor Poretti
Anna Marie Signor

Project Officer
Victor Poretti

Supervisor:
Paul Olsen

**Chief
Bureau of Freshwater & Biological Monitoring
Alfred Korndoerfer, Jr.**

EXECUTIVE SUMMARY

Toxicity tests using the amphipod *Hyalella azteca* were performed on sediments collected from three sites in the Lower Delaware Water Region. This initiative was undertaken by the Bureau of Freshwater and Biological Monitoring and administered under the New Jersey Department of Environmental Protection's Division of Watershed Management. One site tested was situated on the North Branch Pennsauken Creek within Watershed Management Area # 18, and two sites, one each on the North Branch and South Branch Rancocas Creek, were within Watershed Management Area # 19, all within the Lower Delaware Water Region. Suspected toxicity at two of the sites (South Branch Rancocas Creek and North Branch Pennsauken Creek) was based on their "severely impaired" biological assessments (i.e. degraded quality of benthic macroinvertebrate communities) found in previous survey(s) of New Jersey's statewide Ambient Biomonitoring Network (AMNET). A reference site was selected on the North Branch Rancocas Creek because of its "non-impaired" AMNET assessment. The sediment toxicity tests were conducted to provide further data, which could be related to the previous assessments. Tests were conducted in accordance with the Bureau's Standard Operating Procedures, which incorporate protocols recognized by the U.S. Environmental Protection Agency. When test site results were statistically compared to that of the reference site, the test sites did not exhibit acute toxicity as measured by survival, or chronic toxicity as measured by growth, of test organisms.

INTRODUCTION

The Ambient Biomonitoring Network (AMNET) program of the New Jersey Department of Environmental Protection (NJDEP), Bureau of Freshwater and Biological Monitoring (BFBM), is designed to establish a biological database for use in gauging stream quality throughout the state. This database, in turn, can be an invaluable aid to New Jersey's water quality and watershed planning and management efforts. Levels of impairment are shown through the use of Rapid Bioassessment Protocols (RBP) advised by the U.S. Environmental Protection Agency (EPA)(1). The RBP assesses impairment through the collection, identification, categorizing, and quantification of instream macroinvertebrate communities. Although the RBP is an excellent way in which to assess impairment, it may sometimes be difficult to distinguish whether impairment is due to water quality degradation or habitat destruction.

Sediment toxicity testing is an additional tool used, before resorting to costly chemical monitoring, to determine whether toxicity is the cause of impairment. The test organism, *Hyalella azteca* is an epibenthic detritivore, reported to also digest bacteria and algae from ingested sediment particles (2). This amphipod crustacean inhabits lakes, ponds, and streams throughout North and South America, typically burrowing into the sediment surface (3,4). *H. azteca* is a sensitive benchmark species, which can be cultured in the laboratory with relative ease.

In October of 2001, the Bureau of Freshwater and Biological Monitoring conducted sediment toxicity tests on three stream sites, within an area of central New Jersey, which had exhibited varying degrees of impairment in previous AMNET sampling. The new initiative was designed to support management efforts in Watershed Management Areas (WMA) # 18 and # 19. Administratively, these include the Pennsauken and Rancocas Creek drainages, respectively, in the Lower Delaware Water Region".

METHODS

Sample sites were selected based on previous AMNET results(5) (Appendix A), proximity to urban and/or agricultural areas, and proximity of point source discharges (i.e. effluents from facilities with New Jersey Pollutant Discharge Elimination System (NJPDES) permits). The sites selected are as follows (see map):

<u>AMNET STATION#</u>	<u>BIOLOGICAL ASSESSMENT</u>	<u>LOCATION</u> (see map)
AN0149 (control site)	non-impaired	North Branch Rancocas Ck. @ Main St. Pemberton
AN0176S	severely impaired	South Branch Rancocas Ck. @ Route 38, Hainesport Twp.
AN0179	severely impaired	North Branch Pennsauken Ck. @ Fellowship Rd. near 295, Mt. Laurel Twp.

Sediment samples were collected from all sites on October 16, 2001. At each station the sediment was collected in the stream channel using a stainless steel scoop sampler and placed into one liter amber glass bottles, then stored at # 4EC until the start of the test (6).

Prior to test initiation the sample sites were assigned assay numbers, in accordance with our ongoing series of toxicity tests, as follows:

01H002a = AN0149 (reference, nonimpaired site)

01H002b = AN0176S

01H002c = AN0179

Testing methodology followed the NJDEP Biomonitoring Laboratory Standard Operating Procedures(7). 24 hours prior to the start of the test, the sediment from each station was mixed to provide a homogeneous sample, and hand picked of any visible indigenous organisms. For each site, 100 ml of sediment was added to each of the five 300 ml replicate test vessels and topped with laboratory grade freshwater to the 250 ml mark. The test vessels were then held at the test temperature (23EC) for 24 hours to allow the sediment to settle(7). After this time period, the overlying water was siphoned, and fresh water was added. A control set of replicates was also set up using 250 ml of overlying water only.

One to seven- day old *H. azteca* juveniles were collected, from our cultures, and held for one week prior to the start of the test (7).

Testing was initiated on October 23, 2001 at 11:20 hours, by adding ten 7 to 14- day old organisms from the holding chamber to each test replicate series. Each day the overlying water was exchanged, and each test replicate was fed 1.5 ml of YCT (prepared as per USEPA, from a mixture of yeast, CEROPHYLL[®] leaves, and digested trout chow) (8), and 1.5 ml of the green algae *Selenastrum capricornutum* at a concentration of 35×10^6 cells ml⁻¹ (after centrifugation). Mortalities were noted if visible; pH, dissolved oxygen, and conductivity were measured from aliquots of each test series; measurements were made at the start of the test and after each 24 hour period (see Table 1).

The test was concluded after ten days (November 2, 2001). As a measure of acute toxicity, live organisms were counted against those dead or missing (see Table 2). As an indication of chronic toxicity, dry weights of surviving organisms were measured (see Table 3). Statistical analysis was performed following EPA guidelines (8). Results of the reference test were compared against the control, and, providing the reference and the control were statistically similar, the remaining tests were compared to the reference.

RESULTS

The tests were valid by virtue of meeting the acceptability requirements of $\geq 80\%$ survival (see Table 2) in the control test series (7). The survival data was not distributed normally as analyzed by the Shapiro-Wilks test for normality, and therefore, the Wilcoxon Rank Sum Test was used when comparing test survival results. There was no significant difference in survival results between the reference test (01H002a) and the control. All test samples were then compared to the reference. The survival data for test 01H002b was not distributed normally as analyzed by the Shapiro-Wilks test, therefore, the Wilcoxon Rank Sum Test was used. Test 01H002b showed no significant difference in mortality from the reference sample. The survival data of test 01H002c was distributed normally as analyzed by the Shapiro-Wilks test, therefore the F-Test and T-Test were used when comparing survival results. The results for test 01H002c showed no significant difference in survival results from that of the reference sample.

Dry weights of both test samples were then compared to that of the reference (see Table 3). The dry weight data was not distributed normally as analyzed by the Shapiro-Wilks test, therefore, the Wilcoxon Rank Sum Test was used when comparing test dry weight results. In this comparison the test samples showed no significant difference from the reference (see appendix B for statistical printout).

Although all indigenous organisms observed in the samples before the start of the test were removed, some did remain; however, their presence did not invalidate test results. Test chambers 01H002a contained several chironomid, or midge, larvae, and 01H002c contained a few clams and worms.

DISCUSSION

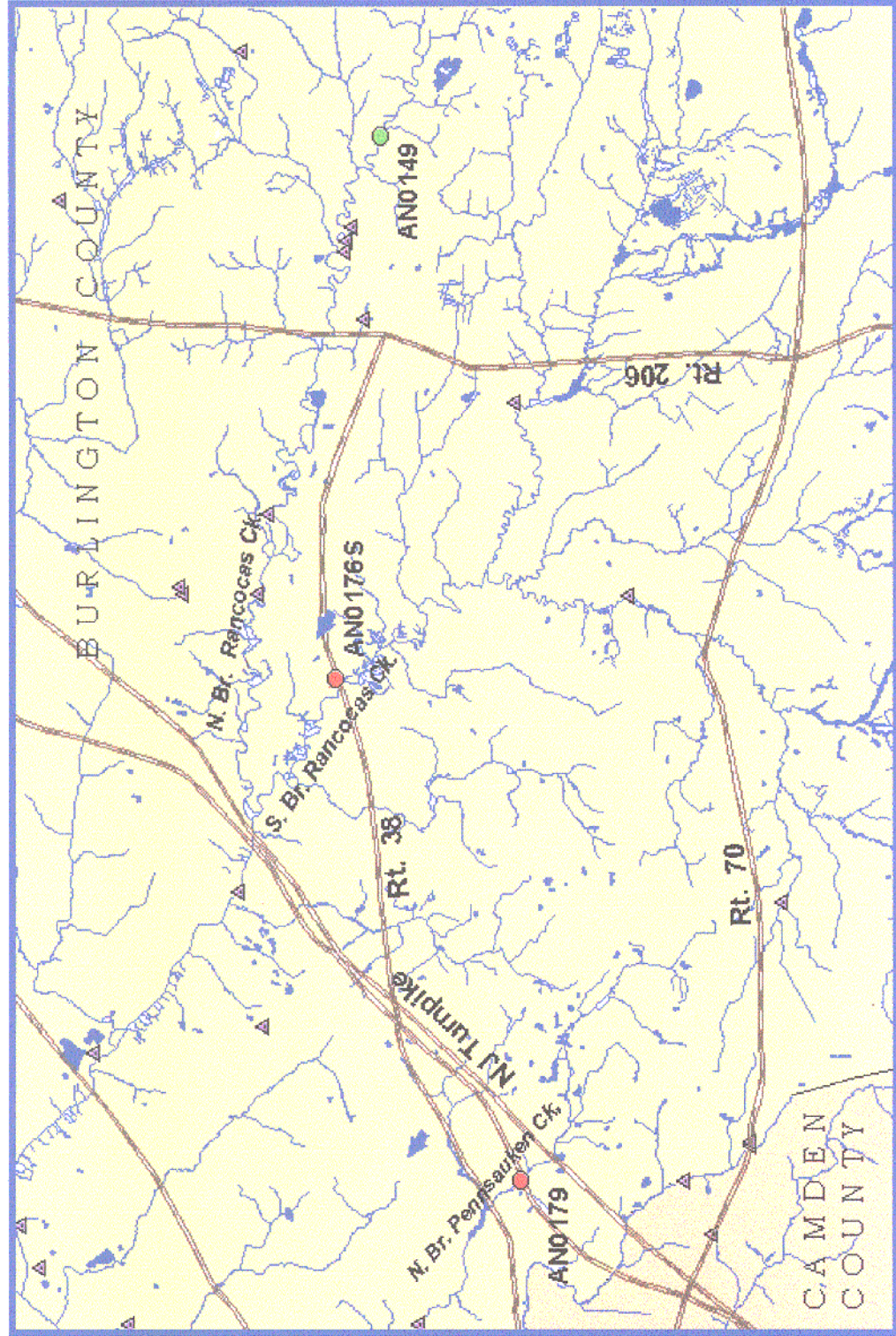
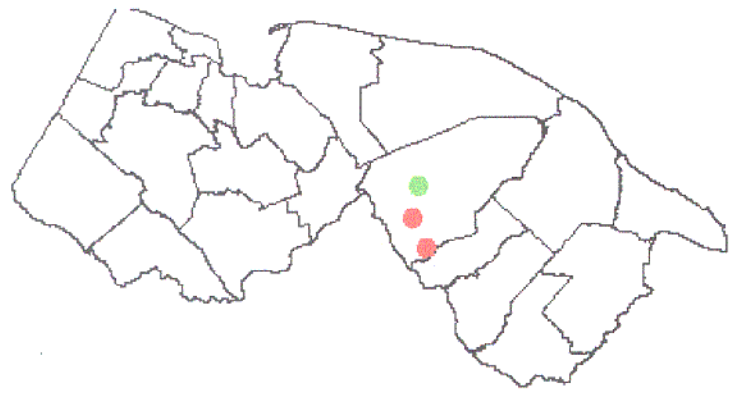
The test sites, North Branch Pennsauken Ck and South Branch Rancocas Ck, in WMA 18 and 19 respectively, were chosen based on the results of previous macroinvertebrate studies and the presence of likely sources of impairment; these included the proximity of NJPDES facilities, and of urbanization or agriculture. The site at North Branch Rancocas Ck in WMA 19, was chosen as a reference site because of its prior nonimpaired bioassessment in the AMNET survey (5, Appendix A), and because it is within the same Water Region (Lower Delaware) as the test sites. Also considered in choosing the reference site, were similarities in stream morphology and position within the New Jersey Ecomap or ecoregion scheme (1,9).






Survival and dry weight results showed no significant differences between the reference and the test site treatments. Since the test site results did not indicate acute toxicity, the severe impairment levels previously found were likely due to other factors including habitat degradation and/or various physiochemical factors (e.g. high temperature, turbidity, low dissolved oxygen, pH, etc...). Impairment may also have been caused by the presence of certain toxic substances at chronically, but not acutely, toxic levels, introduced episodically, rather than continuously, into the stream. Therefore, it is advisable by these study results, that supplemental sampling be performed for target analytes, such as excessive nutrients (usually forms of nitrogen or phosphorus), and pesticides or other known toxic compounds.

REFERENCES

1. Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers - benthic macroinvertebrates and fish. EPA/440/4-89/001. United State Environmental Protection Agency. Washington, D.C. 143pp. and appendices.
2. Hargrave, B.T. 1970. The utilization of benthic microflora by *Hyalella azteca*. J. Animal Ecology. 39:427-437.
3. de March, B.G.E. 1981. *Hyalella azteca* (Saussure). In: S.G. Lawrence (ed), Manual for the culture of selected freshwater invertebrates. Can. Spec. Pub. fish. Aquatic. Sc. No. 54, Department of Fisheries and Oceans.
4. Pennak, Robert William. 1978. Fresh-water invertebrates of the United States. John Wiley and Sons, Inc. New York, New York. 813pp.
5. New Jersey Department of Environmental Protection. Data report, 1998. Ambient biomonitoring network, Delaware region, upper tidal portion. Bureau of Freshwater and Biological Monitoring, Trenton. 13pp. and appendices.
6. New Jersey Department of Environmental Protection. 1992 Field sampling procedures manual, NJDEP, Trenton. 360pp..
7. New Jersey Department of Environmental Protection. Laboratory report. Standard operating procedures, culturing and sediment toxicity testing with *Hyalella azteca*. SM001.0795. 1995. Bureau of Freshwater & Biological Monitoring, Trenton. 7pp and appendices.
8. United State Environmental Protection Agency. 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA/600/4-90/027. Washington, D.C. 293pp.
9. New Jersey Department of Environmental Protection. 1996. New Jersey ECOMAP. State Forestry Services, Trenton.

AMNET SITES
AN0149, AN0176S,
AN0179



-  Nonimpaired AMNET Site
-  Severely Impaired AMNET Site
-  NJPDES Site
-  Major Roads
-  Streams
-  Stream Flow
-  NJDEP
Division of Watershed Management
Bureau of Freshwater &
Biological Monitoring

Bureau of Freshwater and Biological Monitoring

**Sediment Toxicity Tests
Watershed Management Area # 18 and 19
Pennsauken and Rancocas Creeks**

**Table 1
Test Chamber Chemical/Physical Parameters**

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.6	7.1	7.4	0.124	1.694
cond. Fmhos	156	125	146	6.752	4.633
D.O. mg/L	9.0	7.3	8.0	0.505	6.346

01H002a	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.4	6.9	7.2	0.154	2.135
cond. Fmhos	160	121	144	12.750	8.871
D.O. mg/L	8.7	7.3	7.7	0.491	6.366

01H002b	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.4	7.1	7.3	0.108	1.485
cond. Fmhos	163	139	151	8.600	5.689
D.O. mg/L	8.8	7.3	7.9	0.517	6.520

01H002c	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.4	6.9	7.2	0.156	2.170
cond. Fmhos	189	141	165	13.150	7.969
D.O. mg/L	8.3	6.7	7.4	0.464	6.308

Bureau of Freshwater and Biological Monitoring

**Sediment Toxicity Tests
Watershed Management Area # 18 and 19
Pennsauken and Rancocas Creeks**

TABLE 2

**MORTALITY DATA
(number surviving)**

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	9	10	10	10	10	98
01H002a	9	10	7	6	9	82
01H002b	9	9	9	9	8	88
01H002c	2	7	6	10	7	64

Statistical Analysis

Test Endpoint: Survival

Results: Wilcoxon Rank Sum Test
Wilcoxon Rank Sum Test
F-Test and T-Test

01H002a: no significant difference from control
01H002b: no significant difference from reference station
01H002c: no significant difference from reference station

*see appendix B for statistical printout

Bureau of Freshwater and Biological Monitoring

**Sediment Toxicity Tests
Watershed Management Area # 18 and 19
Pennsauken and Rancocas Creeks**

**TABLE 3
WEIGHT DETERMINATION**

Drying Oven Temperature: 105EC

Duration: 2 hours

Analyst: T. Miller

REPLICATE.	WGT. OF BOAT (mg)	DRY WGT: BOAT + ORGANISMS (mg)	TOTAL WGT. OF ORGANISMS (mg)	NUMBER OF ORGANISMS	ORGANISM AVG. DRY WGT. (mg)	GROUP AVG. (mg)	
CONTROL	A	13.25	13.32	0.07	9	0.0078	0.060
	B	10.93	11.65	0.72	10	0.072	
	C	8.86	9.87	1.01	10	0.101	
	D	9.53	9.72	0.19	10	0.019	
	E	8.86	9.87	1.07	10	0.101	
01H002a	A	10.10	10.53	0.43	9	0.048	0.081
	B	8.44	9.58	1.14	9	0.13	
	C	7.87	7.98	0.11	9	0.012	
	D	11.37	12.49	1.12	9	0.12	
	E	10.84	11.61	0.77	8	0.096	
01H002b	A	8.88	9.86	0.98	9	0.11	0.089
	B	14.62	15.57	0.95	10	0.095	
	C	11.23	11.87	0.64	7	0.091	
	D	11.19	11.72	0.53	6	0.088	
	E	6.29	6.83	0.54	9	.06	
01H002c	A	10.21	10.45	.024	2	0.12	0.085
	B	8.28	8.71	0.43	7	0.06	
	C	9.57	10.08	0.51	6	0.085	
	D	6.51	7.35	0.84	10	0.084	
	E	12.48	13.02	0.54	7	0.077	

Statistical Analysis

Test Endpoint: Growth

Test Used:

Results: Wilcoxon Rank Sum Test
Wilcoxon Rank Sum Test
Wilcoxon Rank Sum Test

01H002a: no significant difference from control
01H002b: no significant difference from reference station
01H002c: no significant difference from reference station

*see appendix B for statistical printout

Bureau of Freshwater and Biological Monitoring

**Sediment Toxicity Tests
Watershed Management Area # 18 and 19
Pennsauken and Rancocas Creeks**

**Appendix A
AMNET Data**

Station: AN0149
 North Br Rancocas Ck, Main St., Pemberton, Burlington County
 Pemberton USGS Quadrangle
 Date Sampled: 1/11/01

Family	Family Tolerance Value (FTV)	Number of Individuals
Chironomidae	6	21
Elmidae	4	19
Asellidae	8	10
Hydropsychidae	4	5
Coenagrionidae	9	4
Philopotamidae	3	4
Lumbricidae	10	4
Polycentropodidae	6	4
Limnephilidae	4	3
Sphaeriidae	8	3
Aeshnidae	3	2
Gomphidae	1	2
Naididae	7	2
Taeniopterygidae	2	2
Calopterygidae	5	1
Ephemerellidae	1	1
Corydalidae	0	1
Ceratopogonidae	6	1
Tetrastemmatidae	7	1
Pyralidae	5	1
Heptageniidae	4	1

Statistical Analysis

Number of Taxa: 21
 Total Number of Individuals: 92
 % Contribution of Dominant Family: 22.83 % (Chironomidae)
 Family Biotic Index: 5.48
 Scraper/Filterer Collector Ratio: 1.31
 Shredder/Total Ratio: 0.17
 E+P+T (Ephemeroptera, Plecoptera, Trichoptera): 7
 % EPT: 21.74
 EPT/C: 0.95
 NJIS Rating: 24
 Biological Condition: Nonimpaired
 Habitat Analysis: 168
 Deficiency(s) noted:
 -

Observations

Streamwater: Clear....Flow: Moderate....Width/Depth (ft): 60/2-3
 Substrate: Gravel/sand....StreamBank Vegetation/Stability: Trees, grass/Good
 Canopy: Partly Open....Other: suburban; station downstream of impoundment
 Water temp. 1.8C / pH 5.5SU / DO 13.6mg/L / Cond. 50umhos;

Station: AN0176S
South Branch Rancocas Ck, Route 38, Hainesport Twp., Burlington County
Mt. Holly USGS Quadrangle
Date Sampled: 04/19/01

Family	Family Tolerance Value (FTV)	Number of Individuals
Tubificidae	10	74
Chironomidae	6	16
BloodRed Chironomidae	8	10
Plagiostomidae	4	3
Sphaeriidae	8	2
Ceratopogonidae	6	1
Asellidae	8	1
Corbiculidae	8	1
Gomphidae	1	1

Statistical Analysis

Number of Taxa: 9
Total Number of Individuals: 109
% Contribution of Dominant Family: 67.89 % (Tubificidae)
Family Biotic Index: 8.87
Scraper/Filterer Collector Ratio: 0.00
Shredder/Total Ratio: 0.09
E+P+T (Ephemeroptera, Plecoptera, Trichoptera): 0
% EPT: 0.00
EPT/C: 0.00
NJIS Rating: 3
Biological Condition: Severely Impaired
Habitat Analysis: 116
Deficiency(s) noted: Tubificidae Family Overwhelmingly Dominant -
- Significant Organic Pollution - Paucity of Clean Water Organisms -

Observations

Streamwater: Turbid....Flow: Slow....Width/Depth (ft): 85/2.3
Substrate: Mud, silt....StreamBank Vegetation/Stability: Trees, shrubs/Poor
Canopy: Open....Other: suburban; storm sewers present, debris on banks, cobbles on banks near bridge
Water temp 10.8C / pH 7.3SU / DO 10.5mg/L / Cond 137umhos ;

Station: AN0179
North Br Pennsauken Ck, Fellowship Rd. Near 295, Mt. Laurel Twp., Burlington County
Moorestown USGS Quadrangle
Date Sampled: 3/8/01

Family	Family Tolerance Value (FTV)	Number of Individuals
Tubificidae	10	40
BloodRed Chironomidae	8	7
Tipulidae	3	2
Hydrobiidae	8	1
Libellulidae	9	1
Lumbriculidae	8	1

Statistical Analysis

Number of Taxa: 6
Total Number of Individuals: 52
% Contribution of Dominant Family: 76.92 % (Tubificidae)
Family Biotic Index: 9.37
Scraper/Filterer Collector Ratio: 0.00
Shredder/Total Ratio: 0.13
E+P+T (Ephemeroptera, Plecoptera, Trichoptera): 0
% EPT: 0.00
EPT/C: 0.00
NJIS Rating: 3
Biological Condition: Severely Impaired
Habitat Analysis: 88
Deficiency(s) noted: Tubificidae Family Overwhelmingly Dominant -
- Significant Organic Pollution - Paucity of Clean Water Organisms -

Observations

Streamwater: Slightly Turbid....Flow: Slow....Width/Depth (ft): 3/1
Substrate: Mud....StreamBank Vegetation/Stability: Trees/Poor
Canopy: Open....Other: suburban; iron precipitate; water color grey/brown
black muck on banks and bottom; Water temp. 6.5C / pH 6.8SU / DO 2.9mg/L / Cond. 771umhos

Bureau of Freshwater and Biological Monitoring

**Sediment Toxicity Tests
Watershed Management Area # 18 and 19
Pennsauken and Rancocas Creeks**

**Appendix B
Statistical Data**

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

SURVIVAL RESULTS

CONTROL VS. AN0149 (reference)

Survival Proportions with Arc Sine Square Root Transformation

Blank	AN0149	Blank Trans	AN0149 Trans
0.9	0.9	1.249	1.249
1.0	1.0	1.4127	1.4127
1.0	0.7	1.4127	0.9912
1.0	0.6	1.4127	0.8861
1.0	0.9	1.4127	1.249

Shapiro-Wilks Test for Normality

Blank Trans	AN0149 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.249	1.249	1.249		-0.0198	-0.3827				
1.4127	1.4127	1.4127		0.1439	-0.2776				
1.4127	0.9912	1.4127	1.2688	0.1439	-0.0198	0.3282	0.7695	0.842	Not Normal
1.4127	0.8861	1.4127		0.1439	-0.0198				
1.4127	1.249	1.4127		0.1439	-0.0198				
		1.249		-0.0198	0.1439				
Mean	Mean	1.4127		0.1439	0.1439				
1.38	1.1576	0.9912		-0.2776	0.1439				
		0.8861		-0.3827	0.1439				
		1.249		-0.0198	0.1439				

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

SURVIVAL RESULTS continued...

CONTROL VS. AN0149 (reference)
Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0149	Critical(from Table K=1)	Result
1.249	0.8861	9	1	0	1	19	No Significant Difference
1.4127	0.9912	8	2	0	2		
1.4127	1.249	10	4	0	4		
1.4127	1.249	6	4	0	4		
1.4127	1.249	1	4	4	0		
1.249	1.4127	7	8	0	8		
1.4127	1.4127	5	8	8	0		
0.9912	1.4127	4	8	8	0		
0.8861	1.4127	3	8	8	0		
1.249	1.4127	2	8	8	0		
				Sum	Sum		
				36	19		

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

SURVIVAL RESULTS continued...

AN0149 (reference) VS. AN0176S

Survival Proportions with Arc Sine Square Root Transformation

AN0149	AN0176S	AN0149 Trans	AN0176S Trans
0.9	0.9	1.249	1.249
1.0	0.9	1.4127	1.249
0.7	0.9	0.9912	1.249
0.6	0.9	0.8861	1.249
0.9	0.8	1.249	1.1071

Shapiro-Wilks Test for Normality

AN0149 Trans	AN0176S Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
1.249	1.249	1.249		0.0599	-0.303				
1.4127	1.249	1.4127		0.2236	-0.1979				
0.9912	1.249	0.9912	1.1891	-0.1979	-0.082	0.2092	0.833	0.842	Not Normal
0.8861	1.249	0.8861		-0.303	0.0599				
1.249	1.1071	1.249		0.0599	0.0599				
		1.249		0.0599	0.0599				
Mean	Mean	1.249		0.0599	0.0599				
1.1576	1.2206	1.249		0.0599	0.0599				
		1.249		0.0599	0.0599				
		1.1071		-0.082	0.2236				

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

SURVIVAL RESULTS continued...

AN0149 (reference) VS. AN0176S

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0149	AN0176S	Critical(from Table K=1)	Result
1.249	0.8861	4	1	1	0	19	No Significant Difference
1.4127	0.9912	3	2	2	0		
0.9912	1.1071	10	3	0	3		
0.8861	1.249	9	6.5	0	6.5		
1.249	1.249	8	6.5	0	6.5		
1.249	1.249	7	6.5	0	6.5		
1.249	1.249	6	6.5	0	6.5		
1.249	1.249	5	6.5	6.5	0		
1.249	1.249	1	6.5	6.5	0		
1.1071	1.4127	2	10	10	0		
				Sum	Sum		
				26	29		

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

SURVIVAL RESULTS continued...

AN0149 (reference) VS. AN0179

Survival Proportions with Arc Sine Square Root Transformation

AN0149	AN0179	AN0149 Trans	AN0179 Trans
0.9	0.2	1.249	0.4636
1.0	0.7	1.4127	0.9912
0.7	0.6	0.9912	0.8861
0.6	1.0	0.8861	1.4127
0.9	0.7	1.249	0.9912

Shapiro-Wilks Test for Normality

AN0149 Trans	AN0179 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
1.249	0.4636	1.249		0.1957	-0.5897				
1.4127	0.9912	1.4127		0.3594	-0.1672				
0.9912	0.8861	0.9912	1.0533	-0.0621	-0.1672	0.7502	0.9122	0.842	Normal
0.8861	1.4127	0.8861		-0.1672	-0.0621				
1.249	0.9912	1.249		0.1957	-0.0621				
		0.4636		-0.5897	-0.0621				
Mean	Mean	0.9912		-0.0621	0.1957				
1.1576	0.949	0.8861		-0.1672	0.1957				
		1.4127		0.3594	0.3594				
		0.9912		-0.0621	0.3594				

F-Test and T-Test

AN0149 Var	AN0179 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0458	0.1145	2.5	6.3882	Equal	1.165	6	1.9432	No Significant Difference

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

GROWTH

CONTROL VS. AN0149 (reference)

Average Dry Weight per Replicate (in mg)

BLANK	AN0149
0.0078	0.11
0.072	0.95
0.101	0.091
0.019	0.088
0.101	0.06

Shapiro-Wilks Test for Normality

BLANK	AN0149	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.0078	0.11	0.0078		-0.1522	-0.1522				
0.072	0.95	0.072		-0.088	-0.141				
0.101	0.091	0.101	0.16	-0.059	-0.1	0.7043	0.4828	0.842	Not Normal
0.019	0.088	0.019		-0.141	-0.088				
0.101	0.06	0.101		-0.059	-0.072				
		0.11		-0.05	-0.069				
Mean	Mean	0.95		0.79	-0.059				
0.0602	0.2598	0.091		-0.069	-0.059				
		0.088		-0.072	-0.05				
		0.06		-0.1	0.79				

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

GROWTH RESULTS continued...

CONTROL VS. AN0149 (reference)

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Control	AN0149	Critical(from Table K=1)	Result
0.0078	0.0078	1	1	1	0	19	No Significant Difference
0.072	0.019	4	2	2	0		
0.101	0.06	10	3	0	3		
0.019	0.072	2	4	4	0		
0.101	0.088	9	5	0	5		
0.11	0.091	8	6	0	6		
0.95	0.101	5	7.5	7.5	0		
0.091	0.101	3	7.5	7.5	0		
0.088	0.11	6	9	0	9		
0.06	0.95	7	10	0	10		
				Sum	Sum		
				22	33		

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

GROWTH continued...

AN0149 (reference) VS. AN0176S

Average Dry Weight per Replicate (in mg)

AN0149	AN0176S
0.11	0.048
0.95	0.13
0.091	0.012
0.088	0.12
0.06	0.096

Shapiro-Wilks Test for Normality

AN0149	AN0176S	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.11	0.048	0.11		-0.0605	-0.1585				
0.95	0.13	0.95		0.7795	-0.1225				
0.091	0.012	0.091	0.1705	-0.0795	-0.1105	0.6865	0.4918	0.842	Not Normal
0.088	0.12	0.088		-0.0825	-0.0825				
0.06	0.096	0.06		-0.1105	-0.0795				
		0.048		-0.1225	-0.0745				
Mean	Mean	0.13		-0.0405	-0.0605				
0.2598	0.0812	0.012		-0.1585	-0.0505				
		0.12		-0.0505	-0.0405				
		0.096		-0.0745	0.7795				

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

GROWTH RESULTS continued...

AN0149 (reference) VS. AN0176S

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0149	AN0176S	Critical(from Table K=1)	Result
0.11	0.012	8	1	0	1	19	No Significant Difference
0.95	0.048	6	2	0	2		
0.091	0.06	5	3	3	0		
0.088	0.088	4	4	4	0		
0.06	0.091	3	5	5	0		
0.048	0.096	10	6	0	6		
0.13	0.11	1	7	7	0		
0.012	0.12	9	8	0	8		
0.12	0.13	7	9	0	9		
0.096	0.95	2	10	10	0		
				Sum	Sum		
				29	26		

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

GROWTH continued...

AN0149 (reference) VS. AN0179

Average Dry Weight per Replicate (in mg)

AN0149	AN0179
0.11	0.12
0.95	0.06
0.091	0.085
0.088	0.084
0.06	0.077

Shapiro-Wilks Test for Normality

AN0149	AN0179	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.11	0.12	0.11		-0.0625	-0.1125				
0.95	0.06	0.95		0.7775	-0.1125				
0.091	0.085	0.091	0.1725	-0.0815	-0.0955	0.6749	0.4298	0.842	Not Normal
0.088	0.084	0.088		-0.0845	-0.0885				
0.06	0.077	0.06		-0.1125	-0.0875				
		0.12		-0.0525	-0.0845				
Mean	Mean	0.06		-0.1125	-0.0815				
0.2598	0.0852	0.085		-0.0875	-0.0625				
		0.084		-0.0885	-0.0525				
		0.077		-0.0955	0.7775				

Appendix B

Sediment Toxicity Tests Watershed Management Area # 18 and 19

GROWTH RESULTS continued...

AN0149 (reference) VS. AN0179

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0149	AN0179	Critical(from Table K=1)	Result
0.11	0.06	7	1.5	0	1.5	19	No Significant Difference
0.95	0.06	5	1.5	1.5	0		
0.091	0.077	10	3	0	3		
0.088	0.084	9	4	0	4		
0.06	0.085	8	5	0	5		
0.12	0.088	4	6	6	0		
0.06	0.091	3	7	7	0		
0.085	0.11	1	8	8	0		
0.084	0.12	6	9	0	9		
0.077	0.95	2	10	10	0		
				Sum	Sum		
				32.5	22.5		