NJ Department of Environmental Protection
Division of Science and Research
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WATER MONITORING MANAGEMENT
James Mumman, Administrator

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SEDIMENT TOXICITY TEST USING THE AMPHIPOD *Hyalella azteca* (South Branch Pennsauken Creek, South Branch Raccoon Creek) October/November 1996

Assay Number(s): 96H007a, 96H007f, 96H007g

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EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyalella azteca* was performed on sediments collected from the South Branch Pennsauken River (AN0184) and a reference site on South Branch Raccoon Creek (AN0682) in the Lower Delaware Basin. The reference was selected on South Branch Raccoon Creek due to a "non-impaired" biological assessment by the Ambient Biomonitoring Network (AMNET). The South Branch Pennsauken River site was chosen because it was suspected of toxicity due to a "severely impaired" assessment by the AMNET program. The South Branch Pennsauken River test site did not exhibit acute toxicity , in either survival or growth results, when statistically compared to the reference station. Further routine AMNET bioassessments will determine if additional testing in necessary.

INTRODUCTION

The Ambient Biomonitoring Network (AMNET) program is designed to establish biologically impaired stream segments throughout the state using EPA's Rapid Bioassessment Protocol (RBP). The RBP assesses impairment through the collection, identification, and classification of macroinvertebrates. Although the RBP is an excellent way in which to assess impairment, it may sometimes be difficult to distinguish if impairment is due to water quality or habitat destruction. Sediment Toxicity Testing is an additional tool to narrow down the cause of impairment to an acute toxicity problem before resorting to costly chemical monitoring.

Hyalella azteca is an epibenthic detritovore reported to also digest bacteria and algae from ingested sediment particles (Hargrave, 1970). This amphipod burrows into the sediment surface and inhabits lakes, ponds, and streams throughout North and South America (de March, 1981; Pennak, 1989). *H. azteca* is a sensitive benchmark species that can be cultured in the laboratory with relative ease.

METHODS

Sample sites were selected based on available AMNET data (see appendix a) and proximity to NJPDES facilities.
The sites selected are as follows (see map):

AMNET STATION#	BIOLOGICAL ASSESSMENT	LOCATION(see map)
AN0184	severely impaired	South Branch Pennsauken River @ Rt. 537, Maple Shade
AN0682	non-impaired	South Branch Raccoon Creek @ High St., Harrison Twp.

Sediment samples were collected from these sites AN0682 and AN0184 on October 16, 1996 at 11:45 and 13:46 hours respectively. 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 and stored at less than 4EC until the start of the test (NJDEP, 1992).

Prior to test initiation the sample sites were assigned assay numbers as follows:

96H007a = control
96H007f = AN0184
96H007g = AN0682

Testing methodology followed the Bureau of Water Monitoring Standard Operating Procedures (NJDEP, SM001.0795, 1995). 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 organism. 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(NJDEP, SM001.0795,1995). After this time period, the overlying water was syphoned, and fresh water was added. A control set of replicates was also set up using 250 ml of overlying water only.

1 - 7 day *H. azteca* juveniles were collected and held for one week prior to the start of the test (NJDEP, 1995).

The test was initiated on October 22, 1996 at 10:45 hours, by adding ten 7 - 14 day old organisms from the holding chamber to each test series replicate. Each day the overlying water was exchanged, and each test replicate was fed 1.5 ml of YCT and 1.5 ml of the green algae *Selenastrum capricornutum* at a concentration of 35 X 10^6 cells/ml. 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 3).

The test was concluded after ten days (November 1, 1996). Live organisms were counted (see table 1) and the dry weights measured (see table 2). Statistical analysis was performed, following EPA guidelines (U.S.E.P.A., 1991). The reference test was compared against the control and the remaining tests compared to the reference, providing the reference and the control were statistically the same.

RESULTS

The test was valid by meeting the acceptability requirements of $ 80% survival (see table 1) in the control test series (NJDEP, SM001.0795,1995). The survival data was not normally distributed when analyzed by the Shapiro-Wilks test for normality, and therefore the Wilcoxan Rank Sum Test was used when comparing test survival results. There was no significant difference between the reference test, 96H007g, survival results and the control survival results. Test 96H007f was then compared to the reference test. 96H007f showed no significant difference from the reference test for mortality.

Growth data (see table 2) was not normally distributed when comparing the control and reference station and also when comparing the reference test with 96H007f. Normality was analyzed using the Shapiro-Wilks test for normality, and the Wilcoxan Rank Sum Test was performed when comparing tests. 96H007f exhibited no significant difference from the reference test for growth. (see appendix b for statistical printout)

Clams were observed in both samples before initiating the test. All indigenous organisms seen were removed before the test was started. Bloodred chironomids were observed in test 96H007f at the end of the test.

On day 6, the dissolved oxygen level dropped below the 40 % saturation protocol requirements in the reference test series.

DISCUSSION

The sample sites on South Branch Pennsauken Creek and South Branch Raccoon Creek were chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities and urbanization. Site AN0184 had severely impaired bioassessment results as analyzed in AMNET. The reference site, AN0682, was chosen because it had a nonimpaired bioassessment based on results from the AMNET program and was within the same major drainage basin as the test site. Similar stream morphology and similar ecological region designation to the sample site suspected of toxicity also factored into choosing the reference site.

Survival and growth results showed no significant differences between the control treatment and reference test. Considering the final results of the test, the drop in dissolved oxygen on day 6 did not effect the final test results.

Survival and growth results showed no significant differences between the control treatment and reference test, or between the reference test and 96H007f, South Branch Pennsauken Creek. Toxicity may not have been exhibited at this site if South Branch Pennsauken Creek is in the process of undergoing biological recovery, or the severe impairment, demonstrated by the AMNET program, may be due to a chronic toxicity problem not detectable in this test. Future macroinvertebrate bioassessments will show if biological impairment still exists in the stream and additional testing will be considered at that time.

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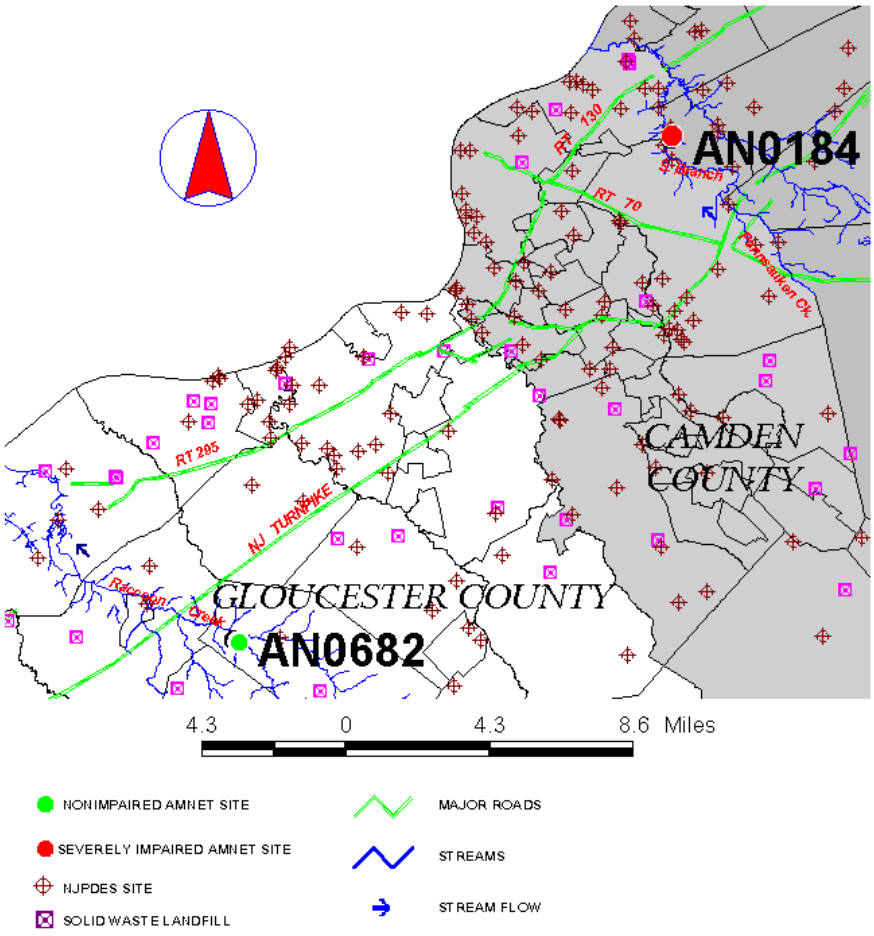
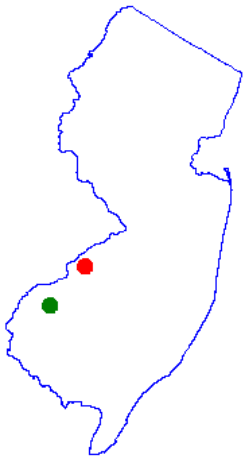


SEDIMENT TOXICITY TEST 96H007a, 96H007f, 96H007g



NJDEP
Division of Science & Research
Water Monitoring Management
Bureau of Water Monitoring

TABLE 1

MORTALITY DATA
(number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	10	10	10	10	10	100
96H007f	10	10	9	10	9	96
96H007g	9	10	10	1	10	80

Statistical Analysis

Test Endpoint: Survival
Test Used: Wilcoxan Rank Sum Test
Results: 96H007g - no significant difference from control
96H007f - no significant difference from reference station

Test Endpoint: Growth
Test Used: Wilcoxan Rank Sum Test
Results: 96H007g - no significant difference from control
96H007f - no significant difference from reference station

*see appendix b for statistical printout

TABLE 2

WEIGHT DETERMINATION

Drying Oven Temperature: 105EC Time/Date Start Drying: 1420 /11-1-96
Time/Date End Drying: 1620 /11-1-96
Analyst: T. Miller

REPLICATE.	WGT. OF BOAT (mg)	DRY WGT: BOAT + LARVAE (mg)	TOTAL WGT. OF LARVAE (mg)	NUMBER OF LARVAE	LARVAE AVG. DRY WGT. (mg)	GROUP AVG. (mg)
CONTROL A	20.61	22.00	1.39	10	0.139	
B	15.49	16.43	0.94	10	0.094	
C	11.71	12.93	1.22	10	0.122	0.119
D	15.59	16.66	1.07	10	0.107	
E	12.10	13.44	1.34	10	1.134	
95H007f A	9.25	11.19	1.94	10	0.194	
B	10.70	12.69	1.99	10	0.199	
C	14.53	16.93	2.40	9	0.267	0.236
D	16.65	19.15	2.50	10	0.250	
E	19.11	21.54	2.43	9	0.270	
95H007g A	13.02	14.75	1.73	9	0.192	
B	13.02	15.18	2.16	10	0.216	
C	10.43	12.91	2.48	10	0.248	0.274
D	9.73	10.22	0.49	1	0.490	
E	14.09	16.35	2.26	10	0.226	

Table 3

Test Chamber Chemical/Physical Parameters

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.2	6.8	7.0	0.170	2.426
cond. Fmhos	141	130	136	3.557	2.609
D.O. mg/L	8.1	6.4	7.2	0.427	5.964

96H007f	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	6.9	6.4	6.6	0.163	2.460
cond. Fmhos	164	141	152	6.700	4.420
D.O. mg/L	7.1	4.0	4.8	1.019	20.49

96H007g	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	6.6	6.2	6.3	0.135	2.131
cond. Fmhos	144	114	131	9.266	7.054
D.O. mg/L	5.9	2.9*	4.4	0.798	18.100

* Day 6 of test D.O. dropped to 33% saturation.

APPENDIX A

AMNET DATA

Delaware Basin - Camden USGS Quadrangle
Station AN0184
South Branch Pennsauken Creek, Route 537, Maple Shade
March 18, 1992

Family	Number of Individuals	Family Tolerance Value (FTV)
Tubificidae	98	10
BloodRedChironomidae	1	8
Hydropsychidae	1	4

Statistical Analysis

Number of Taxa = 3
Total Number of Individuals = 100
% Contribution of Dominant Family = 98.00
Family Biotic Index = 9.92
Scraper/Filterer Collector Ratio = 0.00
Shredder/Total Ratio = 0.01
E+P+T* = 1 *(Ephemeroptera, Plecoptera and Trichoptera)
%EPT = 1.00
EPT/C* = 1.00 *(Chironomidae)
NJIS Rating = 0
Biological Condition = severely impaired
Deficiency(s) noted: paucity of clean water organisms
significant organic pollution
low diversity
Tubificidae overwhelmingly dominant

Observations

Streamwater: green...Flow: slow...Width/Depth(ft): 30/<1...
Substrate: sand/gravel...Streambank Vegetation/Stability: poor/
poor...Canopy: open...Other: tree-lined; filamentous algae;
macrophytes

AN0682 # Raccoon Ck S Br, High St, Harrison Twp, Gloucester Co, Woodstown Quad
August 8, 1995

Taxon (Family Level)	FTV	NOI
PROSTOMATIDAE	7	1
TUBIFICIDAE	10	13
SPHAERIIDAE	8	5
GAMMARIDAE	4	7
HEPTAGENIIDAE	4	12
BAETIDAE	4	3
SIPHLONURIDAE	7	2
EPHEMERELLIDAE	1	1
CAENIDAE	7	1
AESHNIDAE	3	1
ELMIDAE	5	6
HYDROPSYCHIDAE	4	29
TIPULIDAE	3	5
SIMULIIDAE	6	4
CHIRONOMIDAE	6	7
EMPIDIDAE	6	3

Number of Taxa + 16 # Population + 100

Dominant Family(s) + HYDROPSYCHIDAE 29.00%
Family Biotic Index + 5.35
Scraper/Filterer Collector Ratio + 0.00
Shredder/Total Ratio + 0.22
E(phemeroptera)+P(lecoptera)+T(richoptera) + 6
%EPT + 48.00
EPT/Chironomids + 6.86

NJIS/Rating + 27/non-impaired

OBSERVATIONS

Clarity + clear
Flow + fast
Width/Depth(ft) + 10/0.5
Substrate + sand/gravel/some cobble
Streambank Vegetation/Stability + fair/fair
Canopy + mostly closed
Other + tree-lined/rural/agricultural; snags; some macrophytes; goats nr ck

APPENDIX B

STATISTICAL DATA

Survival Proportion with Arc-Sine Square Root Transformation

Blank	AN0682	Blank Trans	AN0682 Trans
1	0.9	1.4127	1.249
1	1	1.4127	1.4127
1	1	1.4127	1.4127
1	0.1	1.4127	0.3218
1	1	1.4127	1.4127

Shapiro-Wilks Test for Normality

Blank Trans	AN0682 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.249	1.4127		0.1255	-0.9654				
1.4127	1.4127	1.4127		0.1255	-0.0382				
1.4127	1.4127	1.4127	1.2872	0.1255	0.1255	1.0595	0.4364	0.842	Not Normal
1.4127	0.3218	1.4127		0.1255	0.1255				
1.4127	1.4127	1.4127		0.1255	0.1255				
		1.249		-0.0382	0.1255				
Mean	Mean	1.4127		0.1255	0.1255				
1.4127	1.1618	1.4127		0.1255	0.1255				
		0.3218		-0.9654	0.1255				
		1.4127		0.1255	0.1255				

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	Blank	AN0682	Critical (from Table K=1)	Result
1.4127	0.3218	9	1	0	1	19	No Significant Difference
1.4127	1.249	6	2	0	2		
1.4127	1.4127	10	6.5	0	6.5		
1.4127	1.4127	8	6.5	0	6.5		
1.4127	1.4127	7	6.5	0	6.5		
1.249	1.4127	5	6.5	6.5	0		
1.4127	1.4127	4	6.5	6.5	0		
1.4127	1.4127	3	6.5	6.5	0		
0.3218	1.4127	2	6.5	6.5	0		
1.4127	1.4127	1	6.5	6.5	0		
				Sum	Sum		
				32.5	22.5		

Survival Proportions with Arc-Sine Square Root Transformation

AN0682	AN0184	AN0682 Trans	AN0184 Trans
0.9	1	1.249	1.4127
1	1	1.4127	1.4127
1	0.9	1.4127	1.249
0.1	1	0.3218	1.4127
1	0.9	1.4127	1.249

Shapiro-Wilks Test for Normality

AN0682 Trans	AN0184 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.249	1.4127	1.249		-0.0055	-0.9327				
1.4127	1.4127	1.4127		0.1582	-0.0055				
1.4127	1.249	1.4127	1.2545	0.1582	-0.0055	1.0202	0.5296	0.842	Not Normal
0.3218	1.4127	0.3218		-0.9327	-0.0055				
1.4127	1.249	1.4127		0.1582	0.1582				
		1.4127		0.1582	0.1582				
Mean	Mean	1.4127		0.1582	0.1582				
1.1618	1.3472	1.249		-0.0055	0.1582				
		1.4127		0.1582	0.1582				
		1.249		-0.0055	0.1582				

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wilcoxan Rank	AN0682	AN0184	Critical (from Table K=1)	Result
1.249	0.3218	4	1	1	0	19	No Significant Difference
1.4127	1.249	10	3	0	3		
1.4127	1.249	8	3	0	3		
0.3218	1.249	1	3	3	0		
1.4127	1.4127	9	7.5	0	7.5		
1.4127	1.4127	7	7.5	0	7.5		
1.4127	1.4127	6	7.5	0	7.5		
1.249	1.4127	5	7.5	7.5	0		
1.4127	1.4127	3	7.5	7.5	0		
1.249	1.4127	2	7.5	7.5	0		
				Sum	Sum		
				26.5	28.5		

Average Dry Weight per Replicate (in mg)

AN0682	AN0184
0.192	0.194
0.216	0.199
0.248	0.267
0.49	0.25
0.226	0.27

Shapiro-Wilks Test for Normality

AN0682	AN0184	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.192	0.194	0.192		-0.0632	-0.0632				
0.216	0.199	0.216		-0.0392	-0.0612				
0.248	0.267	0.248	0.2552	-0.0072	-0.0562	0.0689	0.6748	0.842	Not Normal
0.49	0.25	0.49		0.2348	-0.0392				
0.226	0.27	0.226		-0.0292	-0.0292				
		0.194		-0.0612	-0.0072				
Mean	Mean	0.199		-0.0562	-0.0052				
0.2744	0.236	0.267		0.0118	0.0118				
		0.25		-0.0052	0.0148				
		0.27		0.0148	0.2348				

Wilcoxan Rank Sum Test

Pooled	Sorted	Point	Wicoxan Rank	AN0682	AN0184	Critical (from Table K=1)	Result
0.192	0.192	1	1	1	0	19	No Significant Difference
0.216	0.194	6	2	0	2		
0.248	0.199	7	3	0	3		
0.49	0.216	2	4	4	0		
0.226	0.226	5	5	5	0		
0.194	0.248	3	6	6	0		
0.199	0.25	9	7	0	7		
0.267	0.267	8	8	0	8		
0.25	0.27	10	9	0	9		
0.27	0.49	4	10	10	0		
				Sum	Sum		
				26	29		