



NJ Department of Environmental Protection
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WATER MONITORING MANAGEMENT

James Mumman, Administrator

August 1996

**SEDIMENT TOXICITY TEST
USING THE AMPHIPOD
Hyaella azteca
(Robinsons Brook, Dead River Sediment)
August 1996**

Assay Number(s): 96H005a, 96H005e, 96H005f

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EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyaella azteca* was performed on sediments collected from Robinsons Brook (AN0196) and a reference site on Dead River (AN0226) in the Northeast Basin. The reference was selected on Dead River due to a "non-impaired" biological assessment by the Ambient Biomonitoring Network (AMNET). The Robinsons Brook site was chosen because it was suspected of toxicity due to a "severely impaired" assessment by the AMNET program. The Robinsons Brook site exhibited acute toxicity as a significant difference, in both survival and growth results, when statistically compared to the reference station. Since toxicity test results substantiate AMNET bioassessments, it is recommended that further biological and chemical monitoring be conducted at this site to determine the source and identities of the contamination present.

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 detritivore 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):

<u>AMNET STATION#</u>	<u>BIOLOGICAL ASSESSMENT</u>	<u>LOCATION</u> (see map)
AN0196	severely impaired	Robinsons Brook @ Goodmans Crossing
AN0226	non-impaired	Dead River @ Somerville Road

Sediment samples were collected from these sites AN0226 and AN0196 on August 13, 1996 at 10:30 and 12:15 hours respectively. At each station the sediment was collected in the stream channel using a stainless steel scoop sampler and placed into two 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:

96H005a = control
96H005e = AN0226
96H005f = AN0196

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 August 20, 1996 at 10:00 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 35×10^6 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 (August 30, 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 $\geq 80\%$ 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 Wilcoxon Rank Sum Test was used when comparing test survival results. There was no significant difference between the reference test, 96H005e, survival results and the control survival results. Test 96H005f was then compared to the reference test. 96H005f was significantly different from the reference test for mortality.

Growth data (see table 2) was normally distributed when comparing the control and reference station and also when comparing the reference test with 96H005f. Normality was analyzed using the Shapiro-Wilks test for normality, and an F-test and T-test was performed when comparing tests. 96H005f exhibited a significant difference from the reference test for growth. (see appendix b for statistical printout)

Gammarus, riffle beetles, and dragonfly nymphs were observed in the reference sample before initiating the test. All indigenous organisms seen were removed before the test was started.

At the end of the test the dissolved oxygen level dropped below the 40 % saturation protocol requirements in both test series. In test 96H005e and -f the D.O. dropped to 31 % saturation at test termination.

DISCUSSION

The sample sites on the Robinsons Brook and Dead River were chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities and urbanization. Site AN0196 had severely impaired bioassessment results as analyzed in AMNET. The reference site, AN0226, 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.

Sediment at site AN0196, on Robinsons Brook, had a muck consistency in which the test organisms did not readily burrow, as they had in the reference sediment.

Survival and growth results showed no significant differences between the control treatment and reference test. The drop in dissolved oxygen at the end of the test did not effect the final test results.

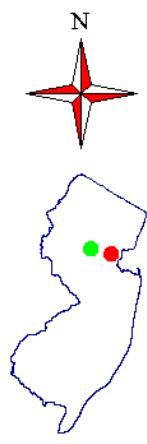
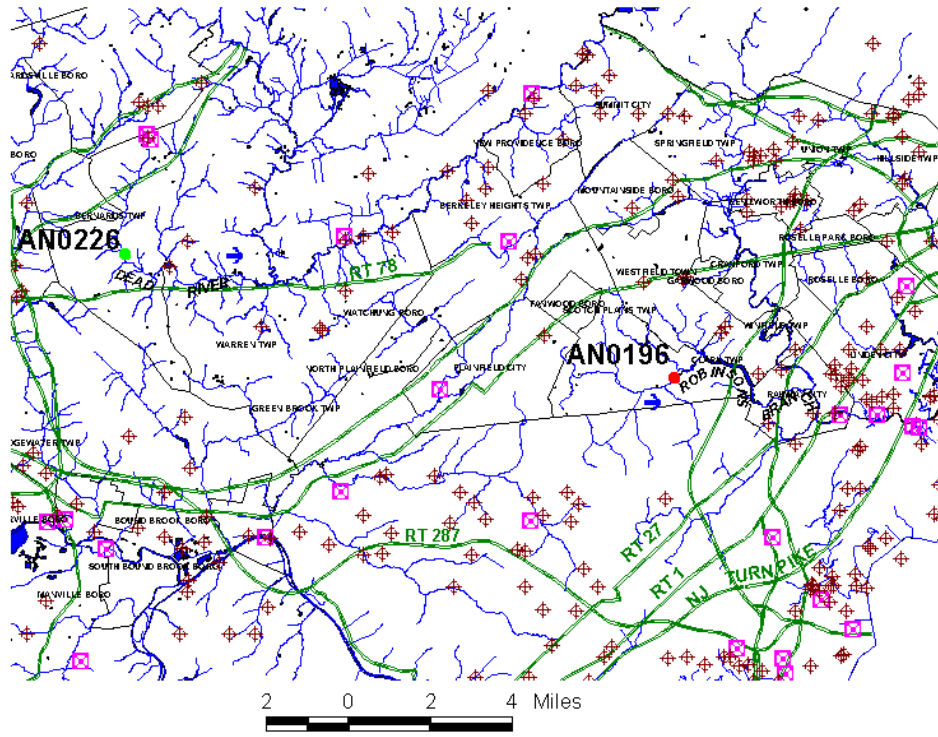
Acute toxicity was exhibited, as a significant difference, when comparing the reference test survival results with mortality results from 96H005f, Robinsons Brook. Acute toxicity was also exhibited, as a significant difference, when comparing the reference test growth results with growth results from 96H005f, Robinsons Brook. Although the growth results in the control and 96H005f were essentially equal, the control does not receive any additional food sources which are present in a highly productive reference sediment. Therefore comparing growth results for the test sediment with the reference demonstrates more specifically what is taking place at the test site. *Hyalella azteca* is reported to digest bacteria and algae from ingested sediment particles (Hargrave, 1970). During the test, the organisms did not readily burrow into Robinsons Brook sediment as they did in the reference sediment. The organisms in the Robinsons Brook sediment were probably either not ingesting any extra food sources, there were no food sources in the sediment, or toxins may have inhibited growth. Robinsons Brook, upstream of the sampling location, is subject to numerous dischargers, and, being in an urban environment is likely impacted by nonpoint sources. Since the dissolved oxygen was maintained above 40 % saturation until test termination, it is unlikely that the drop in D.O. to 31 % saturation at test termination effected the outcome. As observed in the reference test, the D.O. also dropped to 31 % saturation with no significant effects on the test organisms.

Impairment of site AN0196 as assessed in the AMNET program, was indicative of significant organic pollution. The severe impairment assessed in the AMNET program along with the acute toxicity demonstrated in the sediment suggests an impact due to the additive effects of the numerous discharges and/or nonpoint sources, likely due to urbanization, which influence the Saddle River, and probably not the result of a single source. Chemical sampling should be performed to determine the sources and identities of the contamination present.

REFERENCES

- de March, B.G.E. *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, 1981
- Hargrave, B.T. The utilization of benthic microflora by *Hyalella azteca*. J. Animal Ecology. 39:427-437, 1970.
- Nebecker, A.V. and Miller, C.E. Use of the amphipod crustacean Hyalella azteca in freshwater and estuarine sediment toxicity tests. Environ. Toxicol. Chem. 7:1027-1033, 1988.
- NJDEP, Water Monitoring Management, Ambient Biomonitoring Network, Passaic, Hackensack, and Wallkill Drainage Basins, 1993, Benthic Macroinvertebrate Data.
- NJDEP, Field Sampling Procedures Manual, 1992
- NJDEP, Water Monitoring Management, Standard Operating Procedures, Culturing and Sediment Toxicity Testing With *Hyalella azteca*, SM001.0795, REV 0, 1995.
- Roach, R.W., Carr, R.S., Howard, C.L., and Cain, D.W. Assessment of produced water impacts in Galveston Bay System. U.S. Fish and Wildlife Report, Clear Lake Ecological Services Office, Houston, TX, 1992
- U.S.E.P.A., Methods For Measuring The Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, EPA/600/4-90/027, 1991.
- Winger, P.V., Lasier, P.J., Geitner, H. Toxicity of sediments and pore water from Brunswick Estuary, Georgia. Arch. Environ. Contam. Toxicol.: In press, 1993.

**SEDIMENT
TOXICITY TEST
96H005a,
96H005e,96H005f**



- NONIMPAIRED AMHET SITE
- IMPAIRED AMHET SITE
- ➔ STREAM FLOW
- STREAMS
- MAJOR ROADS
- SOLID WASTE LANDFILL
- NJPDES SITES

NJDEP
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TABLE 1

MORTALITY DATA
(number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	10	8	10	10	9	94
96H005e	10	10	10	10	10	100
96H005f	9	7	10	9	9	88

Statistical Analysis

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results: 96H005e - no significant difference from control
96H005f - significant difference from reference station

Test Endpoint: Growth

Test Used: F-test and T-test

Results: 96H005e - no significant difference from control
96H005f - significant difference from reference station

*see appendix b for statistical printout

TABLE 2**WEIGHT DETERMINATION**Drying Oven Temperature: 105ECTime/Date Start Drying: 1415 / 8-30-96Time/Date End Drying: 1615 / 8-30-96Analyst: 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	15.36	16.64	1.28	10	0.128	0.141
B	16.72	17.64	0.92	8	0.115	
C	16.68	18.17	1.49	10	0.149	
D	17.38	18.96	1.58	10	0.158	
E	16.81	18.21	1.40	9	1.156	
95H005e A	14.53	16.68	2.15	10	0.215	0.232
B	16.48	19.15	2.67	10	0.267	
C	20.05	22.20	2.15	10	0.220	
D	17.33	19.53	2.20	10	0.220	
E	19.49	21.94	2.45	10	0.245	
95H005f A	19.11	20.66	1.55	9	0.172	0.141
B	13.21	14.30	1.09	7	0.156	
C	18.44	19.99	1.55	10	0.155	
D	20.76	21.70	0.94	9	0.104	
E	15.86	16.91	1.05	9	0.117	

Table 3**Test Chamber Chemical/Physical Parameters**

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.2	6.9	7.1	0.113	1.601
cond. Fmhos	144	126	136	5.605	4.130
D.O. mg/L	7.4	6.8	7.1	0.382	5.389

96H005e	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.1	6.8	7.0	0.121	1.744
cond. Fmhos	203	162	183	14.358	7.854
D.O. mg/L	6.3	2.6*	4.9	0.986	19.943

96H005f	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.1	6.7	6.9	0.129	1.874
cond. Fmhos	245	179	199	19.323	9.719
D.O. mg/L	5.9	2.6	4.5	0.945	20.956

*31% saturation

APPENDIX A
AMNET DATA

Passaic Basin - Perth Amboy USGS Quadrangle
 Station AN0196
 Robinsons Brook, Goodmans Crossing, Scotch Plains
 February 19, 1992

Family	Number of Individuals	Family Tolerance Value (FTV)
Sphaeriidae	46	8
Tubificidae	34	10
Tipulidae	1	3
Turbellaria	4	4
Gastropoda	5	7
Hydropsychidae	1	4
Chironomidae	7	6
Hirudinea	1	10
Lumbriculidae	1	8

Statistical Analysis

Number of Taxa = 9
 Total Number of Individuals = 100
 % Contribution of Dominant Family = 46.00
 Family Biotic Index = 8.26
 Scraper/Filterer Collector Ratio = 0.08
 Shredder/Total Ratio = 0.04
 E+P+T* = 1 *(Ephemeroptera, Plecoptera and Trichoptera)
 %EPT = 1.00
 EPT/C* = 0.14 *(Chironomidae)
 NJIS Rating = 6
 Biological Condition = severely impaired
 Deficiency(s) noted: paucity of clean water organisms
 significant organic pollution

Observations

Streamwater: slightly turbid...Flow: slow...Width/Depth(ft): 30/1.
 ..Substrate: sand/gravel...Streambank Vegetation/Stability: poor/
 poor...Canopy: open...Other: sparsely tree-lined; apartments

Passaic Basin - Bernardsville USGS Quadrangle
 Station AN0226
 Dead River, Somerville Road, Liberty Corner, Bernards Township
 February 4, 1992

Family	Number of Individuals	Family Tolerance Value (FTV)
EphemereIIDae	14	1
Simuliidae	46	6
Odontoceridae	1	0
Elmidae	3	4
Chironomidae	21	6
Nemouridae	1	2
Hydroptilidae	1	4
Gastropoda	1	7
Limnephilidae	1	4
Turbellaria	2	4
Capniidae	5	1
Gomphidae	1	1
Hydropsychidae	1	4
Taeniopterygidae	1	2
Porifera	1	7

Statistical Analysis

Number of Taxa = 15
 Total Number of Individuals = 100
 % Contribution of Dominant Family = 46.00
 Family Biotic Index = 4.72
 Scraper/Filterer Collector Ratio = 0.06
 Shredder/Total Ratio = 0.23
 E+P+T* = 8 *(Ephemeroptera, Plecoptera and Trichoptera)
 %EPT = 25.00
 EPT/C* = 1.19 *(Chironomidae)
 NJIS Rating = 24
 Biological Condition = non-impaired
 Deficiency(s) noted: none

Observations

Streamwater: turbid, green...Flow: slow...Width/Depth(ft): 20/1...
 Substrate: rock...Streambank Vegetation/Stability: good/good...
 Canopy: open...Other: wood-lined; fish

APPENDIX B
STATISTICAL DATA

Survival Proportions with Arc-Sine Square Root Transformation

Blank	Blank Trans	AN0226	AN0226 Trans
1	1.4127	1	1.4127
0.8	1.1071	1	1.4127
1	1.4127	1	1.4127
1	1.4127	1	1.4127
0.9	1.249	1	1.4127

Shapiro-Wilks Test for Normality

Blank Trans	AN0226 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0469	-0.2587				
1.1071	1.4127	1.1071		-0.2587	-0.1168				
1.4127	1.4127	1.4127	1.3658	0.0469	0.0469	0.0982	0.5352	0.842	Not Normal
1.4127	1.4127	1.4127		0.0469	0.0469				
1.249	1.4127	1.249		-0.1168	0.0469				
		1.4127		0.0469	0.0469				
Mean	Mean	1.4127		0.0469	0.0469				
1.3188	1.4127	1.4127		0.0469	0.0469				
		1.4127		0.0469	0.0469				
		1.4127		0.0469	0.0469				

Wilcoxon Rank Sum Test

Pooled	Sorted	Wilcoxon Rank	Point	Blank	AN0226	Critical(from Table K=1)	Result
1.4127	1.1071	1	2	1	0	19	No Significant Difference
1.1071	1.249	2	5	2	0		
1.4127	1.4127	6.5	1	6.5	0		
1.4127	1.4127	6.5	3	6.5	0		
1.249	1.4127	6.5	4	6.5	0		
1.4127	1.4127	6.5	6	0	6.5		
1.4127	1.4127	6.5	7	0	6.5		
1.4127	1.4127	6.5	8	0	6.5		
1.4127	1.4127	6.5	9	0	6.5		
1.4127	1.4127	6.5	10	0	6.5		
				Sum	Sum		
				22.5	32.5		

Survival Proportions with Arc-Sine Square Root Transformation

AN0226	AN0226 Trans	AN0196	AN0196 Trans
1	1.4127	0.9	1.249
1	1.4127	0.7	0.9912
1	1.4127	1	1.4127
1	1.4127	0.9	1.249
1	1.4127	0.9	1.249

Shapiro-Wilks Test for Normality

AN0226 Trans	AN0196 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.249	1.4127		0.0913	-0.3302				
1.4127	0.9912	1.4127		0.0913	-0.0724				
1.4127	1.4127	1.4127	1.3214	0.0913	-0.0724	0.1748	0.7042	0.842	Not Normal
1.4127	1.249	1.4127		0.0913	-0.0724				
1.4127	1.249	1.4127		0.0913	0.0913				
		1.249		-0.0724	0.0913				
Mean	Mean	0.9912		-0.3302	0.0913				
1.4127	1.2302	1.4127		0.0913	0.0913				
		1.249		-0.0724	0.0913				
		1.249		-0.0724	0.0913				

Wilcoxon Rank Sum Test

Pooled	Sorted	Wilcoxon Rank	Point	AN0226	AN0196	Critical (from Table K=1)	Result
1.4127	0.9912	1	7	0	1	19	Significantly Different
1.4127	1.249	3	6	0	3		
1.4127	1.249	3	9	0	3		
1.4127	1.249	3	10	0	3		
1.4127	1.4127	7.5	1	7.5	0		
1.249	1.4127	7.5	2	7.5	0		
0.9912	1.4127	7.5	3	7.5	0		
1.4127	1.4127	7.5	4	7.5	0		
1.249	1.4127	7.5	5	7.5	0		
1.249	1.4127	7.5	8	0	7.5		
				Sum	Sum		
				37.5	17.5		

Average Dry Weight per Replicate (in mg)

Blank	AN0226
0.128	0.215
0.115	0.267
0.149	0.215
0.158	0.22
0.156	0.245

Shapiro-Wilks Test for Normality

Blank	AN0226	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.128	0.215	0.128		-0.0588	-0.0718				
0.115	0.267	0.115		-0.0718	-0.0588				
0.149	0.215	0.149	0.1868	-0.0378	-0.0378	0.0243	0.9313	0.842	Normal
0.158	0.22	0.158		-0.0288	-0.0308				
0.156	0.245	0.156		-0.0308	-0.0288				
		0.215		0.0282	0.0282				
Mean	Mean	0.267		0.0802	0.0282				
0.1412	0.2324	0.215		0.0282	0.0332				
		0.22		0.0332	0.0582				
		0.245		0.0582	0.0802				

F-test and T-Test

AN0279 Var	AN0291 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0004	0.0005	1.25	6.3882	Equal	-6.7976	7	1.8946	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0226	AN0196
0.215	0.172
0.267	0.156
0.215	0.155
0.22	0.104
0.245	0.117

Shapiro-Wilks Test for Normality

AN0226	AN0196	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.215	0.172	0.215		0.0284	-0.0826				
0.267	0.156	0.267		0.0804	-0.0696				
0.215	0.155	0.215	0.1866	0.0284	-0.0316	0.0264	0.9519	0.842	Normal
0.22	0.104	0.22		0.0334	-0.0306				
0.245	0.117	0.245		0.0584	-0.0146				
		0.172		-0.0146	0.0284				
Mean	Mean	0.156		-0.0306	0.0284				
0.2324	0.1408	0.155		-0.0316	0.0334				
		0.104		-0.0826	0.0584				
		0.117		-0.0696	0.0804				

F-test and T-test

AN0226 Var	AN0196 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0005	0.0008	1.6	6.3882	Equal	5.6808	7	1.8946	Significantly Different