

State of New Jersey
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**SEDIMENT TOXICITY TEST
USING THE AMPHIPOD**
Hyaella azteca
Manapaqua Brook, Ridgeway Branch
(Atlantic Coastal Basin)



New Jersey Department of Environmental Protection
ROBERT C. SHINN, JR.
COMMISSIONER

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WATER MONITORING MANAGEMENT
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May 1997

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USING THE AMPHIPOD
Hyaella azteca
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Assay Number(s): 97H003f, 97H003g

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EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyalella azteca* was performed on sediments collected from selected sites on Manapaqua Brook and a reference site on Ridgeway Branch in the Atlantic Coastal Basin. The reference was selected on Ridgeway Branch because of its "non-impaired" biological assessment as per the Ambient Biomonitoring Network (AMNET). The Manapaqua Brook site was chosen because of suspected toxicity due to "severely impaired" assessments as per AMNET program. Sediment toxicity testing provides further data which can be related to previous assessments. When statistically compared to the reference, the test site did not exhibit acute toxicity, as measured by survival and growth of test organisms.

INTRODUCTION

The Ambient Biomonitoring Network (AMNET) program of the New Jersey Department of Environmental Protection (NJDEP) is designed to establish biologically impaired stream segments throughout the state using the U.S. Environmental Protection Agency (EPA) Rapid Bioassessment Protocol (RBP)(10). The RBP assesses impairment through the collection, identification, categorizing, and quantification 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 degradation 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 (2). This amphipod burrows into the sediment surface and inhabits lakes, ponds, and streams throughout North and South America (1)(8). *H. azteca* is a sensitive benchmark, i.e. established, test species that can be cultured in the laboratory with relative ease.

METHODS

Sample sites were selected based on available AMNET data(5) (see appendix A) and proximity to 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)
AN0532	severely impaired	Manapaqua Brook @ Rt.70, Lakehurst
AN0528	non-impaired	Ridgeway Branch @Rt.70, Manchester Twp.

Sediment samples were collected from sites AN0528 and AN0532 on May 6, 1997 at 10:45 and 11:35 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 (4).

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

97H003a = control
97H003f = AN0528
97H003g = AN0532

Testing methodology followed the Bureau of Water Monitoring Standard Operating Procedures(6). 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(6). 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 and held for one week prior to the start of the test (6).

The test was initiated on May 13, 1997 at 10:45 hours, by adding ten 7 - 14 day old organisms from the holding chamber to each test series replicates. Each day the overlying water was exchanged, and each test replicate was fed 1.5 ml of yeast, CEROPHYLL7, Trout chow(YCT)(11), and 1.5 ml of the green algae *Selenastrum capricornutum* at a concentration of 35×10^6 cells/ml (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 (May 25, 1997). Live organisms were counted (see table 2) and the dry weights measured (see table3). Statistical analysis was performed following EPA guidelines (11). The reference test was compared against the control, and the remaining tests compared to the reference, providing the reference and the control were statistically similar.

RESULTS

The test was valid by virtue of meeting the acceptability requirements of $\geq 80\%$ survival (see table 2) in the control test series (6). 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 between the reference test, 97H003f, survival results and the control survival results. Test 97H003g was then compared to the reference. 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. 97H003g showed no significant difference from the reference for mortality. Test 97H003g was then compared to the reference for dry weight(see table 3). The dry weight data was distributed normally as analyzed by the Shapiro-Wilks test, and therefore an F-Test and T-Test were used when comparing test dry weight results. 97H003g showed no significant difference from the reference for dry weight.(see appendix B for statistical printout)

DISCUSSION

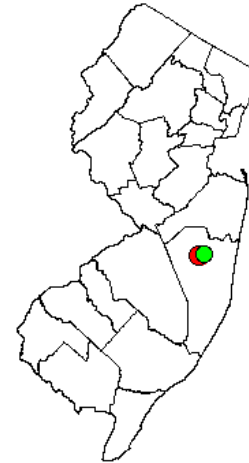
The sample site on Manapaqua Brook was chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities and urbanization. Site AN0532 had a severely impaired bioassessment results as analyzed in AMNET(5). The reference site at Ridgeway Branch, AN0528, was chosen because it had a nonimpaired bioassessment based on results from the AMNET program and was within the same major drainage basin as the test sites. Similar stream morphology and similar New Jersey Ecomap(7) designation (based on geology, soil, and natural vegetation) was also considered in choosing the reference site.

Survival and dry weight results showed no significant differences between the reference and the test site treatments. The fact that these toxicity test results on severely biologically impaired AMNET stations do not indicate the presence of toxic compounds in acutely toxic levels, indicates that the following may be true: the impairments may be due to elevated nutrient concentrations in the water body(ies); the toxic compounds causing the observed impairment are present at chronic, not acute levels; toxic compounds are being introduced into the water body(ies) episodically rather than continuously. Therefore, it is suggested by these study results that supplemental sediment and water column monitoring be performed for target analytes, such as nutrients and known toxic compounds.

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**SEDIMENT
TOXICITY TEST
97H003f, 97H003g**



- | | |
|---|---|
|  NONIMPAIRED AMNET SITE |  MAJOR ROADS |
|  SEVERELY IMPAIRED AMNET SITE |  STREAMS |
|  HJPDES SITE |  STREAM FLOW |
|  SOLID WASTE LANDFILL | |



NJDEP
Water Monitoring Management
Bureau of Freshwater &
Biological Monitoring

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

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.6	6.9	7.2	0.20	2.74
cond. Fmhos	143	125	133	8.33	6.25
D.O. mg/L	7.7	4.1	5.7	1.21	21.32

97H003f	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.0	6.6	6.9	0.11	1.57
cond. Fmhos	148	105	132	12.94	9.78
D.O. mg/L	6.9	3.8	5.1	0.92	17.85

97H003g	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.1	6.8	7.0	0.09	1.25
cond. Fmhos	149	96	134	15.92	11.90
D.O. mg/L	6.7	4.1	5.0	0.74	14.67

TABLE 2

MORTALITY DATA
(number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	10	10	10	10	10	100
97H003f	10	10	10	10	10	100
97H003g	10	10	10	9	7	92

Statistical Analysis

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results: 97H003f - no significant difference from control
97H003g - no significant difference from reference station

*see appendix B for statistical printout

TABLE 3

WEIGHT DETERMINATION

Drying Oven Temperature: 105ECDuration: 2 hoursAnalyst: 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	15.79	16.81	1.02	10	0.102	0.126
B	12.96	14.45	1.49	10	0.149	
C	14.32	15.50	1.18	10	0.118	
D	12.76	13.94	1.18	10	0.118	
E	15.90	17.34	1.44	10	0.144	
97H003f A	10.09	12.10	2.01	10	0.201	0.181
B	11.57	13.87	2.30	10	0.230	
C	9.67	11.42	1.75	10	0.175	
D	10.60	12.53	1.93	10	0.193	
E	6.66	8.66	2.00	10	0.200	
97H003g A	14.07	15.63	1.56	10	0.156	0.200
B	10.41	11.99	1.58	10	0.158	
C	9.12	10.54	1.42	9	0.158	
D	10.07	11.59	1.52	7	0.217	
E	11.98	14.13	2.15	10	0.215	

Statistical Analysis

Test Endpoint: Growth

Test Used: F-test and T-test

Results: 97H003f - no significant difference from control
97H003g - no significant difference from reference station

*see appendix B for statistical printout

APPENDIX A

AMNET DATA(5)

October 19, 1994

Taxon (Family Level)	FTV	NOI
LUMBRICULIDAE	8	3
SPHAERIIDAE	8	1
ASELLIDAE	8	14
METRETOPODIDAE	2	4
LEPTOPHLEBIIDAE	2	12
COENAGRIONIDAE	9	1
PERLIDAE	1	1
CORIXIDAE	7	1
VELIIDAE	9	1
ELMIDAE	5	4
CORYDALIDAE	0	4
PHILOPOTAMIDAE	3	18
HYDROPSYCHIDAE	4	8
PHRYGANEIDAE	4	1
LIMNEPHILIDAE	4	1
ODONTOCERIDAE	0	3
BRACHYCENTRIDAE	1	6
TIPULIDAE	3	1
SIMULIIDAE	6	1
CHIRONOMIDAE	6	4
BLOODREDCHIRONOMIDAE	8	11

Number of Taxa + 21 # Number of Individuals + 100

Dominant Family(s) + PHILOPOTAMIDAE 18.00%

Family Biotic Index + 4.43

Scraper/Filterer Collector Ratio + 0.10

Shredder/Total Ratio + 0.13

E(phemeroptera)+P(lecoptera)+T(richoptera) + 9

%EPT + 54.00

EPT/Chironomids + 3.60

NJIS/Rating + 30/non-impaired

OBSERVATIONS

Clarity + clear

Flow + moderate

Width/Depth(ft) + 15-20/1-2

Substrate + sand

Streambank Vegetation/Stability + good/good

Canopy + partly closed

Other + woods/commercial

FTV = Family Tolerance Value, NOI = Number Of Individuals(per 100 organism subsample)

October 19, 1994

Taxon (Family Level)	FTV	NOI
ASELLIDAE	8	97
ELMIDAE	5	1
PYRALIDAE	5	1
SIMULIIDAE	6	1

Number of Taxa + 4 # Population + 100

Dominant Family(s) + ASELLIDAE 97.00%
Family Biotic Index + 7.92
Scraper/Filterer Collector Ratio + 0.00
Shredder/Total Ratio + 0.00
E(phemeroptera)+P(lecoptera)+T(richoptera) + 0
%EPT + 0.00
EPT/Chironomids + 0.00

NJIS/Rating + 0/severely impaired

Deficiency(s) noted + low diversity
ASELLIDAE overwhelmingly dominant
paucity of clean water organisms
significant organic pollution

OBSERVATIONS

Clarity + slightly turbid lgt brown
Flow + slow-moderate
Width/Depth(ft) + 10-12/~1
Substrate + sand
Streambank Vegetation/Stability + good/good
Canopy + mostly closed
Other + woods/commercial; urban/Lakehurst Naval Air Sta upstr; flow
partially blocked by debris dwnstr of bridge

FTV = Family Tolerance Value, NOI = Number Of Individuals(per 100 organism subsample)

APPENDIX B

STATISTICAL DATA

SURVIVAL RESULTS

Survival Proportion with Arc-Sine Square Root Transformation

Blank	AN0528	Blank Trans	AN0528 Trans
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127

Shapiro-Wilks Test for Normality

Blank Trans	AN0528 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0	0				
1.4127	1.4127	1.4127		0	0				
1.4127	1.4127	1.4127	1.4127	0	0	0	0	0.842	Not Normal
1.4127	1.4127	1.4127		0	0				
1.4127	1.4127	1.4127		0	0				
		1.4127		0	0				
Mean	Mean	1.4127		0	0				
1.4127	1.4127	1.4127		0	0				
		1.4127		0	0				
		1.4127		0	0				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0528	Critical(from Table K=1)	Result
1.4127	1.4127	10	5.5	0	5.5	19	No Significant Difference
1.4127	1.4127	9	5.5	0	5.5		
1.4127	1.4127	8	5.5	0	5.5		
1.4127	1.4127	7	5.5	0	5.5		
1.4127	1.4127	6	5.5	0	5.5		
1.4127	1.4127	5	5.5	5.5	0		
1.4127	1.4127	4	5.5	5.5	0		
1.4127	1.4127	3	5.5	5.5	0		
1.4127	1.4127	2	5.5	5.5	0		
1.4127	1.4127	1	5.5	5.5	0		
				Sum	Sum		
				27.5	27.5		

Survival Proportion with Arc-Sine Square Root Transformation

AN0528	AN0532	AN0528 Trans	AN0532 Trans
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127
1.0	0.9	1.4127	1.249
1.0	0.7	1.4127	0.9912
1.0	1.0	1.4127	1.4127

Shapiro-Wilks Test for Normality

AN0528 Trans	AN0532 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical- W (0.05)	Result
1.4127	1.4127	1.4127		0.0585	-0.363				
1.4127	1.4127	1.4127		0.0585	-0.1052				
1.4127	1.249	1.4127	1.3542	0.0585	0.0585	0.1702	0.514	0.842	Not Normal
1.4127	0.9912	1.4127		0.0585	0.0585				
1.4127	1.4127	1.4127		0.0585	0.0585				
		1.4127		0.0585	0.0585				
Mean	Mean	1.4127		0.0585	0.0585				
1.4127	1.2957	1.249		-0.1052	0.0585				
		0.9912		-0.363	0.0585				
		1.4127		0.0585	0.0585				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0528	AN0532	Critical(from Table K=1)	Result
1.4127	0.9912	9	1	0	1	19	No Significant Difference
1.4127	1.249	8	2	0	2		
1.4127	1.4127	10	6.5	0	6.5		
1.4127	1.4127	7	6.5	0	6.5		
1.4127	1.4127	6	6.5	0	6.5		
1.4127	1.4127	5	6.5	6.5	0		
1.4127	1.4127	4	6.5	6.5	0		
1.249	1.4127	3	6.5	6.5	0		
0.9912	1.4127	2	6.5	6.5	0		
1.4127	1.4127	1	6.5	6.5	0		
				Sum	Sum		
				32.5	22.5		

GROWTH

Average Dry Weight per Replicate (in mg)

Blank	AN0528
0.102	0.201
0.149	0.230
0.118	0.175
0.118	0.193
0.144	0.200

Shapiro-Wilks Test for Normality

Blank	AN0528	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.102	0.201	0.102		-0.061	-0.061				
0.149	0.230	0.149		-0.014	-0.045				
0.118	0.175	0.118	0.163	-0.045	-0.045	0.0167	0.9411	0.842	Normal
0.118	0.193	0.118		-0.045	-0.019				
0.144	0.200	0.144		-0.019	-0.014				
		0.201		0.038	0.012				
Mean	Mean	0.23		0.067	0.03				
0.126	0.200	0.175		0.012	0.037				
		0.193		0.03	0.038				
		0.2		0.037	0.067				

F-Test and T-Test

Blank Var	AN0528 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	0.0004	1	6.3882	Equal	-5.8186	8	1.8595	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0528	AN0532
0.201	0.156
0.230	0.158
0.175	0.158
0.193	0.217
0.200	0.215

Shapiro-Wilks Test for Normality

AN0528	AN0532	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.201	0.156	0.201		0.0107	-0.0343				
0.230	0.158	0.230		0.0397	-0.0323				
0.175	0.158	0.175	0.1903	-0.0153	-0.0323	0.0066	0.9112	0.842	Normal
0.193	0.217	0.193		0.0027	-0.0153				
0.200	0.215	0.200		0.0097	0.0027				
		0.156		-0.0343	0.0097				
Mean	Mean	0.158		-0.0323	0.0107				
0.200	0.181	0.158		-0.0323	0.0247				
		0.217		0.0267	0.0267				
		0.215		0.0247	0.0397				

F-test and T-Test

AN0528 Var	AN0532 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	0.001	2.5	6.3882	Equal	1.1355	6	1.9432	No Significant Difference