

**State of New Jersey
CHRISTINE TODD WHITMAN
GOVERNOR**

**SEDIMENT TOXICITY TEST
USING THE AMPHIPOD**

Hyaella azteca
**Whippany River
(Passaic Basin)**



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

APRIL 1998



Department of Environmental Protection
Division of Science and Research
P.O. Box 427, Trenton, NJ 08625-0427

WATER MONITORING MANAGEMENT
James Mumman, Administrator

April 1998

**SEDIMENT TOXICITY TEST
USING THE AMPHIPOD
Hyalella azteca
Whippany River
(Passaic Basin)**

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

Assay Number(s): 97H006f, 97H006g

Report Prepared By:
Victor Poretti

Analysts:
Thomas Miller
Dean Bryson

Samplers:
Thomas Miller
Dean Bryson

Supervisor:
Paul Olsen

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

EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyaella azteca* was performed on sediments collected from a selected site on the Whippany River, and a reference site also on the Whippany River, in the Passaic Basin. Suspected toxicity at the Whippany River site 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). The reference was selected because of its "non-impaired" biological assessment as per the Ambient Biomonitoring Network (AMNET). 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 a biological database for use in gauging stream quality throughout the state. Levels of impairment are shown through the use of Rapid Bioassessment Protocol (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 if impairment is due to water quality degradation or habitat destruction. Sediment toxicity testing is an additional tool used to determine whether toxicity is the cause of impairment, before resorting to costly chemical monitoring.

Hyalella azteca is an epibenthic detritovore reported to also digest bacteria and algae from ingested sediment particles (2). This amphipod surface and inhabits lakes, ponds, and streams throughout North and South America, typically burrowing into the sediment surface (3,4). *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 of 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(see map)</u>
AN0234	severely impaired	Whippany River @ Ridgedale Ave, Morristown
AN0232	non-impaired	Whippany River @ Mount Pleasant Rd., Mendham Twp.

Sediment samples were collected from sites AN0232 and AN0234 on December 2, 1997 at 12:45 and 13:15 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, 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:

97H006a = control
97H006f = AN0232
97H006g = AN0234

Testing methodology followed the Bureau of Water Monitoring 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 and held for one week prior to the start of the test (7).

The test was initiated on December 9, 1997 at 11:00 hours, by adding ten 7 to 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)(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 (December 19, 1997). Live organisms were counted (see Table 2) and the dry weights measured (see Table 3). Statistical analysis was performed following EPA guidelines (9). 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, 97H006f, survival results and the control survival results. Test 97H006g 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. Test 97H006g showed no significant difference from the reference for mortality. Test 97H006g 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. Test 97H006g showed no significant difference from the reference for dry weight (see appendix B for statistical printout).

All indigenous organisms observed in the samples before the start of the test were removed. Some organisms, however, did remain, although their presence did not invalidate test results. Test chamber 97H006f contained several blood red chironomids*, various worms, and an amphipod.

*pollution tolerant midges of several genera, which possess excess hemoglobin

DISCUSSION

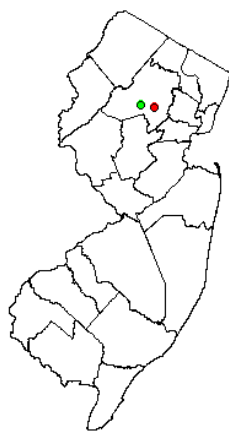
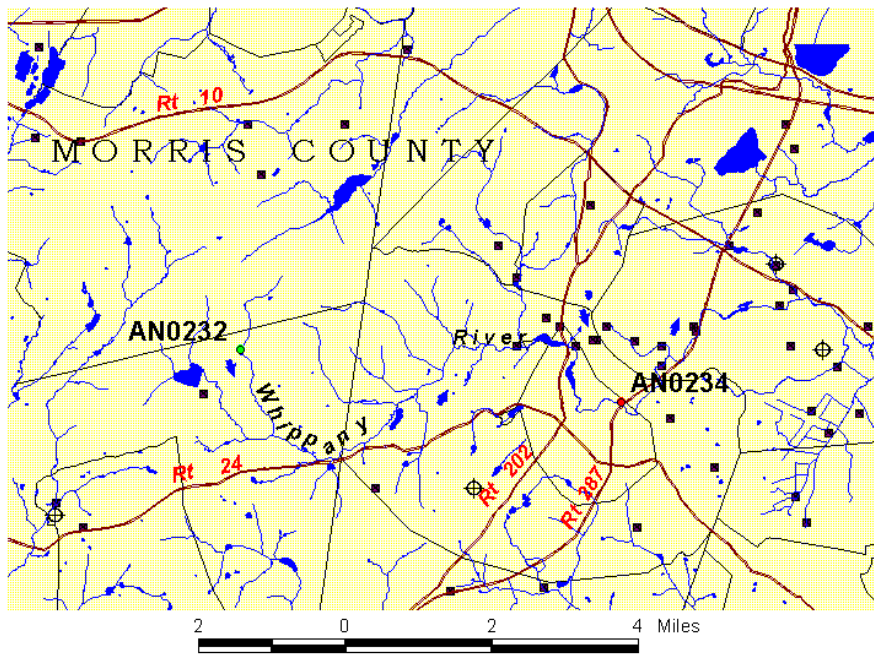
The test site (AN0234) on the Whippany River was chosen based on the results of previous macroinvertebrate studies, the proximity of NJPDES facilities, and urbanization. Site AN0234 had severely impaired bioassessment results as analyzed in AMNET(5). The reference site at Whippany River, AN0232, was chosen because of its nonimpaired bioassessment based on results of the AMNET survey, and being within the same major drainage basin (Passaic River) in proximity to the test site. Similar stream morphology and coinciding position in the New Jersey Ecomap or ecoregion scheme (based on geology, soil, natural vegetation, etc.) (9) 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. Since the test site results did not indicate acute toxicity, the severe impairment level previously found may have been due to elevated nutrient concentrations, or to the presence of other toxic substances at chronically, but not acutely, toxic levels. These could have been 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 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. Ambient biomonitoring network, Passaic/Hackensack/Wallkill drainage basin, benthic macroinvertebrate data, 1992. 14pp. + maps 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.

**Map of Study Area
Whippany River**



- NONIMPAIRED AMHET SITE
- SEVERELY IMPAIRED AMHET SITE
- X IJPDDES SITE*
- + SOLID WASTE LANDFILL
- MAJOR ROADS
- STREAMS
- STREAM FLOW



NJDEP
Water Monitoring Management
Bureau of Freshwater &
Biological Monitoring

*New Jersey Pollutant Discharge Elimination System, outfall site

Table 1
Test Chamber Chemical/Physical Parameters

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.8	7.3	7.6	0.16	2.12
cond. Fmhos	155	131	141	6.75	4.79
D.O. mg/L	7.9	5.6	7.1	0.65	9.16

97H006f	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	8.0	7.4	7.7	0.18	2.28
cond. Fmhos	193	186	178	7.13	4.02
D.O. mg/L	7.7	5.8	7.1	0.59	8.31

97H006g	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.9	7.3	7.6	0.24	3.11
cond. Fmhos	204	166	180	10.59	5.88
D.O. mg/L	8.0	5.0	6.6	0.78	11.88

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
97H006f	4	10	10	9	10	86
97H006g	10	10	10	10	10	100

Statistical Analysis

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results: 97H006f - no significant difference from control

97H006g - no significant difference from reference station

*see appendix B for statistical printout

TABLE 3
WEIGHT DETERMINATION

Drying Oven Temperature: 105EC

Duration: 2 hours

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	16.60	17.29	0.69	10	0.069	0.078
	B	19.87	20.74	0.87	10	0.087	
	C	15.97	16.75	0.78	10	0.078	
	D	16.54	17.41	0.87	10	0.087	
	E	12.75	13.43	0.68	10	0.068	
97H006f	A	15.92	16.40	0.48	4	1.120	0.201
	B	11.17	13.22	2.05	10	0.205	
	C	13.10	15.63	2.53	10	0.253	
	D	13.14	15.10	1.96	9	0.218	
	E	13.91	16.00	2.09	10	0.209	
97H006g	A	17.43	20.20	2.77	10	0.277	0.298
	B	16.97	20.30	3.33	10	0.333	
	C	12.22	14.91	2.69	10	0.269	
	D	11.17	14.60	3.43	10	0.343	
	E	11.86	14.55	2.69	10	0.269	

Statistical Analysis

Test Endpoint: Growth

Results:

Rank Sum 97H006f - no significant difference from control

T-test 97H006g - no significant difference from reference station

*see appendix B for statistical printout

APPENDIX A

AMNET DATA(5)

AN0232 # Whippany River
 Mount Pleasant Rd., Mendham Twp.
 USGS Quad #Mendham
 Latitude / Longitude: 40 48' 43.679"N / 74 34' 09.368"W

July 7, 1993

Taxon (Family Level)	FTV	NOI
HYDROPSYCHIDAE	4	26
PHILOPOTAMIDAE	3	23
SIMULIIDAE	6	10
LEUCTIDAE	0	4
NEMOURIDAE	2	2
EMPIDIDAE	6	1
CHIRONOMIDAE	6	8
CORYDALIDAE	0	4
PSYCHOMYIIDAE	2	1
LUMBRICULIDAE	8	3
RHYACOPHILIDAE	0	6
PERLIDAE	1	1
BAETIDAE	4	2
TIPULIDAE	3	3
GLOSSOSOMATIDAE	0	2
LIMNEPHILIDAE	4	1
LEPTOPHLEBIIDAE	2	1
HEPTAGENIIDAE	4	1
PSEPHENIDAE	4	1

Number of Taxa +19 # Number of Individuals+100

Dominant Family(s) #HYDROPSYCHIDAE = 26%
 Family Biotic Index +3.49
 Scraper/Filterer Collector Ratio + 0.05
 Shredder/Total Ratio + 0.10
 E(phemeroptera)+P(lecoptera)+T(richoptera) +12
 %EPT +70
 EPT/Chironomids + 8.75

NJIS/Rating +30/non-impaired

OBSERVATIONS

Clarity + clear
 Flow +moderate
 Width/Depth(ft) +6/<1
 Substrate + gravel/cobbles
 Streambank Vegetation/Stability + fair/fair
 Canopy +mostly closed
 Other +tree-lined

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

AN0234 # Whippany River, Ridgedale Ave., Morristown
 USGS Quad +Morristown
 Latitude / Longitude: 40 48' 04.916"N / 74 27' 58.620"W

May 22, 1990

Taxon (Family Level)	FTV	NOI
HYDROPSYCHIDAE	4	5
NAIDIDAE	7	87
GAMMARIDAE	4	6
CHIRONOMIDAE	6	6
BLOOD REDCHIRONOMIDAE*	8	1

Number of Taxa +5 # Number of Individuals+ 105

Dominant Family(s) +NAIDIDAE = 82.86%

Family Biotic Index + 6.64

Scraper/Filterer Collector Ratio + 0.00

Shredder/Total Ratio + 0.01

E(phemeroptera)+P(lecoptera)+T(riehoptera) +1

%EPT +4.76

EPT/Chironomids + 0.71

NJIS/Rating +6/severely impaired

Deficiency(s) noted + low diversity

NAIDIDAE overwhelmingly dominant

paucity of clean water organisms

OBSERVATIONS

Clarity +slighty turbid

Flow +moderate

Width/Depth(ft) +30/1

Substrate +boulders/cobbles/gravel

Streambank Vegetation/Stability +goof/good

Canopy +open

Other +commercial/industrial, periphyton, macrophytes

*pollution tollerant midges of several genera, which possess excess hemoglobin

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

APPENDIX B

STATISTICAL DATA

SURVIVAL RESULTS

BLANK VS. AN0232

Survival Proportions with Arc Sine Square Root Transformation

Blank	AN0232	Blank Trans	AN0232 Trans
1.0	0.4	1.4127	0.6847
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	1.0	1.4127	1.4127

Shapiro-Wilks Test for Normality

Blank Trans	AN0232 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	0.6847	1.4127		0.0892	-0.6388				
1.4127	1.4127	1.4127		0.0892	-0.0745				
1.4127	1.4127	1.4127	1.3235	0.0892	0.0892	0.4773	0.4661	0.842	Not Normal
1.4127	1.249	1.4127		0.0892	0.0892				
1.4127	1.4127	1.4127		0.0892	0.0892				
		0.6847		-0.6388	0.0892				
Mean	Mean	1.4127		0.0892	0.0892				
1.4127	1.2344	1.4127		0.0892	0.0892				
		1.249		-0.0745	0.0892				
		1.4127		0.0892	0.0892				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0232	Critical(from Table K=1)	Result
1.4127	0.6847	6	1	0	1	19	No Significant Difference
1.4127	1.249	9	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		
0.6847	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		
1.249	1.4127	2	6.5	6.5	0		
1.4127	1.4127	1	6.5	6.5	0		
				Sum	Sum		
				32.5	22.5		

AN0232 (REFERENCE) VS. AN0234

Survival Proportions with Arc Sine Square Root Transformation

AN0232	AN0234	AN0232 Trans	AN0234 Trans
0.4	1.0	0.6847	1.4127
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127
0.9	1.0	1.249	1.4127
1.0	1.0	1.4127	1.4127

Shapiro-Wilks Test for Normality

AN0232 Trans	AN0234 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.6847	1.4127	0.6847		-0.6388	-0.6388				
1.4127	1.4127	1.4127		0.0892	-0.0745				
1.4127	1.4127	1.4127	1.3235	0.0892	0.0892	0.4773	0.4661	0.842	Not Normal
1.249	1.4127	1.249		-0.0745	0.0892				
1.4127	1.4127	1.4127		0.0892	0.0892				
		1.4127		0.0892	0.0892				
Mean	Mean	1.4127		0.0892	0.0892				
1.2344	1.4127	1.4127		0.0892	0.0892				
		1.4127		0.0892	0.0892				
		1.4127		0.0892	0.0892				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0232	AN0234	Critical(from Table K=1)	Result
0.6847	0.6847	1	1	1	0	19	No Significant Difference
1.4127	1.249	4	2	2	0		
1.4127	1.4127	10	6.5	0	6.5		
1.249	1.4127	9	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		
1.4127	1.4127	6	6.5	0	6.5		
1.4127	1.4127	5	6.5	6.5	0		
1.4127	1.4127	3	6.5	6.5	0		
1.4127	1.4127	2	6.5	6.5	0		
				Sum	Sum		
				22.5	32.5		

GROWTH

BLANK VS. AN0232

Average Dry Weight per Replicate (in mg)

Blank	AN0232
0.069	0.120
0.087	0.205
0.078	0.253
0.087	0.218
0.068	0.209

Shapiro-Wilks Test for Normality

Blank	AN0232	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.069	0.120	0.069		-0.0704	-0.0714				
0.087	0.205	0.087		-0.0524	-0.0704				
0.078	0.253	0.078	0.1394	-0.0614	-0.0614	0.0479	0.8269	0.842	Not Normal
0.087	0.218	0.087		-0.0524	-0.0524				
0.068	0.209	0.068		-0.0714	-0.0524				
		0.120		-0.0194	-0.0194				
Mean	Mean	0.205		0.0656	0.0656				
0.078	0.201	0.253		0.1136	0.0696				
		0.218		0.0786	0.0786				
		0.209		0.0696	0.1136				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0232	Critical(from Table K=1)	Result
0.069	0.068	5	1	1	0	19	No Significant Difference
0.087	0.069	1	2	2	0		
0.078	0.078	3	3	3	0		
0.087	0.087	4	4.5	4.5	0		
0.068	0.087	2	4.5	4.5	0		
0.120	0.120	6	6	0	6		
0.205	0.205	7	7	0	7		
0.253	0.209	10	8	0	8		
0.218	0.218	9	9	0	9		
0.209	0.253	8	10	0	10		
				Sum	Sum		
				15	40		

AN0232 (REFERENCE) VS. AN0234

Average Dry Weight per Replicate (in mg)

AN0232	AN0234
0.120	0.277
0.205	0.333
0.253	0.269
0.218	0.343
0.209	0.269

Shapiro-Wilks Test for Normality

AN0232	AN0234	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.120	0.277	0.120		-0.1296	-0.1296				
0.205	0.333	0.205		-0.0446	-0.0446				
0.253	0.269	0.253	0.250	0.0034	-0.0406	0.0386	0.9505	0.842	Normal
0.218	0.343	0.218		-0.0316	-0.0316				
0.209	0.269	0.209		-0.0406	0.0034				
		0.277		0.0274	0.0194				
Mean	Mean	0.333		0.0834	0.0194				
0.201	0.298	0.269		0.0194	0.0274				
		0.343		0.0934	0.0834				
		0.269		0.0194	0.0934				

F-Test and T-Test

AN0232 Var	AN0234 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0024	0.0013	1.8462	6.3882	Equal	-3.5731	7	1.8946	No Significant Difference