



NJ Department of Environmental Protection
Division of Science and Research
CN 422, Trenton, NJ 08625-0422

WATER MONITORING MANAGEMENT
James Mumman, Administrator

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**SEDIMENT TOXICITY TEST
USING THE AMPHIPOD
Hyaella azteca
(PASSAIC RIVER SEDIMENT)
NOVEMBER 1995**

Assay Number(s): 95H012a, 95H012b, 95H012c

Report Prepared By:

Victor Poretti

Analysts:

**Thomas Miller
Dean Bryson**

Samplers:

**William Honachefsky
Thomas Vernam**

**Acting Chief
Bureau of Water Monitoring**

Alfred Korndoerfer, Jr.

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 water quality problem without the use of costly chemical monitoring.

Hyalella azteca is an epibenthic detritivore that is 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 which 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)
AN0224	non-impaired	Passaic River @ Valley Rd. (reference)
AN0224a	no data	Passaic River @ Rt. 512 & Railroad Bridge
AN0229	severely impaired	Passaic River @ Stanley Ave

Sediment samples were collected from these sites on October 10, 1995 at 10:40, 11:30, and 12:40 hours respectively. At each station the sediment was collected in the stream channel using a PVC core 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:

95H012a = AN0224
95H012b = AN0224a
95H012c = AN0229

Testing methodology followed the Bureau of Water Monitoring Standard Operating Procedures (NJDEP, 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, 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 24, 1995 at 11:00 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 each 24 hour period thereafter (see table 3).

The test was concluded after ten days (November 3, 1995). 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, 1995). The survival data of the tests 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 data. There was no significant difference between the reference test, 95H012a, survival data and the control survival data. Tests 95H012b and 95H012c survival data was then compared to the reference test and both had no significant differences from the reference test.

Growth data (see table 2) was normally distributed as analyzed by the Shapiro-Wilks test for normality, and an F-test and T-test was performed when comparing tests. Again there was no significant differences between the control and the reference test 95H012a, or when comparing 95H012b and 95H012c to the 95H012a. (see appendix b for statistical printout)

The dissolved oxygen level dropped below test specifications ($\geq 40\%$ saturation) in test 95H012c sometime during the final 24 hours of the test (see table 3).

DISCUSSION

The sample sites on the Passaic River were chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities. The reference site, AN0224, was chosen because it had a nonimpaired bioassessment based on the AMNET macroinvertebrate program. Site AN0229 was selected based on a severely impaired bioassessment. Site AN0224a was selected as an additional site between the two (see map) as an attempt to further pinpoint the location where biological impairment was taking place. The habitat of the two downstream sites were similar to the reference site, suggesting that impairment due to habitat destruction is unlikely.

The results of the ten day test showed no toxicity at either downstream site based on statistical comparisons with the control and the reference station. There are several possibilities why this occurred: 1) The facilities discharging into this portion of the river may have been improved, in which case a follow up macroinvertebrate study should also show improvement. 2) The impairment detected by the AMNET program may be due to a nutrient problem resulting from sewage treatment plants that discharge into the stream. This would result in a shortage of clean water organisms over a longer period of time. A 10 day test may mask a nutrient problem because it would increase the available food supply by increasing the indigenous algae and bacteria concentrations. *H. azteca* would have more of a food supply for the short term but show chronic effects over the long term, which a 10 day test would not detect. 3) Sediment samples were taken during a moderately high flow. This may have served to hide the stream channel proper, making the samples not a true representation of the stream channel sediment. 4) Macroinvertebrate samples may have shown

impairment due to an intermittent point or non-point source present at the time of macroinvertebrate collection, that was not present at the time of sediment sample collection.

A more in depth study should be conducted to characterize the stream's biological impairment. Sites AN0224a and AN0229 should be reassessed for impairment through macroinvertebrate analysis. If this still shows impairment, sediment toxicity testing should be conducted at low flow in the stream channel proper. Nutrient analysis should be conducted along with chemical analysis for suspected toxins.

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.

NJDEP, Field Sampling Procedures Manual, 1992

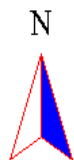
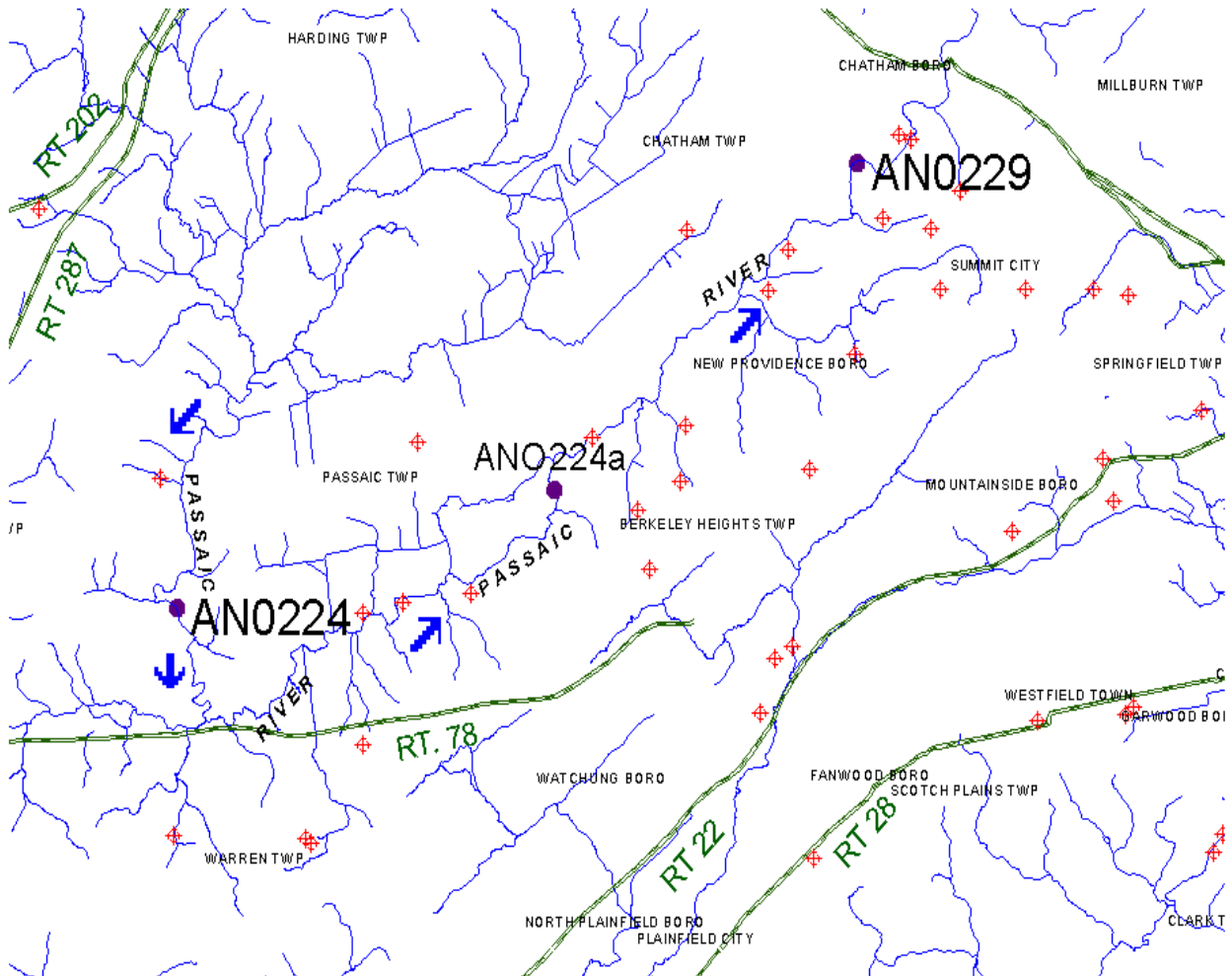
NJDEP, Standard Operating Procedures, Culturing and Sediment Toxicity Testing With *Hyalella azteca*, SM001.0795, REV 0, 1995.

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.

SEDIMENT TOXICITY TESTS

95H012a, 95H012b, 94H012c

SAMPLING STATIONS



● SAMPLING STATIONS

⊕ NJPDES FACILITIES

— MAJOR ROADS

➔ STREAM FLOW

ASSAY NUMBER ASSIGNMENTS

95H012a = AN0224

95H012b = AN0224a

95H012c = AN0229

TABLE 1
MORTALITY DATA
(number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	7	10	9	8	6	80
95H012a	10	9	7	9	9	88
95H012b	10	10	9	10	10	98
95H012c	9	10	9	10	10	96

Statistical Analysis

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results: 95H012a - no significant difference from control
95H012b - no significant difference from reference station
95H012c - no significant difference from reference station

Test Endpoint: Growth

Test Used: F-test and T-test

Results: 95H012a - no significant difference from control
95H012b - no significant difference from reference station
95H012c - no significant difference from reference station

*see appendix b for statistical printout

TABLE 2

WEIGHT DETERMINATION

Drying Oven Temperature: 105EC

Time/Date Start Drying: 1425 / 11-3-95

Time/Date End Drying: 0900 / 11-6-95

Analyst: T. Miller

REPLICATE / WEIGHING BOAT NO.	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(1)	10.09	10.76	0.67	7	0.096	0.067
B(2)	15.09	15.78	0.69	10	0.069	
C(3)	15.23	15.75	0.52	9	0.058	
D(4)	14.14	14.64	0.5	8	0.063	
E(5)	12.67	12.98	0.31	6	0.052	
95H012a A(6)	11.6	12.52	0.92	10	0.092	0.11
B(7)	14.18	15.21	1.03	9	0.114	
C(8)	11.76	12.41	0.65	7	0.093	
D(9)	13.11	14.16	1.05	9	0.117	
E(10)	13.32	14.54	1.22	9	0.136	
95H012b A(11)	14.25	15.55	1.3	10	0.13	0.115
B(12)	16.47	17.72	1.25	10	0.125	
C(13)	13.24	13.97	0.73	9	0.081	
D(14)	13.56	14.8	1.24	10	0.124	
E(15)	13.69	14.85	1.16	10	0.116	
95H012c A(16)	12.97	13.87	0.9	9	0.1	0.092
B(17)	16.27	17.34	1.07	10	0.107	
C(18)	12.34	12.92	0.58	9	0.064	
D(19)	16.52	17.39	0.87	10	0.087	
E(20)	13.42	14.45	1.03	10	0.103	

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

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.9	6.9	7.5	0.32	4.41
cond. Fmhos	147	132	138	5.37	3.88
D.O. mg/L	8	6.2	7.4	0.58	7.9

95H012a	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.9	6.5	7.1	0.36	4.98
cond. Fmhos	180	132	156	13.08	8.37
D.O. mg/L	8	3.9	5.4	1.13	20.7

95H012b	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.9	6.4	7	0.38	5.44
cond. Fmhos	177	132	149	12.72	8.52
D.O. mg/L	8	4.2	5.8	1.1	18.85

95H012c	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.9	6.5	7.3	0.36	4.97
cond. Fmhos	177	132	168	12.62	7.53
D.O. mg/L	8	3.3*	5.1	1.33	25.93

*38% saturation. The D.O. dropped below 40% only on the last day of testing.

**APPENDIX A
AMNET DATA**

Passaic Basin - Bernardsville USGS Quadrangle
 Station AN0224
 Passaic River, Valley Road (Route 512), near Millington
 February 5, 1992

Family	Number of Individuals	Tolerance	Family Value (FTV)
Chironomidae	21		6
Simuliidae	10		6
Gammaridae	15		4
Coenagrionidae	2		9
Elmidae	13		4
Lepidostomatidae	5		1
Hydropsychidae	11		4
Taeniopterygidae	6		2
Psephenidae	4		4
Gastropoda	2		7
Turbellaria	2		4
Perlidae	1		1
Sialidae	1		4
Leptoceridae	2		4
Asellidae	1		8
Tipulidae	1		3
Hydroptilidae	1		4
Sphaeriidae	1		8
Empididae	1		6

Statistical Analysis

Number of Taxa = 19
 Total Number of Individuals = 100
 % Contribution of Dominant Family = 21.00
 Family Biotic Index = 4.57
 Scraper/Filterer Collector Ratio = 0.29
 Shredder/Total Ratio = 0.07
 E+P+T* = 6 *(Ephemeroptera, Plecoptera and Trichoptera)
 %EPT = 26.00
 EPT/C* = 1.24 *(Chironomidae)
 NJIS Rating = 27
 Biological Condition = non-impaired
 Deficiency(s) noted: none

Observations

Streamwater: clear...Flow: moderate...Width/Depth(ft): 50/1...
 Substrate: rocks...Streambank Vegetation/Stability: good/good...
 Canopy: open...Other: wooded

Passaic Basin - Chatham USGS Quadrangle
 Station AN0229
 Passaic River, Stanley Avenue, Chatham
 June 11, 1990

Family	Number of Individuals	Family Tolerance Value (FTV)
Naididae	80	7
Hydropsychidae	4	4
Chironomidae	15	6
BloodRedChironomidae	1	8

Statistical Analysis

Number of Taxa = 4
 Total Number of Individuals = 100
 % Contribution of Dominant Family = 80.00
 Family Biotic Index = 6.74
 Scraper/Filterer Collector Ratio = 0.00
 Shredder/Total Ratio = 0.00
 E+P+T* = 1 *(Ephemeroptera, Plecoptera and Trichoptera)
 %EPT = 4.00
 EPT/C* = 0.25 *(Chironomidae)
 NJIS Rating = 6
 Biological Condition = severely impaired
 Deficiency(s) noted: Naididae overwhelmingly dominant
 paucity of clean water organisms

Observations

Streamwater: ...Flow: moderate...Width/Depth(ft): 50/1.5...
 Substrate: sand/gravel/rocks...Streambank Vegetation/Stability: ..
 .Canopy: ...Other: macrophytes

**APPENDIX B
STATISTICAL DATA**

Survival Proportions with Arc-Sine Square Root Transformation

Blank	Blank Trans	95H012A	95H012A Trans
0.7	0.9912	1	1.4127
1	1.4127	0.9	1.249
0.9	1.249	0.7	0.9912
0.8	1.1071	0.9	1.249
0.6	0.8861	0.9	1.249

Shapiro-Wilks Test for Normality

Blank	95H012A	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.9912	1.4127	0.9912		-0.1885	-0.2936				
1.4127	1.249	1.4127		0.233	-0.1885				
1.249	0.9912	1.249	1.1797	0.0693	-0.1885	0.2903	0.9083	0.842	Normal
1.1071	1.249	1.1071		-0.0726	-0.0726				
0.8861	1.249	0.8861		-0.2936	0.0693				
		1.4127		0.233	0.0693				
Mean	Mean	1.249		0.0693	0.0693				
1.1292	1.2302	0.9912		-0.1885	0.0693				
		1.249		0.0693	0.233				
		1.249		0.0693	0.233				

F-test and T-Test

Blank Var	95H012A Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0433	0.0229	1.8908	6.3882	Equal	-0.8778	7	1.8946	Not Significantly Different

Survival Proportions with Arc-Sine Square Root Transformation

95H012A	95H012A Trans	95H012B	95H012B Trans
1	1.4127	1	1.4127
0.9	1.249	1	1.4127
0.7	0.9912	0.9	1.249
0.9	1.249	1	1.4127
0.9	1.249	1	1.4127

Shapiro-Wilks Test for Normality

95H012A	95H012B	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.1076	-0.3139				
1.249	1.4127	1.249		-0.0561	-0.0561				
0.9912	1.249	0.9912	1.3051	-0.3139	-0.0561	0.169	0.7558	0.842	Not Normal
1.249	1.4127	1.249		-0.0561	-0.0561				
1.249	1.4127	1.249		-0.0561	-0.0561				
		1.4127		0.1076	0.1076				
Mean	Mean	1.4127		0.1076	0.1076				
1.2302	1.38	1.249		-0.0561	0.1076				
		1.4127		0.1076	0.1076				
		1.4127		0.1076	0.1076				

Wilcoxon Rank Sum Test

Pooled	Sorted	Wilcoxon Rank	Point	95H012A	95H012B	Critical (from Table K=1)	Result
1.4127	0.9912	1	3	1	0	19	Not Significantly Different
1.249	1.249	3.5	2	3.5	0		
0.9912	1.249	3.5	4	3.5	0		
1.249	1.249	3.5	5	3.5	0		
1.249	1.249	3.5	8	0	3.5		
1.4127	1.4127	8	1	8	0		
1.4127	1.4127	8	6	0	8		
1.249	1.4127	8	7	0	8		
1.4127	1.4127	8	9	0	8		
1.4127	1.4127	8	10	0	8		
				Sum	Sum		
				19.5	35.5		

Survival Proportions with Arc-Sine Square Root Transformation

95H012A	95H012A Trans	95H012C	95H012C Trans
1	1.4127	0.9	1.249
0.9	1.249	1	1.4127
0.7	0.9912	0.9	1.249
0.9	1.249	1	1.4127
0.9	1.249	1	1.4127

Shapiro-Wilks Test for Normality

95H012A	95H012C	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.249	1.4127		0.124	-0.2975				
1.249	1.4127	1.249		-0.0397	-0.0397				
0.9912	1.249	0.9912	1.2887	-0.2975	-0.0397	0.1579	0.7796	0.842	Not Normal
1.249	1.4127	1.249		-0.0397	-0.0397				
1.249	1.4127	1.249		-0.0397	-0.0397				
		1.249		-0.0397	-0.0397				
Mean	Mean	1.4127		0.124	0.124				
1.2302	1.3472	1.249		-0.0397	0.124				
		1.4127		0.124	0.124				
		1.4127		0.124	0.124				

Wilcoxon Rank Sum Test

Pooled	Sorted	Wilcoxon Rank	Point	95H012A	95H012C	Critical (from Table K=1)	Result
1.4127	0.9912	1	3	1	0	19	Not Significantly Different
1.249	1.249	4	2	4	0		
0.9912	1.249	4	4	4	0		
1.249	1.249	4	5	4	0		
1.249	1.249	4	6	0	4		
1.249	1.249	4	8	0	4		
1.4127	1.4127	8.5	1	8.5	0		
1.249	1.4127	8.5	7	0	8.5		
1.4127	1.4127	8.5	9	0	8.5		
1.4127	1.4127	8.5	10	0	8.5		
				Sum	Sum		
				21.5	33.5		

Average Dry Weight per Replicate (in mg)

Blank	95H012A
0.096	0.092
0.069	0.114
0.058	0.093
0.063	0.117
0.052	0.136

Shapiro-Wilks Test for Normality

Blank	95H012A	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.096	0.092	0.096		0.007	-0.037				
0.069	0.114	0.069		-0.02	-0.031				
0.058	0.093	0.058	0.089	-0.031	-0.026	0.0071	0.9445	0.842	Normal
0.063	0.117	0.063		-0.026	-0.02				
0.052	0.136	0.052		-0.037	0.003				
		0.092		0.003	0.004				
Mean	Mean	0.114		0.025	0.007				
0.0676	0.1104	0.093		0.004	0.025				
		0.117		0.028	0.028				
		0.136		0.047	0.047				

F-test and T-Test

Blank Var	95H012A Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Degs. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0003	0.0003	1	6.3882	Equal	-3.9071	8	1.8595	No Significant Difference

Average Dry Weight per Replicate (in mg)

95H012A	95H012B
0.092	0.13
0.114	0.125
0.093	0.081
0.117	0.124
0.136	0.116

Shapiro-Wilks Test for Normality

95H012A	95H012B	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.092	0.13	0.092		-0.0208	-0.0318				
0.114	0.125	0.114		0.0012	-0.0208				
0.093	0.081	0.093	0.1128	-0.0198	-0.0198	0.003	0.9078	0.842	Normal
0.117	0.124	0.117		0.0042	0.0012				
0.136	0.116	0.136		0.0232	0.0032				
		0.13		0.0172	0.0042				
Mean	Mean	0.125		0.0122	0.0112				
0.1104	0.1152	0.081		-0.0318	0.0122				
		0.124		0.0112	0.0172				
		0.116		0.0032	0.0232				

Ftest and T-Test

95H012A Var	95H012B Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Degs. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0003	0.0004	1.3333	6.3882	Equal	-0.4057	7	1.8946	No Significant Difference

Average Dry Weight per Replicate (in mg)

95H012A	95H012C
0.092	0.1
0.114	0.107
0.093	0.064
0.117	0.087
0.136	0.103

Shapiro-Wilks Test for Normality

95H012A	95H012C	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.092	0.1	0.092		-0.0093	-0.0093				
0.114	0.107	0.114		0.0127	0.0057				
0.093	0.064	0.093	0.1013	0.093	0.0127	0.017	0.8838	0.842	Normal
0.117	0.087	0.117		0.0157	0.0157				
0.136	0.103	0.136		0.0347	0.0347				
		0.1		0.1	0.064				
Mean	Mean	0.107		0.0057	0.087				
0.1104	0.0922	0.064		0.064	0.093				
		0.087		0.087	0.1				
		0.103		0.103	0.103				

F-test and T-Test

95H012A Var	95H012C Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Degs. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0003	0.0003	1	6.3882	Equal	1.6614	8	1.8595	No Significant Difference