

State of New Jersey
CHRISTINE TODD WHITMAN
GOVERNOR

**SEDIMENT TOXICITY TEST
USING THE AMPHIPOD**
Hyaella azteca
(Nichomus Run, Major Run,
South Branch Raccoon Creek)



New Jersey Department of Environmental Protection
ROBERT C. SHINN, JR.
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Division of Science and Research
CN 27, Trenton, NJ 08625-0427

WATER MONITORING MANAGEMENT

James Mumman, Administrator

March 1997

**SEDIMENT TOXICITY TEST
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Hyaella azteca
(Nichomus Run, Major Run,
South Branch Raccoon Creek)**

Assay Number(s): 97H002b, 97H002c, 97H002d

Report Prepared By:

Victor Poretti

Analysts:

Thomas Miller
Dean Bryson

Samplers:

Thomas Miller
Dean Bryson

Acting Chief

Bureau of Freshwater & Biological Monitoring

Alfred Korndoerfer, Jr.

EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyalella azteca* was performed on sediments collected from Nichomus Run (AN0692), Major Run (AN0694) and a reference site on South Branch Raccoon Creek (AN0682) in the Delaware Basin. The reference was selected on South Branch Raccoon Creek because of its "non-impaired" biological assessment as per the Ambient Biomonitoring Network (AMNET). The Nichomus Run and Major Run sites were 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 the previous evaluations. 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 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 New Jersey Pollutant Discharge Elimination System (NJPDDES) facilities.

The sites selected are as follows (see map):

<u>AMNET STATION#</u>	<u>BIOLOGICAL ASSESSMENT</u>	<u>LOCATION(see map)</u>
AN0692	severely impaired	Nichomus Run @ Rt. 45, Pilesgrove Twp.
AN0694	severely impaired	Major Run @ Pointers-Sharpstown Rd., Mannington Twp.
AN0682	non-impaired	S. Branch Raccoon Creek @ High St., Harrison Twp.

Sediment samples were collected from sites AN0682, AN0694, and AN0692 on March 5, 1997 at 10:30, 11:20, and 11:45 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:

97H002a = control
97H002b = AN0682
97H002c = AN0692
97H002d = AN0694

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 at the bottom (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 March 11, 1997 at 10:40 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, CEROPHYLL 7, 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 (March 21, 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, 97H002b, survival results and the control survival results. Test 97H002c and 97H002d were 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. 97H002c and 97H002d showed no significant difference from the reference for mortality. Test 97H002c and 97H002d were 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. 97H002c and 97H002d showed no significant difference from the reference for dry weight.(see appendix B for statistical printout)

On day 2 the dissolved oxygen in 97H002d fell below the test criterion of 40% saturation (to 37%), thus continuous aeration was maintained in all test vessels.

Although all visible indigenous macroinvertebrates had apparently been removed from the sediment samples, some tubifex worms were seen on the sediment surface of 97H002d test vessels.

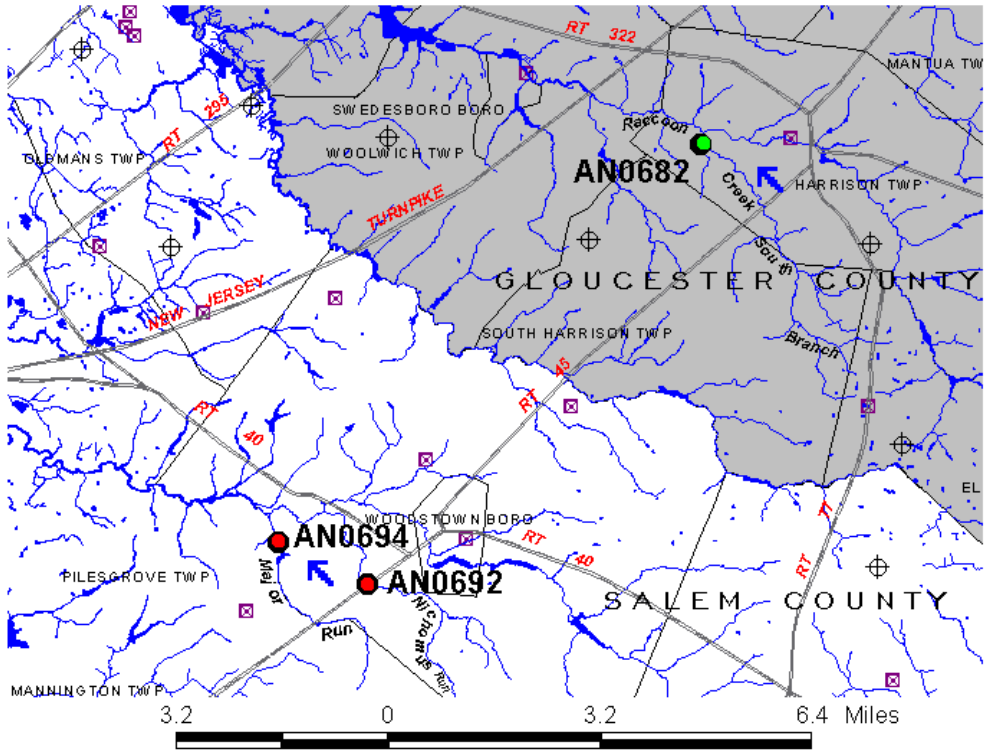
DISCUSSION

The sample sites on Nichomus Run and Major Run were chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities and urbanization. Sites AN0692 and AN0694 had severely impaired bioassessment results as analyzed in AMNET(5). The reference site at South Branch Raccoon Creek, 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 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.

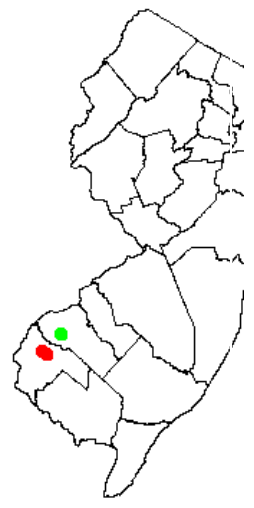
Survival and dry weight results showed no significant differences between the reference and the test site treatments. On day 2, the dissolved oxygen fell below 40% saturation in 97H002d was 37% and continuous aeration was maintained in all test vessels thereafter. Since mortalities were not substantial enough to show toxicity, the drop in D.O. probably did not adversely effect the test results. Toxicity may not have been exhibited at these sites if the streams are in the process of undergoing biological recovery, or the severe impairments, demonstrated by the AMNET program, may be due to a chronic toxicity problem not detectable in this test

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**SEDIMENT TOXICITY TESTS:
97H002b,
97H002c, 97H0**



- NONIM PAIRED AMNET SITE
- SEVERELY IMPAIRED AMNET SITE
- NJPDES SITE
- SOLIDWASTE LANDFILL
- MAJOR ROADS
- STREAMS
- STREAM FLOW



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

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.8	7.2	7.5	0.23	3.04
cond. Fmhos	148	126	140	7.16	5.11
D.O. mg/L	8.1	6.0	7.3	0.81	11.15

97H002b	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.6	7.0	7.3	0.29	4.02
cond. Fmhos	155	130	146	8.59	5.90
D.O. mg/L	8.3	4.8	6.9	1.37	19.71

97H002c	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.7	7.1	7.5	0.23	3.07
cond. Fmhos	157	137	150	6.61	4.41
D.O. mg/L	8.3	4.8	7.4	1.21	16.44

97H002d	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.6	6.6	7.2	0.39	5.36
cond. Fmhos	161	146	151	4.77	3.16
D.O. mg/L	8.4	3.2*	6.6	1.90	28.64

* The D.O. fell below 40% saturation on day 2 of the test.

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
97H002b	10	10	10	10	10	100
97H002c	10	10	8	10	10	96
97H002d	10	10	10	9	10	98

Statistical Analysis

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results: 97H002b - no significant difference from control
97H002c - no significant difference from reference station
97H002d - no significant difference from reference station

*see appendix B for statistical printout

TABLE 3

WEIGHT DETERMINATION

Drying Oven Temperature: 105EC
Analyst: T. Miller

Time/Date Start Drying: 1400 /3-21-97
Time/Date End Drying: 1600 /3-21-97

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	12.05	13.48	1.43	10	0.143	0.109	
	B	10.87	11.51	0.64	10		0.064
	C	9.74	10.99	1.25	10		0.125
	D	8.63	9.48	0.85	10		0.085
	E	9.73	11.01	1.28	10		0.128
97H002b A	9.97	12.12	2.15	10	0.215	0.213	
	B	10.33	12.54	2.21	10		0.221
	C	9.89	12.18	2.29	10		0.229
	D	14.77	16.62	1.85	10		0.185
	E	12.66	14.79	2.13	10		0.213
97H002c A	9.53	11.44	1.91	10	0.191	0.194	
	B	9.94	11.67	1.73	10		0.173
	C	8.28	10.01	1.73	8		0.216
	D	8.52	10.39	1.87	10		0.187
	E	8.23	10.275	2.04	10		0.204
97H002d A	8.61	10.93	2.32	10	0.232	0.191	
	B	10.67	12.40	1.73	10		0.173
	C	9.75	11.51	1.76	10		0.176
	D	9.60	11.25	1.65	9		0.183
	E	9.20	11.10	1.90	10		0.190

Statistical Analysis

Test Endpoint: Growth
 Test Used: F-test and T-test
 Results: 97H002b - no significant difference from control
 97H002c - no significant difference from reference station
 97H002d - no significant difference from reference station

*see appendix B for statistical printout

APPENDIX A
AMNET DATA(5)

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
SIPHONURIDAE	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 # Individuals per subsample + 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

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

August 22, 1995

Taxon (Family Level)	FTV	NOI
DUGESIIDAE	9	1
TUBIFICIDAE	10	13
NAIDIDAE	7	55
ELMIDAE	5	1
CHIRONOMIDAE	6	2
BLOODREDCHIRONOMIDAE	8	28

Number of Taxa +6 #Individuals per subsample + 100

Dominant Family(s) +NAIDIDAE 55.00%
 Family Biotic Index + 7.65
 Scraper/Filterer Collector Ratio + 0.00
 Shredder/Total Ratio + 0.00
 E(phemeroptera)+P(lecoptera)+T(riehoptera) +0
 %EPT + 0.00
 EPT/Chironomids + 0.00

NJIS/Rating +6/severely impaired

Deficiency(s) noted +paucity of clean water organisms
 significant organic pollution

OBSERVATIONS

Clarity +turbid
 Flow +slow
 Width/Depth(ft) +5-6/1-2
 Substrate +mud
 Streambank Vegetation/Stability +fair/fair
 Canopy +mostly open
 Other +tree-lined/residential/agricultural (dairy farm); foul odor
 from sediment; neighbor said...dairy farm has discharged sour
 milk into stream

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

August 22, 1995

Taxon (Family Level)	FTV	NOI
DUGESIIDAE	9	11
TUBIFICIDAE	10	52
SPHAERIIDAE	8	1
TIPULIDAE	3	1
CHIRONOMIDAE	6	5
BLOODREDCHIRONOMIDAE	8	7
PLUMATELLIDAE	7	23

Number of Taxa +7 # Individuals per subsample + 100

Dominant Family(s) +TUBIFICIDAE 52.00%
Family Biotic Index + 8.77
Scraper/Filterer Collector Ratio + 0.00
Shredder/Total Ratio + 0.00
E(phemeroptera)+P(lecoptera)+T(riehoptera) +0
%EPT + 0.00
EPT/Chironomids + 0.00

NJIS/Rating +6/severely impaired

Deficiency(s) noted +paucity of clean water organisms
significant organic pollution

OBSERVATIONS

Clarity +turbid
Flow +slow-moderate
Width/Depth(ft) +4-5/<1
Substrate +sand
Streambank Vegetation/Stability +fair/fair
Canopy +closed
Other +woods/agricultural; many ditches (with water) entering stream

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

APPENDIX B
STATISTICAL DATA

SURVIVAL RESULTS

Survival Proportions with Arc-Sine Square Root Transformation

BLANK	AN0682	BLANK Trans	AN0682 Trans
1	1	1.4127	1.4127
1	1	1.4127	1.4127
1	1	1.4127	1.4127
1	1	1.4127	1.4127
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	1.4127	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	AN0682	Critical(fr om 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 Proportions with Arc-Sine Square Root Transformation

AN0682	AN0692	AN0682 Trans	AN0692 Trans
1	1	1.4127	1.4127
1	1	1.4127	1.4127
1	0.8	1.4127	1.1071
1	1	1.4127	1.4127
1	1	1.4127	1.4127

Shapiro-Wilks Test for Normality

AN0682 Trans	AN0692 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0306	-0.275				
1.4127	1.4127	1.4127		0.0306	0.0306				
1.4127	1.1071	1.4127	1.3821	0.0306	0.0306	0.0841	0.3657	0.842	Not Normal
1.4127	1.4127	1.4127		0.0306	0.0306				
1.4127	1.4127	1.4127		0.0306	0.0306				
		1.4127		0.0306	0.0306				
Mean	Mean	1.4127		0.0306	0.0306				
1.4127	1.3516	1.1071		-0.275	0.0306				
		1.4127		0.0306	0.0306				
		1.4127		0.0306	0.0306				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0682	AN0692	Critical(from Table K=1)	Result
1.4127	1.1071	8	1	0	1	19	No Significant Difference
1.4127	1.4127	10	6	0	6		
1.4127	1.4127	9	6	0	6		
1.4127	1.4127	7	6	0	6		
1.4127	1.4127	6	6	0	6		
1.4127	1.4127	5	6	6	0		
1.4127	1.4127	4	6	6	0		
1.1071	1.4127	3	6	6	0		
1.4127	1.4127	2	6	6	0		
1.4127	1.4127	1	6	6	0		
				Sum	Sum		
				30	25		

Survival Proportions with Arc-Sine Square Root Transformation

AN0682	AN0694	AN0682 Trans	AN0694 Trans
1	1	1.4127	1.4127
1	1	1.4127	1.4127
1	1	1.4127	1.4127
1	0.9	1.4127	1.249
1	1	1.4127	1.4127

Shapiro-Wilks Test for Normality

AN0682 Trans	AN0694 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0164	-0.1473				
1.4127	1.4127	1.4127		0.0164	0.0164				
1.4127	1.4127	1.4127	1.3963	0.0164	0.0164	0.0241	0.3662	0.842	Not Normal
1.4127	1.249	1.4127		0.0164	0.0164				
1.4127	1.4127	1.4127		0.0164	0.0164				
		1.4127		0.0164	0.0164				
Mean	Mean	1.4127		0.0164	0.0164				
1.4127	1.38	1.4127		0.0164	0.0164				
		1.249		-0.1473	0.0164				
		1.4127		0.0164	0.0164				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0682	AN0694	Critical(fr om Table K=1)	Result
1.4127	1.249	9	1	0	1	19	No Significant Difference
1.4127	1.4127	10	6	0	6		
1.4127	1.4127	8	6	0	6		
1.4127	1.4127	7	6	0	6		
1.4127	1.4127	6	6	0	6		
1.4127	1.4127	5	6	6	0		
1.4127	1.4127	4	6	6	0		
1.4127	1.4127	3	6	6	0		
1.249	1.4127	2	6	6	0		
1.4127	1.4127	1	6	6	0		
				Sum	Sum		
				30	25		

GROWTH RESULTS

Average Dry Weight per Replicate (in mg)

BLANK	AN0682
0.143	0.215
0.064	0.221
0.125	0.229
0.085	0.185
0.128	0.213

Shapiro-Wilks Test for Normality

BLANK	AN0682	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.143	0.215	0.143		-0.0178	-0.0968				
0.064	0.221	0.064		-0.0968	-0.0758				
0.125	0.229	0.125	0.1608	-0.0358	-0.0358	0.0323	0.9032	0.842	Normal
0.085	0.185	0.085		-0.0758	-0.0328				
0.128	0.213	0.128		-0.0328	-0.0178				
		0.215		0.0542	0.0242				
Mean	Mean	0.221		0.0602	0.0522				
0.109	0.2126	0.229		0.0682	0.0542				
		0.185		0.0242	0.0602				

F-Test and T-Test

BLANK Var	AN0682 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0011	0.0003	3.6667	6.3882	Equal	-6.1913	6	1.9432	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0682	AN0692
0.215	0.191
0.221	0.173
0.229	0.216
0.185	0.187
0.213	0.204

Shapiro-Wilks Test for Normality

AN0682	AN0692	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.215	0.191	0.215		0.0116	-0.0304				
0.221	0.173	0.221		0.0176	-0.0184				
0.229	0.216	0.229	0.2034	0.0256	-0.0164	0.003	0.9538	0.842	Normal
0.185	0.187	0.185		-0.0184	-0.0124				
0.213	0.204	0.213		0.0096	0.0006				
		0.191		-0.0124	0.0096				
Mean	Mean	0.173		-0.0304	0.0116				
0.2126	0.1942	0.216		0.0126	0.0126				
		0.187		-0.0164	0.0176				
		0.204		0.0006	0.0256				

F-test and T-Test

AN0682 Var	AN0692 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0003	0.0003	1	6.3882	Equal	1.6797	8	1.8595	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0682	AN0694
0.215	0.232
0.221	0.173
0.229	0.176
0.185	0.183
0.213	0.19

Shapiro-Wilks Test for Normality

AN0682	AN0694	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.215	0.232	0.215		0.0133	-0.0287				
0.221	0.173	0.221		0.0193	-0.0257				
0.229	0.176	0.229	0.2017	0.0273	-0.0187	0.0046	0.8912	0.842	Normal
0.185	0.183	0.185		-0.0167	-0.0167				
0.213	0.19	0.213		0.0113	-0.0117				
		0.232		0.0303	0.0113				
Mean	Mean	0.173		-0.0287	0.0133				
0.2126	0.1908	0.176		-0.0257	0.0193				
		0.183		-0.0187	0.0273				
		0.19		-0.0117	0.0303				

F-Test and T-Test

AN0682 Var	AN0694 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0003	0.0006	2	6.3882	Equal	1.6249	7	1.8946	No Significant Difference