

**State of New Jersey
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**SEDIMENT TOXICITY TEST
USING THE AMPHIPOD**

Hyaella azteca

**Cruser Brook, Duck Pond Run,
Heathcote Brook, Green Brook
(Raritan Basin)**



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

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Department of Environmental Protection
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WATER MONITORING MANAGEMENT

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June 1997

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Assay Number(s): 97H004b, 97H004c, 97H004d, 97H004e

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EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyaella azteca* was performed on sediments collected from selected sites on Duck Pond Run, Heathcote Brook, Green Brook, and a reference site on Crusier Brook in the Raritan Basin. The reference was selected on Crusier Brook because of its "non-impaired" biological assessment as per the Ambient Biomonitoring Network (AMNET). The Duck Pond Run, Heathcote Brook, and Green Brook 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 previous assessments. When statistically compared to the reference, the test sites 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(see map)</u>
AN0394	severely impaired	Duck Pond Run @ Route 1., West Windsor
AN0396	severely impaired	Heathcote Brook @ Academy St., Kingston
AN0421	severely impaired	Green Brook @ Raymond Ave., Watchung
AN0403	non-impaired	Cruser Brook @ Route 206, Harlingen

Sediment samples were collected from sites AN0394, AN0396, AN0403, and AN0421 on June 10, 1997 at 9:20, 10:00, 10:40, 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 one liter amber glass bottles and stored at less than 4°C until the start of the test (4).

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

97H004a = control
97H004b = AN0403
97H004c = AN0394
97H004d = AN0396
97H004e = AN0421

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 June 17, 1997 at 10:00 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 (June 27, 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 \$ 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, 97H004b, survival results and the control survival results. Test 97H004c, 97H004d, and 97H004e 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. 97H004c, 97H004d, and 97H004e showed no significant difference from the reference for mortality. Test 97H004c, 97H004d, and 97H004e 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. 97H004c, 97H004d, and 97H004e showed no significant difference from the reference for dry weight.(see appendix B for statistical printout)

On day 5 the dissolved oxygen in 97H004e fell below the test criterion of 40% saturation (to 37.8%). On day 9 the dissolved oxygen in 97H004c and 97H004e fell below the test criterion of 40% saturation (to 39.5% and 39.7% respectively).

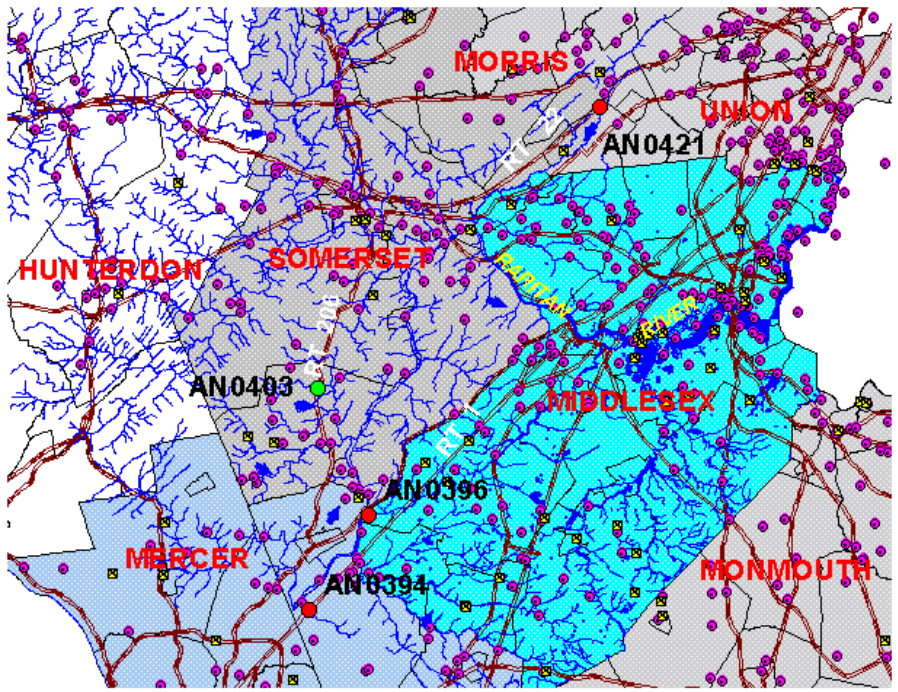
DISCUSSION

The sample sites on Duck Pond Run, Heathcote Brook, and Green Brook were chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities and urbanization. Sites AN0394, AN0396, and AN0421 had *severely impaired* bioassessment results as analyzed in AMNET(5). The reference site at Cruser Brook, AN0401, 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.

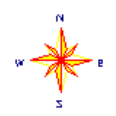
Survival and dry weight results showed no significant differences between the reference and the test site treatments. On day 5 the dissolved oxygen in 97H004e fell below the test criterion of 40% saturation (to 37.8%). On day 9 the dissolved oxygen in 97H004c and 97H004e fell below the test criterion of 40% saturation (to 39.5% and 39.7% respectively). The problem was not consistent thus remedial action was taken. Since mortalities were not substantial enough to show toxicity, the drop in D.O. probably did not adversely affect the test results. The nontoxicity exhibited at these sites may be attributed to the streams are in the process of undergoing biological recovery; albeit 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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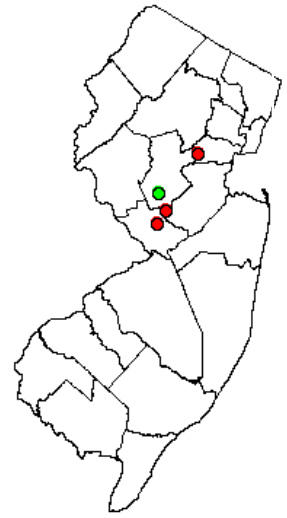
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- NONIMPAIRED AMHET SITE
- SEVERELY IMPAIRED AMHET SITE
- NJPDES SITE
- MAJOR ROADS
- STREAMS



SEDIMENT TOXICITY TEST
 97H004b, 97H004c
 97H004d, 97H004e



NJDEP
 Water Monitoring Management
 Bureau of Freshwater &
 Biological Monitoring

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

Control	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.7	7.1	7.3	0.20	2.78
cond. Fmhos	144	125	133	6.53	4.93
D.O. mg/L	8.3	5.1	6.1	0.95	15.65

97H004b	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.5	7.1	7.3	0.15	1.98
cond. Fmhos	226	160	193	25.81	13.38
D.O. mg/L	7.1	3.7	4.8	0.91	19.03

97H004c	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.2	6.9	7.0	0.08	1.19
cond. Fmhos	175	128	157	13.65	8.71
D.O. mg/L	6.6	3.4*	4.8	0.97	20.29

97H004d	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.1	6.8	7.0	0.08	1.13
cond. Fmhos	151	126	138	7.05	5.11
D.O. mg/L	6.6	4.0	5.2	0.78	15.09

97H004e	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.4	7.0	7.2	0.12	1.63
cond. Fmhos	214	154	180	19.25	10.69
D.O. mg/L	6.6	3.3*	4.6	1.04	22.75

*On day 5 the dissolved oxygen in 97H004e fell below the test criterion of 40% saturation (to 37.8%). On day 9 the dissolved oxygen in 97H004c and 97H004e fell below the test criterion of 40% saturation (to 39.5% and 39.7% respectively).

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
97H004b	10	10	10	10	9	98
97H004c	10	10	10	9	10	98
97H004d	9	10	10	7	10	92
97H004e	10	8	10	10	6	88

Statistical Analysis

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results: 97H004b - no significant difference from control
97H004c - no significant difference from reference station
97H004d - no significant difference from reference station
97H004e - 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	
97H004b A	9.14	10.77	1.63	10	0.163	0.200
B	7.64	9.58	1.94	10	0.194	
C	10.31	12.11	1.80	10	0.180	
D	6.60	8.72	2.12	10	0.212	
E	18.82	20.57	1.75	7	0.250	
97H004c A	15.65	17.45	1.80	10	0.180	0.200
B	16.96	18.81	1.85	10	0.185	
C	13.28	15.10	1.82	9	0.202	
D	15.67	17.79	2.12	10	0.212	
E	13.84	16.03	2.19	10	0.219	
97H004d A	9.98	11.90	1.92	10	0.192	0.212
B	8.65	11.03	2.38	10	0.238	
C	10.38	12.43	2.05	10	0.205	
D	11.60	13.84	2.24	10	0.224	
E	11.20	13.19	1.99	10	0.199	
97H004e A	13.25	15.27	2.02	10	0.202	0.226
B	11.85	13.82	1.97	10	0.197	
C	13.55	15.89	2.34	10	0.234	
D	15.17	17.43	2.26	9	0.251	
E	11.02	13.47	2.45	10	0.245	

Statistical Analysis

Test Endpoint: Growth

Test Used: F-test and T-test

Results:

97H004b - no significant difference from control

97H004c - no significant difference from reference station

97H004d - no significant difference from reference station

97H004e - no significant difference from reference station

*see appendix B for statistical printout

APPENDIX A

AMNET DATA(5)

Raritan Basin - Princeton USGS Quadrangle
 Station AN0394
 Duck Pond Run, Route 1, West Windsor
 April 12, 1994

Family	Number	Family of Individuals	Tolerance Value (FTV)
Tubificidae	45		10
Lumbricidae	5		10
Chironomidae	43		6
Naididae	2		7
Sphaeriidae	1		8
Gammaridae	2		4
Hirudinea	1		10
Ceratopogonidae	1		6

Statistical Analysis

Number of Taxa: 8
 Total Number of Individuals: 100
 % Contribution of Dominant Family: 45.00
 Family Biotic Index: 8.04
 Scraper/Filterer Collector Ratio: 0.00
 Shredder/Total Ratio: 0.14
 E+P+T*: 0 *(Ephemeroptera, Plecoptera and Trichoptera)
 %EPT: 0.00
 EPT/C*: 0.00 *(Chironomidae)
 NJIS Score: 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): 6-8/1...
 Substrate: sand/muck/cobble...Streambank Vegetation/Stability: fair/fair
 ...Canopy: mostly open...Other: wooded/commercial; filamentous algae;
 minnows

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

Raritan Basin - Hightstown USGS Quadrangle
 Station AN0396
 Heathcote Brook, Academy Street, Kingston
 October 6, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Hydropsychidae	1	4
Hirudinea	1	10
Gastropoda	83	7
Sphaeriidae	8	8
Tipulidae	4	3
Simuliidae	1	6
Coenagrionidae	1	9
Lumbricidae	4	10
Turbellaria	1	4

Statistical Analysis

Number of Taxa: 9
 Total Number of Individuals: 104
 % Contribution of Dominant Family: 79.81
 Family Biotic Index: 7.02
 Scraper/Filterer Collector Ratio: 5.30
 Shredder/Total Ratio: 0.03
 E+P+T*: 1 *(Ephemeroptera, Plecoptera and Trichoptera)
 %EPT: 0.96
 EPT/C*: 0.00 *(Chironomidae)
 NJIS Score: 3
 Biological Condition: severely impaired
 Deficiency(s) noted: Gastropoda overwhelmingly dominant
 paucity of clean water organisms
 significant organic pollution

Observations

Streamwater: clear...Flow: moderate...Width/Depth(ft): 20/<1...Substrate:
 sand/gravel/cobble...Streambank Vegetation/Stability: good/good...Canopy:
 half open...Other: tree-lined; macrophytes

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

Raritan Basin - Rocky Hill USGS Quadrangle
 Station AN0403
 Crusier Brook, Route 206, Harlingen
 November 10, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Hydropsychidae	31	4
Lumbriculidae	3	8
Elmidae	12	4
Tubificidae	3	10
Helicopsychidae	10	3
Caenidae	1	7
Psephenidae	10	4
Heptageniidae	7	4
Gammaridae	2	4
Chironomidae	2	6
Turbellaria	4	4
Gastropoda	5	7
Sphaeriidae	6	8
BloodRedChironomidae	1	8
Hydrophilidae	1	5
Sialidae	1	4
Coenagrionidae	1	9

Statistical Analysis

Number of Taxa: 17
 Total Number of Individuals: 100
 % Contribution of Dominant Family: 31.00
 Family Biotic Index: 4.76
 Scraper/Filterer Collector Ratio: 0.54
 Shredder/Total Ratio: 0.02
 E+P+T*: 4 *(Ephemeroptera, Plecoptera and Trichoptera)
 %EPT: 49.00
 EPT/C*: 16.33 *(Chironomidae)
 NJIS Score: 27
 Biological Condition: non-impaired
 Deficiency(s) noted: none

Observations

Streamwater: slightly turbid...Flow: slow...Width/Depth(ft): 15/1...
 Substrate: sand/rubble/cobble...Streambank Vegetation/Stability: good/good.
 ...Canopy: open...Other: tree-lined; mosses; darters

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

Raritan Basin - Chatham USGS Quadrangle
 Station AN0421
 Green Brook, Raymond Avenue, Watchung
 February 27, 1992

Family	Number of Individuals	Family Tolerance Value (FTV)
Lumbricidae	3	10
Chironomidae	2	6
Gastropoda	4	7
Hydropsychidae	1	4

Statistical Analysis

Number of Taxa: 4
 Total Number of Individuals: 10
 % Contribution of Dominant Family: 40.00
 Family Biotic Index: 7.40
 Scraper/Filterer Collector Ratio: 0.00
 Shredder/Total Ratio: 0.00
 E+P+T*: 1 *(Ephemeroptera, Plecoptera and Trichoptera)
 %EPT: 10.00
 EPT/C*: 0.50 *(Chironomidae)
 NJIS Score: 6
 Biological Condition: severely impaired
 Deficiency(s) noted: low diversity
 paucity of clean water organisms
 significant organic pollution

Observations

Streamwater: clear...Flow: moderate...Width/Depth(ft): 50/<1...Substrate:
 sand/gravel/rubble...Streambank Vegetation/Stability: poor/poor...Canopy:
 open...Other: residential/commercial

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	AN0403	Blank Trans	AN0403 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	0.9	1.4127	1.249

Shapiro-Wilks Test for Normality

Blank Trans	AN0403 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	1.4127	1.4127		0.0164	0.0164				
1.4127	1.249	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				
		1.4127		0.0164	0.0164				
		1.249		-0.1473	0.0164				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0403	Critical(from Table K=1)	Result
1.4127	1.249	10	1	0	1	19	No Significant Difference
1.4127	1.4127	9	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		
1.4127	1.4127	2	6	6	0		
1.249	1.4127	1	6	6	0		
				Sum	Sum		
				30	25		

Survival Proportions with Arc Sine Square Root Transformation

AN0403	AN0394	AN0403 Trans	AN0394 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	0.9	1.4127	1.249
0.9	1.0	1.249	1.4127

Shapiro-Wilks Test for Normality

AN0403 Trans	AN0394 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0327	-0.131				
1.4127	1.4127	1.4127		0.0327	-0.131				
1.4127	1.4127	1.4127	1.38	0.0327	0.0327	0.0429	0.5093	0.842	Not Normal
1.4127	1.249	1.4127		0.0327	0.0327				
1.249	1.4127	1.249		-0.131	0.0327				
		1.4127		0.0327	0.0327				
Mean	Mean	1.4127		0.0327	0.0327				
1.38	1.38	1.4127		0.0327	0.0327				
		1.249		-0.131	0.0327				
		1.4127		0.0327	0.0327				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0403	AN0394	Critical(from Table K=1)	Result
1.4127	1.249	9	1.5	0	1.5	19	No Significant Difference
1.4127	1.249	5	1.5	1.5	0		
1.4127	1.4127	10	6.5	0	6.5		
1.4127	1.4127	8	6.5	0	6.5		
1.249	1.4127	7	6.5	0	6.5		
1.4127	1.4127	6	6.5	0	6.5		
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		
				27.5	27.5		

Survival Proportions with Arc Sine Square Root Transformation

AN0403	AN0396	AN0403 Trans	AN0396 Trans
1.0	0.9	1.4127	1.249
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127
1.0	0.7	1.4127	0.9912
0.9	1.0	1.249	1.4127

Shapiro-Wilks Test for Normality

AN0403 Trans	AN0396 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.249	1.4127		0.0749	-0.3466				
1.4127	1.4127	1.4127		0.0749	-0.0888				
1.4127	1.4127	1.4127	1.3378	0.0749	-0.0888	0.1752	0.6247	0.842	Not Normal
1.4127	0.9912	1.4127		0.0749	0.0749				
1.249	1.4127	1.249		-0.0888	0.0749				
		1.249		-0.0888	0.0749				
Mean	Mean	1.4127		0.0749	0.0749				
1.38	1.2957	1.4127		0.0749	0.0749				
		0.9912		-0.3466	0.0749				
		1.4127		0.0749	0.0749				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0403	AN0396	Critical(from Table K=1)	Result
1.4127	0.9912	9	1	0	1	19	No Significant Difference
1.4127	1.249	6	2.5	0	2.5		
1.4127	1.249	5	2.5	2.5	0		
1.4127	1.4127	10	7	0	7		
1.249	1.4127	8	7	0	7		
1.249	1.4127	7	7	0	7		
1.4127	1.4127	4	7	7	0		
1.4127	1.4127	3	7	7	0		
0.9912	1.4127	2	7	7	0		
1.4127	1.4127	1	7	7	0		
				Sum	Sum		
				30.5	24.5		

Survival Proportions with Arc Sine Square Root Transformation

AN0403	AN0421	AN0403 Trans	AN0421 Trans
1.0	1.0	1.4127	1.4127
1.0	0.8	1.4127	1.1071
1.0	1.0	1.4127	1.4127
1.0	1.0	1.4127	1.4127
0.9	0.6	1.249	0.8861

Shapiro-Wilks Test for Normality

AN0403 Trans	AN0421 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0996	-0.427				
1.4127	1.1071	1.4127		0.0996	-0.206				
1.4127	1.4127	1.4127	1.3131	0.0996	-0.0641	0.2983	0.6426	0.842	Not Normal
1.4127	1.4127	1.4127		0.0996	0.0996				
1.249	0.8861	1.249		-0.0641	0.0996				
		1.4127		0.0996	0.0996				
Mean	Mean	1.1071		-0.206	0.0996				
1.38	1.2463	1.4127		0.0996	0.0996				
		1.4127		0.0996	0.0996				
		0.8861		-0.427	0.0996				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0403	AN0421	Critical(from Table K=1)	Result
1.4127	0.8861	10	1	0	1	19	No Significant Difference
1.4127	1.1071	7	2	0	2		
1.4127	1.249	5	3	3	0		
1.4127	1.4127	9	7	0	7		
1.249	1.4127	8	7	0	7		
1.4127	1.4127	6	7	0	7		
1.1071	1.4127	4	7	7	0		
1.4127	1.4127	3	7	7	0		
1.4127	1.4127	2	7	7	0		
0.8861	1.4127	1	7	7	0		
				Sum	Sum		
				31	24		

GROWTH

Average Dry Weight per Replicate (in mg)

Blank	AN0403
0.047	0.086
0.051	0.120
0.050	0.140
0.066	0.158
0.090	0.102

Shapiro-Wilks Test for Normality

Blank	AN0403	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.047	0.086	0.047		-0.044	-0.044				
0.051	0.120	0.051		-0.04	-0.041				
0.050	0.140	0.050	0.091	-0.041	-0.04	0.0137	0.9265	0.842	Normal
0.066	0.158	0.066		-0.025	-0.025				
0.090	0.102	0.090		-0.001	-0.005				
		0.086		-0.005	-0.001				
Mean	Mean	0.120		0.029	0.011				
0.061	0.121	0.140		0.049	0.029				
		0.158		0.067	0.049				
		0.102		0.011	0.067				

F-Test and T-Test

Blank Var	AN0403 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0003	0.0008	2.6667	6.3882	Equal	-4.0722	6	1.9432	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0403	AN0394
0.086	0.103
0.120	0.127
0.140	0.175
0.158	0.143
0.102	0.176

Shapiro-Wilks Test for Normality

AN0403	AN0394	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.086	0.103	0.086		-0.047	-0.047				
0.120	0.127	0.120		-0.013	-0.031				
0.140	0.175	0.140	0.133	0.007	-0.03	0.0087	0.9473	0.842	Normal
0.158	0.143	0.158		0.025	-0.013				
0.102	0.176	0.102		-0.031	-0.006				
		0.103		-0.03	0.007				
Mean	Mean	0.127		-0.006	0.01				
0.121	0.145	0.175		0.042	0.025				
		0.143		0.01	0.042				
		0.176		0.043	0.043				

F-Test and Ttest

AN0403 Var	AN0394 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0008	0.001	1.25	6.3882	Equal	-1.2438	7	1.8946	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0403	AN0396
0.086	0.068
0.120	0.136
0.140	0.223
0.158	0.193
0.102	0.221

Shapiro-Wilks Test for Normality

AN0403	AN0396	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.086	0.068	0.086		-0.0587	-0.0767				
0.120	0.136	0.120		-0.0247	-0.0587				
0.140	0.223	0.140	0.145	-0.0047	-0.0427	0.0263	0.9453	0.842	Normal
0.158	0.193	0.158		0.0133	-0.0247				
0.102	0.221	0.102		-0.0427	-0.0087				
		0.068		-0.0767	-0.0047				
Mean	Mean	0.136		-0.0087	0.0133				
0.121	0.168	0.223		0.0783	0.0483				
		0.193		0.0483	0.0763				
		0.221		0.0763	0.0783				

F-Test and T-Test

AN0403 Var	AN0396 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variance s	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0008	0.0044	5.5	6.3882	Equal	-1.4574	5	2.015	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0403	AN0421
0.086	0.140
0.120	0.120
0.140	0.133
0.158	0.178
0.102	0.082

Shapiro-Wilks Test for Normality

AN0403	AN0421	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.086	0.140	0.086		-0.0399	-0.0439				
0.120	0.120	0.120		-0.0059	-0.0399				
0.140	0.133	0.140	0.126	0.0141	-0.0239	0.0084	0.962	0.842	Normal
0.158	0.178	0.158		0.0321	-0.0059				
0.102	0.082	0.102		-0.0239	-0.0059				
		0.140		0.0141	0.0071				
Mean	Mean	0.120		-0.0059	0.0141				
0.121	0.131	0.133		0.0071	0.0141				
		0.178		0.0521	0.0321				
		0.082		-0.0439	0.0521				

F-Test and T-Test

AN0403 Var	AN0421 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variations	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0008	0.0012	1.5	6.3882	Equal	-0.47	7	1.8946	No Significant Difference