

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

Hyaella azteca

**Willow Brook, Big Brook,
Debois Creek, Stan Brook
(Atlantic Coastal Basin)**



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

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Assay Number(s): 97H003b, 97H003c, 97H003d, 97H003e

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EXECUTIVE SUMMARY

A toxicity test using the amphipod *Hyaella azteca* was performed on sediments collected from selected sites on Willow Brook, Big Brook, Debois Creek, and a reference site on Stan Brook in the Atlantic Coastal Basin. The reference was selected on Stan Brook because of its "non-impaired" biological assessment as per the Ambient Biomonitoring Network (AMNET). The Willow Brook, Big Brook, and Debois Creek 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 effluents from facilities with to 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)
AN0468	severely impaired	Willow Brook @ Willow Brook Rd., Colts Neck Twp.
AN0469	severely impaired	Big Brook @ Rt. 79, Marlboro Twp.
AN0487	severely impaired	Debois Creek @ Strickland Rd., Howell Twp.
AN0496	non-impaired	Stan Brook @ Easy St., Howell Twp.

Sediment samples were collected from sites AN0487 and AN0496 on May 6, 1997 at 12:15 and 13:25 hours respectively. Sediment samples were collected from sites AN0468 and AN0469 on May 8, 1997 at 12:00 and 12:25 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
97H003b = AN0496
97H003c = AN0468
97H003d = AN0469
97H003e = AN0487

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, 97H003b, survival results and the control survival results. Test 97H003c, 97H003d, and 97H003e 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. 97H003c, 97H003d, and 97H003e showed no significant difference from the reference for mortality. Test 97H003c, 97H003d, and 97H003e 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. 97H003c, 97H003d, and 97H003e showed no significant difference from the reference for dry weight.(see appendix B for statistical printout)

On day 6 the dissolved oxygen in 97H003d fell below the test criterion of 40% saturation (to 36%). On day 7 the dissolved oxygen in 97H003c and 97H003e fell below the test criterion of 40% saturation (to 38.5% and 39.5% respectively).

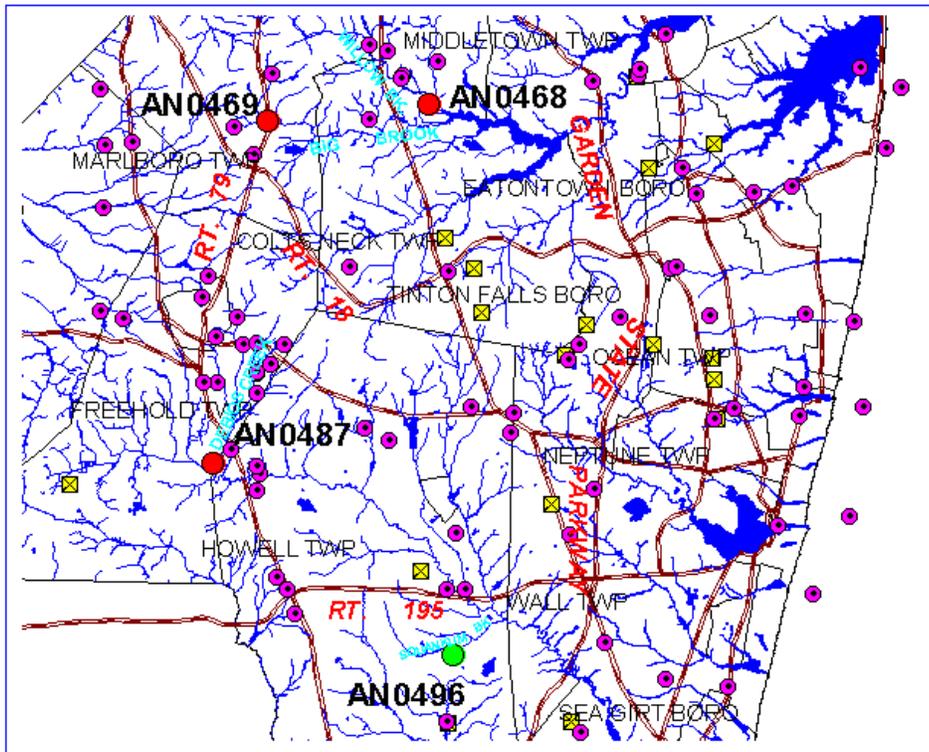
DISCUSSION

The sample sites on Willow Brook, Big Brook, and Debois Creek were chosen based on the results of macroinvertebrate studies and the proximity of NJPDES facilities and urbanization. Sites AN0468, AN0469, and AN0487 had severely impaired bioassessment results as analyzed in AMNET(5). The reference site at Stan Brook, AN0496, 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.

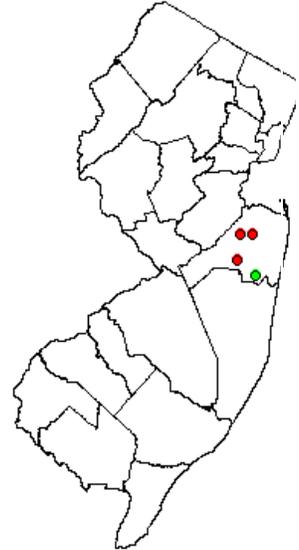
Survival and dry weight results showed no significant differences between the reference and the test site treatments. On day 6 the dissolved oxygen in 97H003d fell below the test criterion of 40% saturation (to 36%). On day 7 the dissolved oxygen in 97H003c and 97H003e fell below the test criterion of 40% saturation (to 38.5% and 39.5% respectively). The problem did not persist thus no remedial action was taken. Since mortalities were not substantial enough to show toxicity, the drop in D.O. probably did not adversely effect the test results. 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
97H003b, 97H003
96H006d, 96H006**



● NONIMPAIRED AMHET SITE

— MAJOR ROADS



NJDEP
Water Monitoring Manage

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

97H003b	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.12	6.5	6.9	0.17	2.44
cond. Fmhos	156	104	140	15.72	11.17
D.O. mg/L	5.8	3.4	4.6	0.70	15.32

97H003c	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.4	6.9	7.1	0.18	2.54
cond. Fmhos	151	132	144	6.40	4.45
D.O. mg/L	7.1	3.3	4.7	1.19	25.52

97H003d	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.2	6.8	6.9	0.14	1.97
cond. Fmhos	157	137	148	6.10	4.11
D.O. mg/L	6.6	3.1	4.5	1.00	22.50

97H003e	HIGH	LOW	AVG.	STD. DEV.	% CV
pH	7.2	6.8	7.0	0.13	1.82
cond. Fmhos	147	127	137	6.66	4.86
D.O. mg/L	6.9	3.4	4.7	1.02	21.73

*On day 6 the dissolved oxygen in 97H003d fell below the test criterion of 40% saturation (to 36%).
 On day 7 the dissolved oxygen in 97H003c and 97H003e fell below the test criterion of 40% saturation (to 38.5% and 39.5% 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
97H003b	10	10	10	10	7	94
97H003c	10	10	10	10	10	100
97H003d	10	10	10	9	10	98
97H003E	10	10	10	9	10	98

Statistical Analysis

Test Endpoint: Survival

Test Used: Wilcoxon Rank Sum Test

Results:
97H003b - no significant difference from control
97H003c - no significant difference from reference station
97H003d - no significant difference from reference station
97H003e - 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	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	
97H003b 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	
97H003c 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	
97H003d 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	
97H003e 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:

- 97H003b - no significant difference from control
- 97H003c - no significant difference from reference station
- 97H003d - no significant difference from reference station
- 97H003e - no significant difference from reference station

*see appendix B for statistical printout

APPENDIX A

AMNET DATA(5)

October 30, 1990

Taxon (Family Level)	FTV	NOI
LUMBRICULIDAE	8	12
SPHAERIIDAE	8	4
ASELLIDAE	8	13
ENTOMOBRYIDAE	10	1
LEPTOPHLEBIIDAE	2	3
SIALIDAE	4	1
CORYDALIDAE	0	1
PSYCHOMYIIDAE	2	1
HYDROPSYCHIDAE	4	20
LIMNEPHILIDAE	4	8
MOLANNIDAE	6	2
CALAMOCERATIDAE	0	2
POLYCENTROPODIDAE	6	1
TIPULIDAE	3	3
SIMULIIDAE	6	1
CHIRONOMIDAE	6	18
TABANIDAE	6	2

Number of Taxa + 17 # Number of Individuals+ 93

Dominant Family(s) + HYDROPSYCHIDAE 21.51%

Family Biotic Index + 5.58

Scraper/Filterer Collector Ratio + 0.00

Shredder/Total Ratio + 0.13

E(phemeroptera)+P(lecoptera)+T(richoptera) + 7

%EPT + 39.78

EPT/Chironomids + 2.06

NJIS/Rating + 27/non-impaired

OBSERVATIONS

Clarity + clear cedar

Flow + slow

Width/Depth(ft) + 2/<1

Substrate + gravel/sand

Streambank Vegetation/Stability + good/good

Canopy + partly open

Other + woods/marsh/agricultural

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

August 3, 1994

Taxon (Family Level)	FTV	NOI
TUBIFICIDAE	10	5
HEPTAGENIIDAE	4	1
HYDROPSYCHIDAE	4	10
CHIRONOMIDAE	6	84

Number of Taxa + 4 # Number of Individuals+ 100

Dominant Family(s) + CHIRONOMIDAE 84.00%

Family Biotic Index + 5.98

Scraper/Filterer Collector Ratio + 0.00

Shredder/Total Ratio + 0.66

E(phemeroptera)+P(lecoptera)+T(richoptera) + 2

%EPT + 11.00

EPT/Chironomids + 0.13

NJIS/Rating + 6/severely impaired

Deficiency(s) noted + low diversity

CHIRONOMIDAE overwhelmingly dominant

paucity of clean water organisms

OBSERVATIONS

Clarity + clear

Flow + slow

Width/Depth(ft) + 20/<1

Substrate + sand

Streambank Vegetation/Stability + good/good

Canopy + mostly open

Other + woods/agricultural; ongoing housing construction upstr

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

August 9, 1994

Taxon (Family Level)	FTV	NOI
TUBIFICIDAE	10	66
LUMBRICIDAE	10	1
SIALIDAE	4	1
HYDROPSYCHIDAE	4	4
SIMULIIDAE	6	1
CHIRONOMIDAE	6	7
BLOODREDCHIRONOMIDAE	8	2

Number of Taxa + 7 # Number of Individuals+ 82

Dominant Family(s) + TUBIFICIDAE 80.49%
 Family Biotic Index + 9.20
 Scraper/Filterer Collector Ratio + 0.00
 Shredder/Total Ratio + 0.02
 E(phemeroptera)+P(lecoptera)+T(riehoptera) + 1
 %EPT + 4.88
 EPT/Chironomids + 0.44

NJIS/Rating + 3/severely impaired

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

OBSERVATIONS

Clarity + clear
 Flow + slow-moderate
 Width/Depth(ft) + 30/<1
 Substrate + sand/gravel
 Streambank Vegetation/Stability + good/good
 Canopy + half open
 Other + tree-lined/agricultural; commercial area upstr

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

September 14, 1994

Taxon (Family Level)	FTV	NOI
PROSTOMATIDAE	7	1
TUBIFICIDAE	10	7
NAIDIDAE	7	2
CAMBARIDAE	5	1
HYDROPSYCHIDAE	4	7
PHRYGANEIDAE	4	1
TIPULIDAE	3	2
SIMULIIDAE	6	4
CHIRONOMIDAE	6	75

Number of Taxa + 9 # Number of Individuals+ 100

Dominant Family(s) + CHIRONOMIDAE 75.00%

Family Biotic Index + 6.08

Scraper/Filterer Collector Ratio + 0.07

Shredder/Total Ratio + 0.02

E(phemeroptera)+P(lecoptera)+T(richoptera) + 2

%EPT + 8.00

EPT/Chironomids + 0.11

NJIS/Rating + 6/severely impaired

Deficiency(s) noted + CHIRONOMIDAE overwhelmingly dominant
paucity of clean water organisms

OBSERVATIONS

Clarity + clear w grey-green tint

Flow + moderate

Width/Depth(ft) + 15/<1

Substrate + sand/some gravel

Streambank Vegetation/Stability + good/good

Canopy + open

Other + brush-lined/suburban; commercial area upstr; undersides of
stones black; oil rising from substrate; trash

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	AN0496	Blank Trans	AN0496 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	0.7	1.4127	0.9912

Shapiro-Wilks Test for Normality

Blank Trans	AN0496 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0421	-0.3794				
1.4127	1.4127	1.4127		0.0421	0.0421				
1.4127	1.4127	1.4127	1.3706	0.0421	0.0421	0.1599	0.3659	0.842	Not Normal
1.4127	1.4127	1.4127		0.0421	0.0421				
1.4127	0.9912	1.4127		0.0421	0.0421				
		1.4127		0.0421	0.0421				
Mean	Mean	1.4127		0.0421	0.0421				
1.4127	1.3284	1.4127		0.0421	0.0421				
		1.4127		0.0421	0.0421				
		0.9912		-0.3794	0.0421				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	Blank	AN0496	Critical(from Table K=1)	Result
1.4127	0.9912	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		
0.9912	1.4127	1	6	6	0		
				Sum	Sum		
				30	25		

Survival Proportion with Arc-Sine Square Root Transformation

AN0496	AN0468	AN0496 Trans	AN0468 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
0.7	1.0	0.9912	1.4127

Shapiro-Wilks Test for Normality

AN0496 Trans	AN0468 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0421	-0.3794				
1.4127	1.4127	1.4127		0.0421	0.0421				
1.4127	1.4127	1.4127	1.3706	0.0421	0.0421	0.1599	0.3659	0.842	Not Normal
1.4127	1.4127	1.4127		0.0421	0.0421				
0.9912	1.4127	0.9912		-0.3794	0.0421				
		1.4127		0.0421	0.0421				
Mean	Mean	1.4127		0.0421	0.0421				
1.3284	1.4127	1.4127		0.0421	0.0421				
		1.4127		0.0421	0.0421				
		1.4127		0.0421	0.0421				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0496	AN0468	Critical(from Table K=1)	Result
1.4127	0.9912	5	1	1	0	19	No Significant Difference
1.4127	1.4127	10	6	0	6		
1.4127	1.4127	9	6	0	6		
1.4127	1.4127	8	6	0	6		
0.9912	1.4127	7	6	0	6		
1.4127	1.4127	6	6	0	6		
1.4127	1.4127	4	6	6	0		
1.4127	1.4127	3	6	6	0		
1.4127	1.4127	2	6	6	0		
1.4127	1.4127	1	6	6	0		
				Sum	Sum		
				25	30		

Survival Proportion with Arc-Sine Square Root Transformation

0	AN0469	AN0496 Trans	AN0469 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	1.0	1.4127	1.4127
0.7	1.0	0.9912	1.4127

Shapiro-Wilks Test for Normality

AN0496 Trans	AN0469 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	1.4127	1.4127		0.0585	0.0585				
0.9912	1.4127	0.9912		-0.363	0.0585				
		1.4127		0.0585	0.0585				
Mean	Mean	1.4127		0.0585	0.0585				
1.3284	1.38	1.249		-0.1052	0.0585				
		1.4127		0.0585	0.0585				
		1.4127		0.0585	0.0585				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0496	AN0469	Critical(from Table K=1)	Result
1.4127	0.9912	5	1	1	0	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	9	6.5	0	6.5		
0.9912	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		
1.249	1.4127	3	6.5	6.5	0		
1.4127	1.4127	2	6.5	6.5	0		
1.4127	1.4127	1	6.5	6.5	0		
				Sum	Sum		
				27	28		

Survival Proportion with Arc-Sine Square Root Transformation

AN0496	AN0487	AN0496 Trans	AN0487 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
0.7	1.0	0.9912	1.4127

Shapiro-Wilks Test for Normality

AN0496 Trans	AN0487 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	1.4127	1.4127	1.3542	0.0585	0.0585	0.1702	0.514	0.842	Not Normal
1.4127	1.249	1.4127		0.0585	0.0585				
0.9912	1.4127	0.9912		-0.363	0.0585				
		1.4127		0.0585	0.0585				
Mean	Mean	1.4127		0.0585	0.0585				
1.3284	1.38	1.4127		0.0585	0.0585				
		1.249		-0.1052	0.0585				
		1.4127		0.0585	0.0585				

Wilcoxon Rank Sum Test

Pooled	Sorted	Point	Wilcoxon Rank	AN0496	AN0487	Critical(from Table K=1)	Result
1.4127	0.9912	5	1	1	0	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		
0.9912	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	28		

GROWTH

Average Dry Weight per Replicate (in mg)

Blank	AN0496
0.102	0.163
0.149	0.194
0.118	0.180
0.118	0.212
0.144	0.250

Shapiro-Wilks Test for Normality

Blank	AN0496	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.102	0.163	0.102		-0.061	-0.061				
0.149	0.194	0.149		-0.014	-0.045				
0.118	0.180	0.118	0.163	-0.045	-0.045	0.0195	0.964	0.842	Normal
0.118	0.212	0.118		-0.045	-0.019				
0.144	0.250	0.144		-0.019	-0.014				
		0.163		0	0				
Mean	Mean	0.194		0.031	0.017				
0.126	0.200	0.180		0.017	0.031				
		0.212		0.049	0.049				
		0.250		0.087	0.087				

F-test and T-Test

Blank Var	AN0496 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0004	0.0011	2.75	6.3882	Equal	-4.2493	6	1.9432	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0496	AN0468
0.163	0.192
0.194	0.238
0.180	0.205
0.212	0.224
0.250	0.199

Shapiro-Wilks Test for Normality

AN0496	AN0468	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.163	0.192	0.163		-0.0427	-0.0427				
0.194	0.238	0.194		-0.0117	-0.0257				
0.180	0.205	0.180	0.2057	-0.0257	-0.0137	0.0062	0.9891	0.842	Normal
0.212	0.224	0.212		0.0063	-0.0117				
0.250	0.199	0.250		0.0443	-0.0067				
		0.192		-0.0137	-0.0007				
Mean	Mean	0.238		0.0323	0.0063				
0.200	0.212	0.205		-0.0007	0.0183				
		0.224		0.0183	0.0323				
		0.199		-0.0067	0.0443				

F-Test and T-Test

AN0496 Var	AN0468 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0011	0.0004	2.75	6.3882	Equal	-0.6813	6	1.9432	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0496	AN0469
0.163	0.180
0.194	0.185
0.180	0.202
0.212	0.212
0.250	0.219

Shapiro-Wilks Test for Normality

AN0496	AN0469	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.163	0.180	0.163		-0.0367	-0.0367				
0.194	0.185	0.194		-0.0057	-0.0197				
0.180	0.202	0.180	0.1997	-0.0197	-0.0197	0.0056	0.9579	0.842	Normal
0.212	0.212	0.212		0.0123	-0.0147				
0.250	0.219	0.250		0.0503	-0.0057				
		0.180		-0.0197	0.0023				
Mean	Mean	0.185		-0.0147	0.0123				
0.200	0.200	0.202		0.0023	0.0123				
		0.212		0.0123	0.0193				
		0.219		0.0193	0.0503				

F-Test and T-Test

AN0496 Var	AN0469 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	0.012	6	1.9432	No Significant Difference

Average Dry Weight per Replicate (in mg)

AN0496	AN0487
0.163	0.202
0.194	0.197
0.180	0.234
0.212	0.251
0.250	0.245

Shapiro-Wilks Test for Normality

AN0496	AN0487	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.163	0.202	0.163		-0.0498	-0.0498				
0.194	0.197	0.194		-0.0188	-0.0328				
0.180	0.234	0.180	0.2128	-0.0328	-0.0188	0.0086	0.9291	0.842	Normal
0.212	0.251	0.212		-0.0008	-0.0158				
0.250	0.245	0.250		0.0372	-0.0108				
		0.202		-0.0108	-0.0008				
Mean	Mean	0.197		-0.0158	0.0212				
0.200	0.226	0.234		0.0212	0.0322				
		0.251		0.0382	0.0372				
		0.245		0.0322	0.0382				

F-Test and T-Test

AN0496 Var	AN0487 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0011	0.0006	1.8333	6.3882	Equal	-1.41	7	1.8946	No Significant Difference