State of New Jersey CHRISTINE TODD WHITMAN GOVERNOR

SEDIMENT TOXICITY TEST USING THE AMPHIPOD

Hyalella azteca Watershed Management Area 19 (Delaware Basin)



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

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

WATER MONITORING MANAGEMENT

James Mumman, Administrator

September 1998

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Hyalella azteca Watershed Management Area 19 (Delaware Basin)

Bureau of Freshwater and Biological Monitoring Biomonitoring Section

Assay Number(s): 98H004a, 98H004b, 98H004c, 98H004d, 98H004e, 98H004f, 98H004g,

Report Prepared By:

Victor Poretti

Analysts:

Thomas Miller Dean Bryson Victor Poretti

Samplers:

Thomas Miller Dean Bryson

Supervisor:

Paul Olsen

Chief

Bureau of Freshwater & Biological Monitoring Alfred Korndoerfer, Jr.

EXECUTIVE SUMMARY

Toxicity tests using the amphipod *Hyalella azteca* were performed on sediments collected from seven sites in the Lower Delaware Basin. This initiative was undertaken in conjunction with New Jerseys Watershed Management Program administered by the Department of Environmental Protections Office of Environmental Planning. The sites tested are situated within Watershed Area # 19, which encompasses the entire drainage area from the Rancocas Creek southwestward to the Cooper River. Suspected toxicity at six of the sites (one each on Budds Run, North Branch Rancocas Creek, Barton Run, Haynes Creek, North Branch Pennsauken Creek, and South Branch Pennsauken Creek)was based on their Aseverely impaired@or Amoderately impaired@biological assessments (i.e. degraded quality of benthic macroinvertebrate communities) found in previous survey(s) of New Jerseys statewide Ambient Biomonitoring Network (AMNET). A reference site was selected at Burrs Mill Brook because of its "non-impaired" AMNET assessment. The sediment toxicity tests were conducted to provide further data, which could be related to the 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 a biological database for use in guaging stream quality throughout the state. This database, in turn, can be an invaluable aid to New Jerseys water quality and watershed planning and management efforts. 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 whether 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. The test organism, *Hyalella azteca* is an epibenthic detritivore, reported to also digest bacteria and algae from ingested sediment particles (2). This amphipod inhabits lakes, ponds, and streams throughout North and South America, typically burrowing into the sediment surface (3,4). *H. azteca* is a sensitive Abenchmark@species, which can be cultured in the laboratory with relative ease.

In spring of 1998, the Bureau of Freshwater and Biological Monitoring conducted sediment toxicity tests on seven stream sites, within an area of southern New Jersey, which had exhibited varying degrees of impairment in previous AMNET sampling. The new initiative was designed to support management efforts in Watershed Area # 19, which encompasses the entire drainage from Rancocas Creek southwestward to Cooper River in the Delaware Basin.

METHODS

Sample sites were selected based on previous AMNET results(5) (see appendix A), proximity to urban and/or agricultural areas, and proximity of point source discharges (i.e. effluents from facilities with New Jersey Pollutant Discharge Elimination System (NJPDES) permits). The sites selected are as follows (see map):

BIOLOGICAL	
<u>ASSESSMENT</u>	<u>LOCATION</u> (see map)
non-impaired	Burrs Mill Brook @ Sooey Place Rd.
moderately impaired	Budds Run @ Main St., Pemberton
moderately impaired	North Branch Rancocas @ Pine St. Park, Mount
	Holly
severely impaired	Barton Run @ Tuckerton Rd., Hoot Owl Estates
non-impaired	Haynes Creek @ Himmelein Rd., Oliphant Mills
moderately impaired	North Branch Pennsauken Ck. @ Rt. 537, Maple
	Shade
moderately impaired	South Branch Pennsauken Ck. @ Greentree Rd.,
	Cherry Hill
	ASSESSMENT non-impaired moderately impaired moderately impaired severely impaired non-impaired moderately impaired

Sediment samples were collected from all sites on May 27, 1998, except AN0151 which was sampled on May 28, 1998. 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:

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98H004a = control

98H004b = AN0154(reference, nonimpaired site)

98H004c = AN0150

98H004d = AN0151

98H004e = AN0166

98H004f = AN0168

98H004g = AN0180

98H004h = AN0184
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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 June 2, 1998 at 9:40 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 X 10⁶ 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 (June 12, 1998). Live organisms were counted (see Table 2) and the dry weights measured (see Table 3). Statistical analysis was performed following EPA guidelines (8). 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 (7). The survival data was not distributed normally as analyzed by the Shapiro-Wilks test for normality, and therefore, the Wilcoxan Rank Sum Test was used when comparing test survival results. There was no significant difference in survival results between the reference test (98H004b), and the control (98H004a). All test samples 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 Wilcoxan Rank Sum Test was used when comparing test survival results. The test samples showed no significant difference in mortality from the reference sample. Dry weights of the test samples were then compared to that of the reference (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. In this comparison the test samples showed no significant difference from the reference (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 98H004b contained several chironomids; test chamber 98H004e contained a few freshwater clams, and test chamber 98H004g, some ostracods.

DISCUSSION

The test sites (one each on Budds Run, North Branch Rancocas Creek, Barton Run, Haynes Creek, North Branch Pennsauken Creek, and South Branch Pennsauken Creek) in Watershed Management Area 19 were chosen based on the results of previous macroinvertebrate studies; also, the proximity of NJPDES facilities, urbanization and/or agriculture. The reference site at Burrs Mill Branch, AN0154, was chosen because of its prior Anonimpaired@bioassessment in the AMNET survey(5), and because it is within the same Watershed Management Area as the test sites. 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.

Site AN0151 (N. Branch Rancocas Creek), and site AN0150 (Budds Run), were chosen for testing based on Amoderately impaired@bioassessment results, upstream presence of NJPDES facilities, and to the high agricultural land use in the area. Sites AN0166 (Barton Run), and AN0168 (Haynes Creek) had Aseverely impaired@and Anonimpaired@bioassessment results, respectively, and were chosen for testing because of historical and active cranberry bog operations upstream of these sites. Sites AN0180 (N. Branch Pennsauken Creek) and AN0184 (S. Branch Pennsauken Creek) both had Amoderately impaired@bioassessment results and were also chosen for testing because of high urban land use in the area, with NJPDES facilities upstream of the 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 levels 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.

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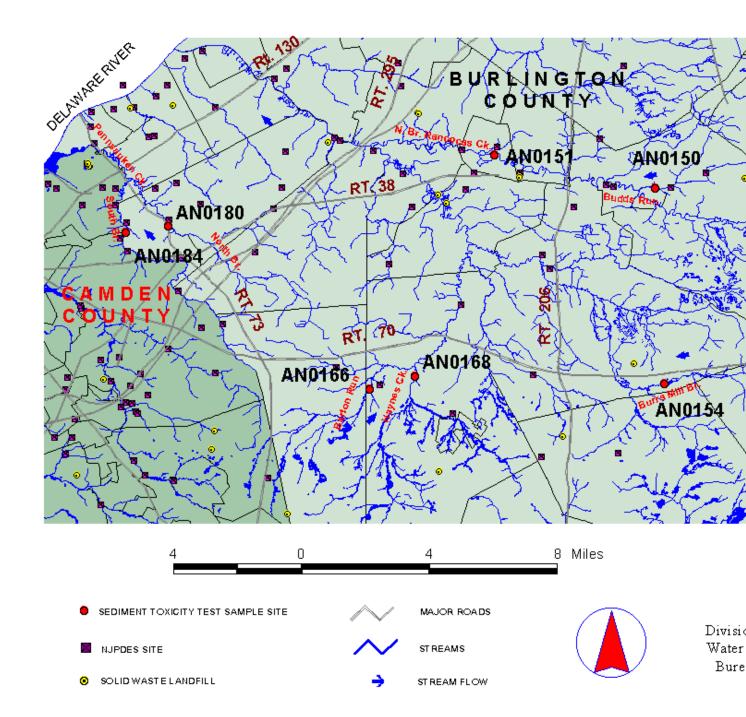


Table 1
Test Chamber Chemical/Physical Parameters

	Т				
Control	HIGH	LOW	AVG.	STD. DEV.	% CV
рН	8.0	7.1	7.7	0.24	3.11
cond. Fmhos	158	136	144	8.10	5.60
D.O. mg/L	8.3	7.2	8.0	0.30	3.75
98H004b	HIGH	LOW	AVG.	STD. DEV.	% CV
рН	7.7	6.6	7.4	0.32	4.39
cond. Fmhos	153	86	129	18.54	14.42
D.O. mg/L	8.1	7.5	7.8	0.22	2.87
_					
98Н004с	HIGH	LOW	AVG.	STD. DEV.	% CV
рН	7.9	7.4	7.6	0.14	1.86
cond. Fmhos	163	135	149	8.70	5.86
D.O. mg/L	8.3	7.5	8.0	0.27	3.42
98H004d	HIGH	LOW	AVG.	STD. DEV.	% CV
рН	7.7	7.1	7.4	0.19	2.63
cond. Fmhos	153	103	129	14.71	11.43
D.O. mg/L	8.3	6.6	7.7	0.53	6.92
98H004e	HIGH	LOW	AVG.	STD. DEV.	% CV
рН	7.9	7.2	7.6	0.21	2.74
cond. Fmhos	182	110	149	21.74	14.58
D.O. mg/L	8.3	7.5	8.0	0.24	3.00

Table 1 continued...

98H004f	HIGH	LOW	AVG.	STD. DEV.	% CV
	7.8	7.1	7.6	0.25	3.24
cond. Fmhos	171	141	154	9.56	6.21
D.O. mg/L	8.5	6.0	7.6	0.73	9.56

98H004g	HIGH	LOW	AVG.	STD. DEV.	% CV
рН	7.8	7.1	7.5	0.24	3.20
cond. Fmhos	177	101	148	26.86	18.20
D.O. mg/L	8.2	6.6	7.7	0.55	7.13

98H004h	HIGH	LOW	AVG.	STD. DEV.	% CV
рН	8.0	7.0	7.6	0.28	3.62
cond. Fmhos	192	162	179	8.84	4.91
D.O. mg/L	8.4	6.6	7.7	0.55	7.07

TABLE 2

MORTALITY DATA (number surviving)

ASSAY #	REP. A	REP. B	REP. C	REP. D	REP. E	%survival
Control	10	9	10	9	10	96
98H004b	10	9	10	10	10	98
98H004c	10	10	10	10	9	98
98H004d	10	10	10	10	10	100
98H004e	9	10	8	9	9	90
98H004f	10	8	9	8	10	90
98H004g	10	10	10	8	10	96
98H004h	10	10	10	10	10	100

Statistical Analysis

Test Endpoint: Survival

Test Used: Wilcoxan Rank Sum Test

Results: 98H004b: no significant difference from control

98H004c-h: 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 DRY WGT: TOTAL WGT. NUMBER OF LARVAE AVG. GROUP AVG. BOAT (mg) BOAT+ OF LARVAE LARVAE DRY WGT. (mg) LARVAE (mg) (mg) (mg) CONTROL 26.89 27.98 1.09 10 0.109 A В 20.37 21.51 1.14 9 0.127 C 22.60 1.93 10 0.193 0.143 20.67 D 19.26 20.55 1.29 9 0.143 E 22.61 24.03 1.42 10 0.142 98H004b A 18.59 19.94 1.35 10 0.135 9 В 22.14 23.84 1.70 0.189 C 17.61 19.04 1.43 10 0.143 0.168 D 15.35 17.22 1.87 10 0.187 Е 26.42 1.87 10 0.187 24.55 98H004c A 18.00 20.04 2.04 10 0.204 В 16.18 18.62 2.44 10 0.244 C 14.96 17.49 2.53 10 0.253 0.230 D 17.01 19.14 2.13 10 0.213 Е 17.89 20.09 2.11 9 0.234 98H004d A 14.72 16.66 1.94 10 0.194 В 19.28 20.88 1.60 10 0.160 C 20.08 10 0.166 0.180 21.74 1.66 D 17.68 19.60 1.92 10 0.192 Е 18.59 20.47 1.88 10 0.188

TABLE 3 continued....

REPLICAT	E.	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)
98H004e	A	21.35	22.88	1.53	9	0.170	
	В	17.60	20.06	2.46	10	0.246	
	С	15.77	17.17	1.40	8	0.175	0.213
	D	20.13	22.26	2.13	9	0.237	
	Е	17.11	19.24	2.13	9	0.237	
98H004f	A	26.56	28.85	2.29	10	0.229	
	В	22.44	24.08	1.64	8	0.205	
	С	21.03	23.27	2.24	9	0.249	.0223
	D	19.17	20.68	1.51	8	0.189	
	Е	14.40	16.84	2.44	10	0.244	
98H004g	A	15.13	17.19	2.06	10	0.206	
	В	14.18	16.19	2.01	10	0.201	
	С	17.46	19.34	1.88	10	0.188	0.192
	D	16.74	18.31	1.57	8	0.196	
	Е	17.76	19.44	1.68	10	0.168	
97H006h	A	21.66	23.80	2.14	10	0.214	
	В	21.17	23.26	2.09	10	0.209	
	C	20.03	21.97	1.94	10	0.194	0.187
	D	20.18	22.03	1.85	10	0.185	
	Е	22.73	24.05	1.32	10	0.132	

Statistical Analysis Test Endpoint: Growth Results:

Rank Sum

98H004b: no significant difference from control 98H004c-h: no significant difference from reference station

^{*}see appendix B for statistical printout

APPENDIX A

AMNET DATA(5)

Sediment Toxicity Tests Watershed Management Area # 19 Delaware Basin - Pemberton USGS Quadrangle Station AN0150 Budds Run, Main Street, Pemberton

February 23, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Simuliidae	40	6
Gammaridae	27	4
Chironomidae	13	6
Asellidae	4	8
Tubificidae	12	10
Hydropsychidae	1	4
Gastropoda	1	7
Turbellaria	1	4
Tipulidae	1	3

Statistical Analysis

Number of Taxa = 9

Total Number of Individuals = 100

% Contribution of Dominant Family = 40.00

Family Biotic Index = 5.96

Scraper/Filterer Collector Ratio = 0.00

Shredder/Total Ratio = 0.03

E+P+T* = 1 * (Ephemeroptera, Plecoptera and Trichoptera)

%EPT = 1.00

EPT/C* = 0.08 *(Chironomidae)

NJIS Rating = 9

Biological Condition = moderately impaired

Deficiency(s) noted: paucity of clean water organisms

Observations

Streamwater: clear...Flow: moderate...Width/Depth(ft): 25/1... Substrate: sand...Streambank Vegetation/Stability: poor/poor

Canopy: open...Other: sparsely tree-lined; darter

6

Delaware Basin - Mount Holly USGS Quadrangle Station AN0151

North Branch Rancocas Creek, Pine Street Park, Mount Holly January 26, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Chironomidae	29	6
Gammaridae	15	4
Lumbricidae	3	10
Hydropsychidae	4	4
Heptageniidae	1	4
Brachycentridae	2	1
Naididae	1	7
Nemertea	1	5
Taeniopterygidae	2	2
Tubificidae	2	10

Statistical Analysis

Number of Taxa = 10

Total Number of Individuals = 60

% Contribution of Dominant Family = 48.33

Family Biotic Index = 5.37

Scraper/Filterer Collector Ratio = 0.00

Shredder/Total Ratio = 0.05

E+P+T* = 4*(Ephemeroptera, Plecoptera and Trichoptera)

%EPT = 15.00

EPT/C* = 0.31 *(Chironomidae)

NJIS Rating = 15

Biological Condition = moderately impaired

Deficiency(s) noted: none

Observations

Streamwater: clear/dark cedar...Flow: fast...Width/Depth(ft): 75/1 ...Substrate: sand/gravel...Streambank Vegetation/Stability: poor/

poor...Canopy: open...Other: tree-lined

Delaware Basin - Pemberton USGS Quadrangle Station AN0154 Burrs Mill Brook, Sooey Place Road (off Route 70)

March 2, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Simuliidae	37	6
Hydropsychidae	33	4
Leptophlebiidae	7	2
Philopotamidae	3	3
Asellidae	6	8
Heptageniidae	1	4
Chironomidae	3	6
Ceratopogonidae	1	6
Limnephilidae	3	4
Lumbriculidae	1	8
Nematoda	2	6
Molannidae	1	6
Leptoceridae	1	4
Aeshnidae	1	3

Statistical Analysis

Number of Taxa = 14

Total Number of Individuals = 100

% Contribution of Dominant Family = 37.00

Family Biotic Index = 4.98

Scraper/Filterer Collector Ratio = 0.00

Shredder/Total Ratio = 0.11

E+P+T* = 7 * (Ephemeroptera, Plecoptera and Trichoptera)

%EPT = 49.00

EPT/C* = 16.33 *(Chironomidae)

NJIS Rating = 30

Biological Condition = non-impaired

Deficiency(s) noted: none

Observations

Streamwater: clear/cedar...Flow: slow...Width/Depth(ft): 20/1... Substrate: sand/gravel...Streambank Vegetation/Stability: poor/

poor...Canopy: open...Other: wooded

Delaware Basin - Mount Holly USGS Quadrangle Station AN0166 Barton Run, Tuckerton Road, near Hoot Owl Estates

Barton Run, Tuckerton Road, near Hoot Owl Estates April 14, 1993

Family	Number of Individuals	Family Tolerance Value (FTV)
Tipulidae	1	3
Elmidae	5	4
Gastropoda	22	7
Sphaeriidae	61	8
Unionidae	2	8
Hydropsychidae	5	4
Nemertea	1	5
Turbellaria	2	4

Statistical Analysis

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10

Number of Taxa = 9

Tubificidae

Total Number of Individuals = 100

% Contribution of Dominant Family = 61.00

Family Biotic Index = 7.24

Scraper/Filterer Collector Ratio = 0.31

Shredder/Total Ratio = 0.00

E+P+T* = 1 *(Ephemeroptera, Plecoptera and Trichoptera)

%EPT = 5.00

EPT/C* = 0.00 * (Chironomidae)

NJIS Rating = 3

Biological Condition = severely impaired

Deficiency(s) noted: paucity of clean water organisms

significant organic pollution

Sphaeriidae overwhelmingly dominant

Observations

Streamwater: very turbid...Flow: slow...Width/Depth(ft): 20/1...
Substrate: sand/gravel/pieces of concrete...Streambank Vegetation/
Stability: poor/poor...Canopy: open...Other: tree-lined;
filamentous algae; muddy water coming in from storm drain (from

road construction by Burlington County Highway Department, no hay bales, etc. being used)

Delaware Basin - Mount Holly USGS Quadrangle Station AN0168 Haynes Creek, Himmelein Road, Oliphant Mills April 14, 1993

	Numbe	r Family
	of	Tolerance
Family	Individuals	Value (FTV)
Sphaeriidae	 19	8
BloodRedChirono	-	8
Hydropsychidae	34	4
Naididae	9	7
Gastropoda	1	7
Turbellaria	6	4
Heptageniidae	1	4
Elmidae	7	4
Tubificidae	4	10
Chironomidae	4	6
Caenidae	2	7
Nemertea	3	5
Lumbriculidae	1	8

10

Statistical Analysis

Lumbricidae

Number of Taxa = 14

Total Number of Individuals = 100

% Contribution of Dominant Family = 34.00

Family Biotic Index = 5.89

Scraper/Filterer Collector Ratio = 0.02

Shredder/Total Ratio = 0.05

E+P+T* = 3 * (Ephemeroptera, Plecoptera and Trichoptera)

%EPT = 37.00

EPT/C* = 3.08 *(Chironomidae)

NJIS Rating = 24

Biological Condition = non-impaired

Deficiency(s) noted: none

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Observations

Streamwater: clear/cedar...Flow: moderate-fast...Width/Depth(ft): 30/1.5...Substrate: sand/gravel/rocks...Streambank Vegetation/

Stability: good/good... Canopy: open... Other: tree-lined;

macrophytes; filamentous green algae

Delaware Basin - Camden USGS Quadrangle Station AN0180

North Branch Pennsauken Creek, Route 537, Maple Shade March 18, 1992

Family	Number of Individuals	Family Tolerance Value (FTV)
Chironomidae	53	6
Naididae	13	7
Hirudinea	5	10
Gammaridae	12	4
Turbellaria	3	4
Tubificidae	5	10
Gastropoda	3	7
Haplotaxidae	1	8
Asellidae	3	8
Sphaeriidae	1	8
Coenagrionidae	1	9

Statistical Analysis

Number of Taxa = 11

Total Number of Individuals = 100

% Contribution of Dominant Family = 53.00

Family Biotic Index = 6.39

Scraper/Filterer Collector Ratio = 0.00

Shredder/Total Ratio = 0.00

E+P+T* = 0 * (Ephemeroptera, Plecoptera and Trichoptera)

%EPT = 0.00

EPT/C* = 0.00 * (Chironomidae)

NJIS Rating = 12

Biological Condition = moderately impaired

Deficiency(s) noted: paucity of clean water organisms

Observations

Streamwater: turbid/green...Flow: moderate...Width/Depth(ft): 50/ <1...Substrate: sand/gravel/mud/cobbles...Streambank Vegetation/ Stability: poor/poor...Canopy: open...Other: commercial area; substrate covered with mosses, silt, and 20 shopping carts.

6

Delaware Basin - Camden USGS Quadrangle Station AN0184 South Branch Pennsauken Creek, Route 537, Maple Shade

March 18, 1992

Family	Individu	Number of als	Family Tolerance Value (FTV)
Tubificidae	omidae	98	10
BloodRedChiron		1	8
Hydropsychidae		1	4

Statistical Analysis

Number of Taxa = 3

Total Number of Individuals = 100

% Contribution of Dominant Family = 98.00

Family Biotic Index = 9.92

Scraper/Filterer Collector Ratio = 0.00

Shredder/Total Ratio = 0.01

E+P+T* = 1 * (Ephemeroptera, Plecoptera and Trichoptera)

%EPT = 1.00

EPT/C* = 1.00 * (Chironomidae)

NJIS Rating = 0

Biological Condition = severely impaired

Deficiency(s) noted: paucity of clean water organisms

significant organic pollution

low diversity

Tubificidae overwhelmingly dominant

Observations

Streamwater: green...Flow: slow...Width/Depth(ft): 30/<1... Substrate: sand/gravel...Streambank Vegetation/Stability: poor/ poor...Canopy: open...Other: tree-lined; filamentous algae; macrophytes

6

APPENDIX B STATISTICAL DATA

Sediment Toxicity Tests Watershed Management Area # 19

SURVIVAL RESULTS

AN0154 vs. Blank Control Survival Proportions with Arc Sine Square Root Transformation

Blank	AN0154	Blank Trans	AN0154 Trans
1.0	1.0	1.4127	1.4127
0.9	0.9	1.249	1.249
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

	U- WIIKS I CS	. 101 1 101 11	iuiity						
Blank Trans	AN0154 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0491	-0.1146				
1.249	1.249	1.249		-0.1146	-0.1146				
1.4127	1.4127	1.4127	1.3636	0.0491	-0.1146	0.0563	0.594	0.842	Not Normal
1.249	1.4127	1.249		-0.1146	0.0491				
1.4127	1.4127	1.4127		0.0491	0.0491				
		1.4127		0.0491	0.0491				
Mean	Mean	1.249		-0.1146	0.0491				
1.3472	1.38	1.4127		0.0491	0.0491				
		1.4127		0.0491	0.0491				
		1.4127		0.0491	0.0491				

Pooled	Sorted	Point	Wilcoxan Rank	Blank	AN0154	Critical(from Table K=1)	Result
1.4127	1.249	7	2	0	2	19	No Significant Difference
1.249	1.249	4	2	2	0		
1.4127	1.249	2	2	2	0		
1.249	1.4127	10	7	0	7		
1.4127	1.4127	9	7	0	7		
1.4127	1.4127	8	7	0	7		
1.249	1.4127	6	7	0	7		
1.4127	1.4127	5	7	7	0		
1.4127	1.4127	3	7	7	0		
1.4127	1.4127	1	7	7	0		
				Sum	Sum		
				25	30		

Survival Proportions with Arc Sine Square Root Transformation

AN0154	AN0150	AN0154 Trans	AN0150 Trans
1.0	1.0	1.4127	1.4127
0.9	1.0	1.249	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

Snap	Shapiro-Wilks Test for Normality								
AN0154 Trans	AN0150 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0327	-0.131				
1.249	1.4127	1.249		-0.131	-0.131				
1.4127	1.4127	1.4127	1.38	0.0327	0.0327	0.0429	0.5093	0.842	Not Normal
1.4127	1.4127	1.4127		0.0327	0.0327				
1.4127	1.249	1.4127		0.0327	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				
		1.4127		0.0327	0.0327				
		1.249		-0.131	0.0327				

Pooled	Sorted	Point	Wilcoxan Rank	AN0154	AN0150	Critical(from Table K=1)	Result
1.4127	1.249	10	1.5	0	1.5	19	No Significant Difference
1.249	1.249	2	1.5	1.5	0		
1.4127	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	4	6.5	6.5	0		
1.4127	1.4127	3	6.5	6.5	0		
1.249	1.4127	1	6.5	6.5	0		
				Sum	Sum		
				27.5	27.5		

Survival Proportions with Arc Sine Square Root Transformation

AN0154	AN0151	AN0154 Trans	AN0151 Trans
1.0	1.0	1.4127	1.4127
0.9	1.0	1.249	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

Shapiro-Wilks Test for Normality

Snapiro	- wilks Test 10	r Norma	шц						
AN0154 Trans	AN0151 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0164	-0.1473				
1.249	1.4127	1.249		-0.1473	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	1.4127	1.4127		0.0164	0.0164				
		1.4127		0.0164	0.0164				
Mean	Mean	1.4127		0.0164	0.0164				
1.38	1.4127	1.4127		0.0164	0.0164				
		1.4127		0.0164	0.0164				
		1.4127		0.0164	0.0164				

Pooled	Sorted	Point	Wilcoxan Rank	AN0154	AN0151	Critical(from Table K=1)	Result
1.4127	1.249	2	1	1	0	19	No Significant Difference
1.249	1.4127	10	6	0	6		
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	1	6	6	0		
				Sum	Sum		
				25	30		

Survival Proportions with Arc Sine Square Root Transformation

AN0154	AN0166	AN0154 Trans	AN0166 Trans
1.0	0.9	1.4127	1.249
0.9	1.0	1.249	1.4127
1.0	0.8	1.4127	1.1071
1.0	0.9	1.4127	1.249
1.0	0.9	1.4127	1.249

Shapiro-Wilks Test for Normality

Sna	piro-wiik	s resulor	Norman	ιy					
AN0154 Trans	AN0166 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.249	1.4127		0.096	-0.2096				
1.249	1.4127	1.249		-0.0677	-0.0677				
1.4127	1.1071	1.4127	1.3167	0.096	-0.0677	0.1083	0.7812	0.842	Not Normal
1.4127	1.249	1.4127		0.096	-0.0677				
1.4127	1.249	1.4127		0.096	-0.0677				
		1.249		-0.0677	0.096				
Mean	Mean	1.4127		0.096	0.096				
1.38	1.2534	1.1071		-0.2096	0.096				
		1.249		-0.0677	0.096				
		1.249		-0.0677	0.096				

Pooled	Sorted	Point	Wilcoxan Rank	AN0154	AN0166	Critical(from Table K=1)	Result
1.4127	1.1071	8	1	0	1	19	No Significant Difference
1.249	1.249	10	3.5	0	3.5		
1.4127	1.249	9	3.5	0	3.5		
1.4127	1.249	6	3.5	0	3.5		
1.4127	1.249	2	3.5	3.5	0		
1.249	1.4127	7	8	0	8		
1.4127	1.4127	5	8	8	0		
1.1071	1.4127	4	8	8	0		
1.249	1.4127	3	8	8	0		
1.249	1.4127	1	8	8	0		
				Sum	Sum		
				35.5	19.5		

Survival Proportions with Arc Sine Square Root Transformation

AN0154	AN0168	AN0154 Trans	AN0168 Trans
1.0	1.0	1.4127	1.4127
0.9	0.8	1.249	1.1071
1.0	0.9	1.4127	1.249
1.0	0.8	1.4127	1.1071
1.0	1.0	1.4127	1.4127

Shapiro-Wilks Test for Normality

Sna)11'0- v v 11K	s rest for	Norman	ιy					
AN0154 Trans	AN0168 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0939	-0.2117				
1.249	1.1071	1.249		-0.0698	-0.2117				
1.4127	1.249	1.4127	1.3188	0.0939	-0.0698	0.1523	0.7196	0.842	Not Normal
1.4127	1.1071	1.4127		0.0939	-0.0698				
1.4127	1.4127	1.4127		0.0939	0.0939				
		1.4127		0.0939	0.0939				
Mean	Mean	1.1071		-0.2117	0.0939				
1.38	1.2577	1.249		-0.0698	0.0939				
		1.1071		-0.2117	0.0939				
		1.4127		0.0939	0.0939				

Pooled	Sorted	Point	Wilcoxan Rank	AN0154	AN0168	Critical(from Table K=1)	Result
1.4127	1.1071	9	1.5	0	1.5	19	No Significant Difference
1.249	1.1071	7	1.5	0	1.5		
1.4127	1.249	8	3.5	0	3.5		
1.4127	1.249	2	3.5	3.5	0		
1.4127	1.4127	10	7.5	0	7.5		
1.4127	1.4127	6	7.5	0	7.5		
1.1071	1.4127	5	7.5	7.5	0		
1.249	1.4127	4	7.5	7.5	0		
1.1071	1.4127	3	7.5	7.5	0		
1.4127	1.4127	1	7.5	7.5	0		
				Sum	Sum		
				33.5	21.5		

Survival Proportions with Arc Sine Square Root Transformation

AN0154	AN0180	AN0154 Trans	AN0180 Trans
1.0	1.0	1.4127	1.4127
0.9	1.0	1.249	1.4127
1.0	1.0	1.4127	1.4127
1.0	0.8	1.4127	1.1071
1.0	1.0	1.4127	1.4127

Shapiro-Wilks Test for Normality

Shap	711 U- YY 11IK	3 1 CSt 101	Norman	ιy					
AN0154 Trans	AN0180 Trans	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0.0469	-0.2587				
1.249	1.4127	1.249		-0.1168	-0.1168				
1.4127	1.4127	1.4127	1.3658	0.0469	0.0469	0.0982	0.5352	0.842	Not Normal
1.4127	1.1071	1.4127		0.0469	0.0469				
1.4127	1.4127	1.4127		0.0469	0.0469				
		1.4127		0.0469	0.0469				
Mean	Mean	1.4127		0.0469	0.0469				
1.38	1.3516	1.4127		0.0469	0.0469				
		1.1071		-0.2587	0.0469				
		1.4127		0.0469	0.0469				

Pooled	Sorted	Point	Wilcoxan Rank	AN0154	AN0180	Critical(from Table K=1)	Result
1.4127	1.1071	9	1	0	1	19	No Significant Difference
1.249	1.249	2	2	2	0		
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		
1.4127	1.4127	6	6.5	0	6.5		
1.4127	1.4127	5	6.5	6.5	0		
1.4127	1.4127	4	6.5	6.5	0		
1.1071	1.4127	3	6.5	6.5	0		
1.4127	1.4127	1	6.5	6.5	0		
				Sum	Sum		

		28	27	

Survival Proportions with Arc Sine Square Root Transformation

AN0154	AN0184	AN0154 Trans	AN0184 Trans
1.0	1.0	1.4127	1.4127
0.9	1.0	1.249	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

Shapiro-Wilks Test for Normality

			TOTIII	- ,	1		Т		
AN0154 Trans	AN0184 Trans	AN0184 Trans	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
1.4127	1.4127	1.4127		0	0				
1.249	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				
1.38	1.4127	1.4127		0	0				
		1.4127		0	0				
		1.4127		0	0				

Pooled	Sorted	Point	Wilcoxan Rank	AN0154	AN0184	Critical(from Table K=1)	Result
1.4127	1.249	2	1	1	0	19	No Significant Difference
1.249	1.4127	10	6	0	6		
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	1	6	6	0	
				Sum	Sum	
				25	30	

GROWTH

AN0154 vs. Blank Control

Average Dry Weights per Replicate (in mg)

11,614	gezij
Blank	AN0154
0.109	0.135
0.127	0.189
0.193	0.143
0.143	0.187
0.142	0.187

Shapiro-Wilks Test for Normality

Blank	AN0154	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.109	0.135	0.109		-0.0465	-0.0465				
0.127	0.189	0.127		-0.0285	-0.0285				
0.193	0.143	0.193	0.1555	0.0375	-0.0205	0.0084	0.8652	0.842	Normal
0.143	0.187	0.143		-0.0125	-0.0135				
0.142	0.187	0.142		-0.0135	-0.0125				
		0.135		-0.0205	-0.0125				
Mean	Mean	0.189		0.0335	0.0315				
0.1428	0.1682	0.143		-0.0125	0.0315				
		0.187		0.0315	0.0335				
		0.187		0.0315	0.0375				

F-Test and T-Test

Blank Var	AN0154 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.001	0.0007	1.4286	6.3882	Equal	-1.3775	7	1.8946	No Significant Difference

Average Dry Weights per Replicate (in mg)

AN0154	AN0150
0.135	0.204
0.189	0.244
0.143	0.253
0.187	0.213
0.187	0.234

Shapiro-Wilks Test for Normality

Snap	711 U- VV 11IK	3 1 636 101	Norman	ı y					
AN0154	AN0150	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.135	0.204	0.135		-0.0639	-0.0639				
0.189	0.244	0.189		-0.0099	-0.0559				
0.143	0.253	0.143	0.1989	-0.0559	-0.0119	0.014	0.9414	0.842	Normal
0.187	0.213	0.187		-0.0119	-0.0119				
0.187	0.234	0.187		-0.0119	-0.0099				
		0.204		0.0051	0.0051				
Mean	Mean	0.244		0.0451	0.0141				
0.1682	0.2296	0.253		0.0541	0.0351				
		0.213		0.0141	0.0451				
		0.234		0.0351	0.0541				

F-Test and T-Test

AN0154 Var	AN0150 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0007	0.0004	1.75	6.3882	Equal	-4.1396	7	1.8946	No Significant Difference

Average Dry Weights per Replicate (in mg)

AN0154	AN0151
0.135	0.194
0.189	0.160
0.143	0.166
0.187	0.192
0.187	0.188

Shapiro-Wilks Test for Normality

Sila)11'0- v v 11K	5 1 (51 101	TOTIHAII	ιy					
AN0154	AN0151	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.135	0.194	0.135		-0.0391	-0.0391				
0.189	0.160	0.189		0.0149	-0.0311				
0.143	0.166	0.143	0.1741	-0.0311	-0.0141	0.0042	0.8257	0.842	Not Normal
0.187	0.192	0.187		0.0129	-0.0081				
0.187	0.188	0.187		0.0129	0.0129				
		0.194		0.0199	0.0129				
Mean	Mean	0.16		-0.0141	0.0139				
0.168	0.180	0.166		-0.0081	0.0149				
		0.192		0.0179	0.0179				
		0.188		0.0139	0.0199				

Pooled	Sorted	Point	Wilcoxan Rank	AN0154	AN0151	Critical(from Table K=1)	Result
0.135	0.135	1	1	1	0	19	No Significant Difference
0.189	0.143	3	2	2	0		
0.143	0.160	7	3	0	3		
0.187	0.166	8	4	0	4		
0.187	0.187	5	5.5	5.5	0		
0.194	0.187	4	5.5	5.5	0		
0.160	0.188	10	7	0	7		
0.166	0.189	2	8	8	0		
0.192	0.192	9	9	0	9		
0.188	0.194	6	10	0	10		
				Sum	Sum		
				22	33		

Average Dry Weights per Replicate (in mg)

AN0154	AN0166
0.135	0.170
0.189	0.246
0.143	0.175
0.187	0.237
0.187	0.237

Shapiro-Wilks Test for Normality

Shap	IITO- VV IIK	5 1 (51 101	TOTIHAII	ıy					
AN0154	AN0166	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result
0.135	0.17	0.135		-0.0556	-0.0556				
0.189	0.246	0.189		-0.0016	-0.0476				
0.143	0.175	0.143	0.1906	-0.0476	-0.0206	0.0134	0.9145	0.842	Normal
0.187	0.237	0.187		-0.0036	-0.0156				
0.187	0.237	0.187		-0.0036	-0.0036				
		0.17		-0.0206	-0.0036				
Mean	Mean	0.246		0.0554	-0.0016				
0.1682	0.213	0.175		-0.0156	0.0464				
		0.237		0.0464	0.0464				
		0.237		0.0464	0.0554				

F-Test and T-Test

1-10	st and 1-	1 CSt						
AN0154 Var	AN0166 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0007	0.0014	2	6.3882	Equal	-2.186	7	1.8946	No Significant Difference

Average Dry Weights per Replicate (in mg)

AN0154	AN0168
0.135	0.229
0.189	0.205
0.143	0.249
0.187	0.189
0.187	0.244

Shapiro-Wilks Test for Normality

Shap	Snapiro-wirks Test for Normanty											
AN0154	AN0168	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result			
0.135	0.229	0.135		-0.0607	-0.0607							
0.189	0.205	0.189		-0.0067	-0.0527							
0.143	0.249	0.143	0.1957	-0.0527	-0.0087	0.0131	0.9213	0.842	Normal			
0.187	0.189	0.187		-0.0087	-0.0087							
0.187	0.244	0.187		-0.0087	-0.0067							
		0.229		0.0333	-0.0067							
Mean	Mean	0.205		0.0093	0.0093							
0.1682	0.2232	0.249		0.0533	0.0333							
		0.189		-0.0067	0.0483							
		0.244		0.0483	0.0533							

F-Test and T-Test

AN0154 Var	AN0168 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0007	0.0007	1	6.3882	Equal	-3.2869	8	1.8595	No Significant Difference

Average Dry Weights per Replicate (in mg)

AN0154	AN0180
0.135	0.206
0.189	0.201
0.143	0.188
0.187	0.196
0.187	0.168

Shapiro-Wilks Test for Normality

	Shaph 6- which rest for restrict the maney											
AN0154	AN0180	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result			
0.135	0.206	0.135		-0.045	-0.045							
0.189	0.201	0.189		0.009	-0.037							
0.143	0.188	0.143	0.18	-0.037	-0.012	0.0052	0.8406	0.842	Not Normal			
0.187	0.196	0.187		0.007	0.007							
0.187	0.168	0.187		0.007	0.007							
		0.206		0.026	0.008							
Mean	Mean	0.201		0.021	0.009							
0.1682	0.1918	0.188		0.008	0.016							
		0.196		0.016	0.021							
		0.168		-0.012	0.026							

Pooled	Sorted	Point	Wilcoxan Rank	AN0154	AN0180	Critical(from Table K=1)	Result
0.135	0.135	1	1	1	0	19	No Significant Difference
0.189	0.143	3	2	2	0		
0.143	0.168	10	3	0	3		
0.187	0.187	5	4.5	4.5	0		
0.187	0.187	4	4.5	4.5	0		
0.206	0.188	8	6	0	6		
0.201	0.189	2	7	7	0		
0.188	0.196	9	8	0	8		
0.196	0.201	7	9	0	9		
0.168	0.206	6	10	0	10		
				Sum	Sum		
				19	36		

Average Dry Weights per Replicate (in mg)

AN0154	AN0184
0.135	0.214
0.189	0.209
0.143	0.194
0.187	0.185
0.187	0.132

Shapiro-Wilks Test for Normality

Sha	Snapiro-whks Test for Normanty											
AN0154	AN0184	Pooled	Mean	Centered	Ordered	D-value	W-value	Critical-W (0.05)	Result			
0.135	0.214	0.135		-0.0425	-0.0455							
0.189	0.209	0.189		0.0115	-0.0425							
0.143	0.194	0.143	0.1775	-0.0345	-0.0345	0.008	0.8574	0.842	Normal			
0.187	0.185	0.187		0.0095	0.0075							
0.187	0.132	0.187		0.0095	0.0095							
		0.214		0.0365	0.0095							
Mean	Mean	0.209		0.0315	0.0115							
0.1682	0.1868	0.194		0.0165	0.0165							
		0.185		0.0075	0.0315							
		0.132		-0.0455	0.0365							

F-Test and T-Test

1-10	st and 1-	1 CSt						
AN0154 Var	AN0184 Var	F-Value	Critical-F (Two-Tailed 0.05)	Variances	T-value	Deg. of Freedom	Critical-T (One-Tailed 0.05)	Result
0.0007	0.0011	1.5714	6.3882	Equal	-0.9803	7	1.8946	No Significant Difference