

Attachment B

Biotoxicity Study Results



ASci Corporation
Environmental Testing Laboratory
Submitted to E&E/USACE-Buffalo – 11/05
ASci-ETL Study ID #3600-006

**Results of Ten-Day
Hyalella azteca and *Chironomus tentans*
Toxicity Tests with Whole Sediments
from Buffalo River
Samples Received September 8, 2005**

Prepared by

**ASci Corporation
Environmental Testing Laboratory
4444 Airpark Boulevard
Duluth, Minnesota 55811-5712**

Submitted to


**Engineering & Environment, Inc.
Rosemont Interstate Center, 1, Suite 118
195 South Rosemont Road
Virginia Beach, VA 23452**

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ASci Corporation
Environmental Testing Laboratory
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REPORT APPROVAL

Name (signed):  Date: 11/22/05

Name (typed): Clayton Allen

Title: Biologist

* * * * *

Name (signed):  Date: 11/22/05

Name (typed): Kurt Anderson

Title: Senior Biologist



TABLE OF CONTENTS

INTRODUCTION.....	1
STUDY SUMMARY.....	1
METHODS AND MATERIALS.....	1
General Test Methods	2
Test Organism Culturing, Holding, and Acclimation	2
Overlying Water Characteristics	3
Exposure System.....	3
Test Performance	4
Treatment of Results	6
RESULTS	7
Overlying Water Characteristics	7
Biological Exposure Results	9
CONCLUSIONS.....	11
REFERENCES	12
TABLE 1. Flow Rates (ml/min) of Overlying Water and Daily Turnover Rates to Buffalo River Sediments Test Chambers During Ten-Day <i>Hyaella</i> and <i>Chironomus</i> Exposures	
TABLE 2. Overlying Water Temperature Values (°C) for Buffalo River Sediments During Ten-Day <i>Hyaella</i> Exposures	
TABLE 3. Overlying Water Temperature Values (°C) for Buffalo River Sediments During Ten-Day <i>Chironomus</i> Exposures	
TABLE 4. Overlying Water Dissolved Oxygen Values (mg/L) for Buffalo River Sediments During Ten-Day <i>Hyaella</i> Exposures	



Table of Contents (cont.)

TABLE 5.	Overlying Water Dissolved Oxygen Values (mg/L) for Buffalo River Sediments During Ten-Day <i>Chironomus</i> Exposures
TABLE 6.	Overlying Water pH Values for Buffalo River Sediments During Ten-Day <i>Hyalella</i> Exposures
TABLE 7.	Overlying Water pH Values for Buffalo River Sediments During Ten-Day <i>Chironomus</i> Exposures
TABLE 8.	Overlying Water Conductivity Values (μ mhos/cm) for Buffalo River Sediments During Ten-Day <i>Hyalella</i> Exposures
TABLE 9.	Overlying Water Conductivity Values (μ mhos/cm) for Buffalo River Sediments During Ten-Day <i>Chironomus</i> Exposures
TABLE 10.	Overlying Water Alkalinity Values (mg/L) for Buffalo River Sediments During Ten-Day <i>Hyalella</i> Exposures
TABLE 11.	Overlying Water Alkalinity Values (mg/L) for Buffalo River Sediments During Ten-Day <i>Chironomus</i> Exposures
TABLE 12.	Overlying Water Hardness Values (mg/L) for Buffalo River Sediments During Ten-Day <i>Hyalella</i> Exposures
TABLE 13.	Overlying Water Hardness Values (mg/L) for Buffalo River Sediments During Ten-Day <i>Chironomus</i> Exposures
TABLE 14.	Overlying Water Ammonia Values (mg/L) for Buffalo River Sediments During Ten-Day <i>Hyalella</i> Exposures
TABLE 15.	Overlying Water Ammonia Values (mg/L) for Buffalo River Sediments During Ten-Day <i>Chironomus</i> Exposures
TABLE 16.	<i>Hyalella</i> Survival Results and <i>Chironomus</i> Survival and Growth Results Following Ten-Day Exposure to Buffalo River Sediments



ASci Corporation
Environmental Testing Laboratory
Submitted to EEI – 11/05
ASci-ETL Study ID #3600-006

Table of Contents (cont.)

TABLE 17. Precision of *Hyaella* 96-Hour NaCl Reference Toxicant Testing

TABLE 18. Precision of *Chironomus* 96-Hour NaCl Reference Toxicant Testing

APPENDIX A -- Chain of Custody Forms

APPENDIX B -- Raw Data Sheets

APPENDIX C -- Statistical Analyses



INTRODUCTION

At the request of the United States Army Corp of Engineers (USACE), Buffalo District and Engineering and Environment, Inc. (EEI), ASci-Environmental Testing Laboratory (ASci-ETL) performed toxicity tests with bulk sediment samples collected from Buffalo River. The 10-day tests were performed to measure the toxicity of selected sediment samples to *Hyaella azteca* (amphipod) and larval *Chironomus tentans* (midge). The *Hyaella* test endpoint was survival, and the *Chironomus* endpoints were survival and growth (ash-free dried weight (AFDW)).

STUDY SUMMARY

The table below summarizes survival and growth (as weight) for each Buffalo River sediment and the West Bearskin control. The test sediments were compared to the West Bearskin control to measure effect.

Endpoint	WBS	11-695	11-733	11-730	11-712	11-610	11-690	11-673	11-658	11-647	11-785	11-780	11-773	11-760
<i>H. azteca</i> Survival (%)	98	90	98	98	92	94	90	80*	88	92	88	96	90	90
<i>C. tentans</i> Survival (%)	90	78	90	88	88	78	92	80	84	82	70	96	84	94
<i>C. tentans</i> AFDW (mg/org)	1.44	0.87*	1.51	1.16	1.25	1.55	1.29	1.34	1.44	1.63	1.25	1.56	1.49	1.54

* Statistically different than the West Bearskin control results.



METHODS AND MATERIALS

General Test Methods

Exposures to determine the toxicity of whole sediment samples from Buffalo River were performed following suggested United States Environmental Protection Agency (USEPA)/USACE methods (USEPA/USACE 1998). Ten-day tests exposing *Hyaella* and *Chironomus* were conducted in a manner to determine the effect of each test sediment on organism survival and *Chironomus* growth. Effect was determined by comparison to organism performance following exposure to the selected reference site sediment. Exposure conditions were maintained using an intermittent flow system for renewal of overlying water. Following are detailed descriptions of test performance, test results, data reduction, and results interpretation.

Test Organism Culturing, Holding, and Acclimation

Hyaella and *Chironomus* were obtained from Environmental Consulting and Testing (ECT), Superior, Wisconsin. Culture conditions were maintained according to suggested EPA methods (EPA 2000). The *Hyaella* were cultured in a static-renewal system with overlying water renewed twice per week, and the *Chironomus* were cultured in a recirculating system. Culture temperature is maintained near the test temperature of 23°C.

The batches of test organisms were hand delivered to ASci-ETL. Upon arrival at ASci-ETL, the batches of organisms were logged in and quarantined in glass containers. Diets during holding were the same as used during the toxicity exposures. The organisms were not crowded or subjected to daily temperature changes greater than 3°C per day during holding. The holding tanks were lightly aerated during the pre-test period. At test initiation the *Hyaella* were 7 to 14 days old. The *Chironomus* were third instar larvae or younger.



Overlying Water Characteristics

Overlying water supplied to the test chambers was dechlorinated City of Duluth tap water. The City draws its water from Lake Superior. The tap water was dechlorinated and metals were removed with treatment through two, 1.5 cubic-foot activated carbon beds.

Exposure System

Sediment from each site tested included five replicates for each species. Exposure chambers were 300-ml Berzilius® glass beakers with 1.5 cm diameter side-wall ports screened with a stainless steel mesh. The ports were located approximately 8 cm above the base of the beaker. The screens were fixed to the beakers using aquarium-grade silicone adhesive. Ten replicate test chambers (five for each species) of each sediment exposure were held in a single all glass 12-L aquarium constructed with silicone adhesive. The 12-L aquaria were fitted with a self-starting siphon drain positioned 10 cm above the base of the tank and provided a water volume of 8 L.

Dechlorinated tap water was fed to a 5-gallon stainless steel headbox where the water was heated and then aerated to reduce supersaturated levels of dissolved gasses. The water was gravity fed to an intermediate polyethylene delivery tank. The intermediate tank contained a submersible pump controlled by a timer. The timer was set to activate the pump at 4-hour intervals (6 times per day). The pump was activated for 5 minutes to deliver an appropriate volume of overlying water to the test system. This volume was rapidly pumped to splitter tubes that delivered fresh overlying water to each holding aquarium. The configuration resulted in two turnovers of overlying water per day. Test temperature ($23^{\circ} \pm 1^{\circ}\text{C}$) was maintained using a constant temperature water bath. Test photoperiod was maintained at 16 hours light and 8 hours darkness per day. Light was supplied by cool-white fluorescent bulbs at an intensity of 50 to 100 ft-candles.



Test Performance

Sediment samples were collected by EEI/ USACE personnel September 6, 2005. The samples were delivered to ASci-ETL by express courier on September 8, 2005. The samples were labeled as 11-730-00-L00, 11-785-00-R00, 11-780-00-C00, 11-773-00-R00, 11-760-00-R00, 11-690-00-C00, 11-673-00-L00, 11-658-00-L00, 11-647-00-C00, 11-610-00-C00, 11-695-00-R00, 11-733-00-R00, and 11-712-00-L00. The Chain of Custody forms were completed upon the samples' arrival. Sample log-in included visual inspection of the shipping coolers, sample container integrity, sediment temperature and appearance. Following log-in procedures, the samples were stored in darkness at 1-4°C until use. Appendix A contains a copy of the Chain of Custody forms.

Laboratory control sediment was collected on July 11, 2005, from West Bearskin Lake, located in Cook County, Minnesota. The sediment sample (5-gallon) was placed in two new polyethylene containers and cooled immediately. Upon arrival at the laboratory, the sample was logged-in and stored under refrigeration (1-4°C) until use. Before use in the tests, the laboratory control sediment was thoroughly homogenized, then sieved through a 2-mm screen to remove indigenous organisms.

The toxicity exposures with both test species were originally performed simultaneously. Twenty-four hours before toxicity test initiation each sample was thoroughly homogenized with a stainless steel auger, and 100-ml portions were transferred to each of the 10 designated replicate exposure chambers. Each set of replicate test chambers were then placed into an assigned 12-L holding chamber containing 8 L of overlying water. The toxicity tests were initiated approximately 24 hours later, after the sediments were allowed to settle. The organisms were introduced into the test system on October 28, 2005.



To start the tests, ten *Hyalella* (7 to 14 days old), and ten *Chironomus* (third instar or younger) were impartially distributed to designated intermediate holding cups. The organisms were handled with a wide-bore glass pipette. The organisms were then transferred from the intermediate vessels to an assigned test replicate.

At test initiation and each daily observation, head flow rate was measured, and any flows found to be outside the range of $\pm 10\%$ from target flow were adjusted. Measurements of overlying water pH, total ammonia, conductivity, hardness, and alkalinity were performed on day 0 and day 10. The temperature and dissolved oxygen were measured daily. The total residual chlorine concentration of the post-carbon water was measured periodically during the test to check for breakthrough.

The test organisms were fed a diet based on EPA methods and recommendations from the culturing laboratory (Aquatic Biosystems). The *Hyalella* were fed a mixture of yeast, Cerophyl®, and fermented trout chow (YCT) prepared to contain 1,800 mg/L total solids. *Chironomus* test chambers received a Tetrafin® slurry. The slurry was prepared to contain 4 g/L total solids. Each test replicate received 1.5 ml of the respective dietary component daily.

The tests were terminated following 10 days of exposure. Any organisms in the overlying water were removed first. The sediments were then removed from the test chambers in a layered fashion using a gentle stream of post-carbon treated water. The sediments were collected in a US Standard #40 sieve. The contents retained on the sieve were rinsed into a white polyethylene pan, placed on a light source, and the sieved contents were searched for test organisms. Numbers of live organisms and dead organisms found were counted and recorded. Organisms not found were recorded as dead. These organisms were assumed to have died early in the exposures and the remains had decayed.

The live *Chironomus* from each replicate were pooled, rinsed, and placed in pre-ashed, pre-weighed aluminum weigh boats. The organisms pooled from each individual test replicate were then dried at 96°C for 23 hours. The dried, pooled organisms were then weighed to the nearest 0.01 mg to determine mean dried weights. Organisms were then ashed at 550°C for two hours, and then



weighed to determine ash-free dry weight (AFDW). AFDW equals the weight of dried larvae minus weight of ashed larvae.

Any pupae that were recovered were included in survival measurements but not growth measurements. For replicates found to contain pupae, the mean weight was calculated by dividing the pooled dry weight of the replicate by the number of organisms exposed less the number of pupae recovered.

Treatment of Results

All data were split into two groups prior to analysis to accommodate TOXSTAT's grouping limits.

The cumulative number of surviving organisms for each test sediment exposure was compared to cumulative survival of organisms exposed to the selected reference site sediment exposure to measure effect. The survival data were analyzed using the TOXSTAT Version 3.5 software package (West Inc., and University of Wyoming, Laramie, Wyoming). The survival data were arc-sine transformed before analysis then checked for normality and equality of variance. The appropriate parametric or non-parametric test was then performed to determine significant effect ($p=0.05$) as compared to the reference site results.

The growth data was not transformed before analysis. Mean ash-free dry weights of organisms were checked for normality and equality of variance. The ash-free dry weight data were then analyzed for significant effect ($p=0.05$) using the appropriate parametric or non-parametric test. Mean growth at each test site was compared to the reference site result to determine effect.



RESULTS

Overlying Water Characteristics

Headbox flow rates were measured daily. The daily values, calculated test chamber flow rates, and volume exchanges are in Table 1. The overall mean flow rate for each of the holding tanks during the test period was 9.3 ml/minute. The mean flow rate shows overlying water was renewed at a rate that averaged 2.0 tank volumes per day.

Tables 2 and 3 summarize the overlying water temperature values measured daily from the *Hyaella* and *Chironomus* exposure chambers. All the individual values were within the proposed range of $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Mean test temperatures were maintained at 22.4°C to 22.7°C .

Overlying water dissolved oxygen (DO) concentrations in the *Hyaella* and *Chironomus* test chambers are in Tables 4 and 5. DO values ranged from 3.5 to 8.7 mg/L during the exposures. The percentage of dissolved oxygen saturation ranged from 41% to 100%. At no time was feeding suspended for either species.

Overlying water pHs for the *Hyaella* and *Chironomus* test chambers are in Tables 6 and 7. The pH of overlying water in the *Hyaella* and *Chironomus* exposures ranged from 6.90-7.70. None of the pH values were outside of the organism's physiologically tolerable range.

Tables 8 and 9 contain the overlying water conductivity values for the *Hyaella* and *Chironomus* exposures. The overall range of conductivity values for both exposures was from 83-323 $\mu\text{mhos/cm}$. None of the values indicated that a significant amount of ionized material was released from the test sediments.



Tables 10 and 11 contain overlying water alkalinity values for the *Hyaella* and *Chironomus* exposures, respectively. Concentrations ranged from 24-158 mg/L as CaCO₃.

Tables 12 and 13 contain the overlying total hardness values for the exposures. Concentrations ranged from 26-118 mg/L as CaCO₃.

Tables 14 and 15 contain the results of total ammonia measurements for the exposures. Ammonia concentrations for the test sediments ranged from <1 mg/L to 7.3 mg/L.

The routine chemistry values indicated the test system maintained suitable water quality to allow assessment of sediment toxicity for both test species.

Biological Exposure Results

All organisms were observed to burrow into all test sediments.

Hyaella azteca Survival -

Table 16 summarizes the *Hyaella* survival results for the 10-day exposures. The laboratory control sediment (West Bearskin) supported acceptable 10-day mean survival of $98 \pm 5\%$. The Buffalo River sediments had survival rates from 80-98%.

Chironomus tentans Survival and Growth Results -

Table 16 summarizes the *Chironomus* survival results for the 10-day exposures. The laboratory control sediment (West Bearskin) supported acceptable 10-day mean survival of $98 \pm 5\%$. The Buffalo River sediments had survival rates from 70-96%.



Chironomus ash-free dried weight results are given in Table 16. Final mean ash-free dried weight for organisms exposed to the laboratory control sediment was an acceptable 1.44 ± 0.14 mg/organism.. Organisms exposed to the Buffalo River sediments had final mean ash-free dry weights ranging from 0.87-1.63 mg/organism.

Hyaella azteca Statistical Results -

Survival Analysis - The *Hyaella* survival data for the management units were compared to the laboratory control (WBS). Results of the analysis showed that only one sediment, 11-673-00-L00, caused significant ($p=0.05$) organism lethality when compared to the West Bearskin sediment results. Printouts of the analysis are contained in Appendix C.

Chironomus tentans Statistical Results -

Survival Analysis - The *Chironomus* survival data for the management units were compared to the laboratory control (WBS). Results of the analysis showed that none of the Buffalo River sediments caused significant ($p=0.05$) organism lethality when compared to the West Bearskin sediment results. Printouts of the analysis are contained in Appendix C.

Ash-free Dried Weight Analysis - The *Chironomus* ash-free dried weight data for the management units were compared to the laboratory control (WBS). Results of the analysis showed that one test sediment, 11-695-00-R00 caused a significant ($p=0.05$) decrease in organism ash-free dried weight when compared to the West Bearskin sediment results. Printouts of the analysis are contained in Appendix C.



CONCLUSIONS

The following conclusions can be drawn from the study results.

- The laboratory control sediment used for this study supported acceptable organism survival for both test species and acceptable *Chironomus* growth.
- One sediment collected from the Buffalo River, 11-673-00-L00, caused significant mortality to *Hyaella* when compared to the laboratory control (West Bearskin).
- None of the sediments collected from the Buffalo River caused significant mortality to *Chironomus* when compared to the laboratory control (West Bearskin).
- Sediment collected from the Buffalo River management unit 11-695-00-R00 caused significant growth impairment to *Chironomus* when compared to the laboratory control (West Bearskin).



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Table 1. Flow Rates (ml/min) of Overlying Water and Daily Turnover Rates to Buffalo River Sediments Test Chambers During 10-Day *Hyalella* and *Chironomus* Exposures

	Test Day													
	0	1	2	3	4	5	6	7	8	9	10	Mean	low	high
Head Flow Rate	320	318	322	312	316	312	316	308	314	318	320	316	308	322
Test Chamber Flow Rate	9.4	9.4	9.5	9.2	9.3	9.2	9.3	9.1	9.2	9.4	9.4	9.3	9.1	9.5
Volume Exchanges	2.0	2.0	2.0	1.9	2.0	1.9	2.0	1.9	1.9	2.0	2.0	2.0	1.9	2.0

17 Oct 87

TABLE 2. Overlying Water Temperature Values (°C) for Buffalo River Sediments During 10-Day Hyalella Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	23.0	22.3	22.3	22.3	22.2	22.2	22.3	22.4	22.3	22.4	22.2	22.4	22.3	22.5
1	22.5	22.3	22.3	22.4	22.3	22.3	22.3	22.6	22.3	22.4	22.3	22.3	22.5	22.3
2	22.8	22.5	22.5	22.5	22.7	22.5	22.6	22.5	22.6	22.6	22.8	22.5	22.6	22.5
3	22.8	22.5	22.5	22.5	22.7	22.6	22.6	22.8	22.8	22.7	22.7	22.8	22.7	22.8
4	22.8	22.5	22.6	22.6	22.4	22.6	22.6	22.7	22.6	22.7	22.8	22.8	22.8	22.8
5	22.9	22.4	22.4	22.8	22.8	22.7	22.7	22.6	22.6	22.7	22.7	22.8	23.0	22.9
6	22.6	22.1	22.4	22.4	22.5	22.4	22.5	22.5	22.4	22.4	22.4	22.4	22.4	22.5
7	22.5	22.6	22.4	22.7	22.6	22.7	22.6	22.6	22.6	22.6	22.6	22.6	22.5	22.6
8	22.5	22.5	22.1	22.4	22.3	22.5	22.5	22.4	22.5	22.3	22.5	22.4	22.5	22.4
9	22.4	22.1	22.3	22.3	22.3	22.5	22.5	22.3	22.5	22.3	22.3	22.3	22.3	22.5
10	22.5	22.5	22.4	22.4	22.3	22.6	22.6	22.5	22.5	22.4	22.4	22.4	22.5	22.4
Low	22.4	22.1	22.1	22.3	22.2	22.2	22.3	22.3	22.3	22.3	22.2	22.3	22.3	22.3
High	23.0	22.6	22.6	22.8	22.8	22.7	22.7	22.8	22.8	22.7	22.8	22.8	23.0	22.9
Mean	22.7	22.4	22.4	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.6	22.6

TABLE 4. Overlying Water Dissolved Oxygen Values (mg/L) for Buffalo River Sediments During 10-Day Hyalella Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	7.7	7.8	7.7	7.3	7.1	7.2	7.3	7.2	6.9	7.1	7.1	6.6	7.0	7.6
1	8.7	8.1	7.3	7.3	7.1	7.2	7.3	7.2	6.5	6.7	6.9	6.6	7.0	7.5
2	7.8	7.9	7.1	7.0	6.7	6.9	6.8	6.9	6.5	6.3	6.8	6.4	6.9	7.1
3	7.7	7.0	6.8	6.5	6.6	6.8	6.6	6.8	5.3	6.5	5.1	6.5	6.7	7.0
4	8.0	7.2	7.3	7.3	7.4	7.3	7.1	7.2	5.3	6.9	5.2	6.7	7.4	8.6
5	7.8	7.3	7.1	7.4	7.4	7.4	7.0	7.0	6.1	5.2	5.1	6.5	7.1	7.4
6	7.2	7.3	6.5	6.4	6.3	6.6	6.4	6.5	6.2	6.5	6.6	5.8	6.6	6.9
7	7.0	7.0	6.4	6.0	6.2	6.3	6.3	6.2	6.2	6.1	6.0	5.9	5.8	6.2
8	7.1	7.2	6.6	6.5	6.6	6.5	6.5	6.5	6.5	6.0	6.3	5.6	5.7	6.4
9	7.0	7.1	6.1	6.6	6.5	6.2	6.1	6.6	6.1	5.2	5.1	5.9	5.8	6.1
10	7.0	7.0	6.2	6.5	6.4	6.4	6.2	6.0	6.4	6.2	6.4	6.1	6.0	6.0
Low	7.0	7.0	6.1	6.0	6.2	6.2	6.1	6.0	5.3	5.2	5.1	5.6	5.7	6.0
High	8.7	8.1	7.7	7.4	7.4	7.4	7.3	7.2	6.9	7.1	7.1	6.7	7.4	8.6
Mean	7.5	7.4	6.9	6.8	6.8	6.8	6.7	6.7	6.2	6.2	6.1	6.2	6.5	7.0

18/8/21

TABLE 3. Overlying Water Temperature Values (°C) for Buffalo River Sediments During 10-Day Chironomus Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	23.0	22.3	22.3	22.3	22.2	22.2	22.3	22.4	22.3	22.4	22.2	22.4	22.3	22.5
1	22.5	22.3	22.3	22.4	22.4	22.3	22.3	22.3	22.3	22.3	22.3	22.4	22.5	22.4
2	22.6	22.6	22.7	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.7	22.5	22.8
3	22.8	22.7	22.5	22.5	22.7	22.6	22.6	22.8	22.8	22.7	22.7	22.8	22.7	22.8
4	22.8	22.5	22.6	22.6	22.7	22.7	22.7	22.6	22.6	22.7	22.8	22.8	22.8	22.8
5	22.9	22.7	22.7	22.8	22.8	22.7	22.7	22.6	22.6	22.7	22.7	22.8	23.0	22.9
6	22.6	22.3	22.4	22.5	22.5	22.7	22.6	22.4	22.5	22.4	22.3	22.4	22.5	22.6
7	22.5	22.6	22.7	22.5	22.6	22.6	22.6	22.6	22.6	22.7	22.6	22.5	22.5	22.6
8	22.5	22.5	22.4	22.4	22.3	22.5	22.5	22.4	22.5	22.3	22.5	22.4	22.5	22.4
9	22.4	22.4	22.3	22.4	22.3	22.5	22.5	22.3	22.5	22.3	22.3	22.3	22.3	22.5
10	22.5	22.5	22.4	22.4	22.3	22.6	22.6	22.5	22.5	22.4	22.4	22.4	22.5	22.4
Low	22.4	22.3	22.3	22.3	22.2	22.2	22.3	22.3	22.3	22.3	22.2	22.3	22.3	22.4
High	23.0	22.7	22.7	22.8	22.8	22.7	22.7	22.8	22.8	22.7	22.8	22.8	23.0	22.9
Mean	22.6	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.6	22.6

TABLE 5. Overlying Water Dissolved Oxygen Values (mg/L) for Buffalo River Sediments During 10-Day Chironomus Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	7.7	7.8	7.7	7.3	7.1	7.2	7.3	7.2	6.9	7.1	7.1	6.6	7.0	7.6
1	7.3	7.0	7.3	7.3	7.1	6.6	7.1	7.1	6.8	6.8	6.0	6.5	6.8	7.5
2	6.9	7.0	7.3	7.3	6.6	6.5	6.4	6.4	6.6	6.5	6.0	6.5	6.5	6.9
3	5.2	6.4	6.4	5.0	5.0	5.0	7.1	5.1	4.2	5.5	4.3	4.2	4.0	4.1
4	6.7	6.7	6.9	6.5	6.5	5.0	7.0	5.7	5.8	4.9	3.5	5.4	4.4	7.1
5	7.1	6.8	6.8	6.7	6.7	6.6	7.1	6.3	5.3	6.7	4.5	5.2	5.1	6.9
6	6.6	6.5	6.3	5.5	5.4	5.5	6.6	6.0	5.5	5.5	5.0	5.2	5.5	5.4
7	5.5	5.4	5.5	5.3	5.5	6.0	5.2	6.3	6.2	5.4	5.0	5.3	5.4	5.8
8	6.7	5.8	6.0	5.8	5.2	5.6	6.0	6.1	5.4	5.0	5.0	5.2	5.2	5.4
9	6.9	5.5	6.1	5.6	5.2	5.8	6.5	6.4	5.8	5.2	5.2	5.1	5.0	5.2
10	6.5	6.5	6.0	5.4	5.5	5.5	5.4	5.0	5.0	4.8	4.9	4.8	4.4	6.0
Low	5.2	5.4	5.5	5.0	5.0	5.0	5.2	5.0	4.2	4.8	3.5	4.2	4.0	4.1
High	7.7	7.8	7.7	7.3	7.1	7.2	7.3	7.2	6.9	7.1	7.1	6.6	7.0	7.6
Mean	6.6	6.5	6.6	6.2	6.0	5.9	6.5	6.1	5.8	5.8	5.1	5.5	5.4	6.2

68 for 6/87

TABLE 6. Overlying Water pH Values for Buffalo River Sediments During 10-Day Hyalella Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	6.90	7.48	7.27	7.25	7.31	7.24	7.29	7.16	7.26	7.24	7.29	7.28	7.13	7.70
10	7.03	7.38	7.39	7.44	7.46	7.49	7.41	7.35	7.41	7.42	7.46	7.49	7.51	7.50

TABLE 8. Overlying Water Conductivity Values (umhos/cm) for Buffalo River Sediments During 10-Day Hyalella Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	83	255	273	231	240	274	229	219	314	323	273	210	301	175
10	141	192	188	189	193	199	172	192	223	179	287	183	189	142

TABLE 10. Overlying Water Alkalinity Values (mg/L) for Buffalo River Sediments During 10-Day Hyalella Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	24	100	110	86	82	96	74	76	120	158	106	80	120	52
10	44	60	70	66	68	68	70	62	110	74	130	68	74	58

TABLE 12.

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	26	88	86	96	116	88	88	84	112	106	106	76	100	64
10	44	80	68	64	74	74	66	64	98	74	118	76	70	68

TABLE 14.

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	<1.0	3.1	5.5	2.5	3.6	4.8	3.4	2.1	6.5	7.3	1.4	1.5	<1.0	4.3
10	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.2	<1.0	<1.0	<1.0	<1.0	<1.0

28 p. 00 64

TABLE 7. Overlying Water pH Values for Buffalo River Sediments During 10-Day *Chironomus* Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	6.90	7.48	7.27	7.25	7.31	7.24	7.29	7.16	7.36	7.24	7.29	7.28	7.13	7.70
10	7.90	7.24	7.21	7.22	7.22	7.21	7.23	7.21	7.20	7.23	7.22	7.27	7.19	7.49

TABLE 8. Overlying Water Conductivity Values (umhos/cm) for Buffalo River Sediments During 10-Day *Chironomus* Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	83	255	273	231	240	274	229	219	314	323	273	210	301	175
10	143	194	192	190	190	189	179	189	222	196	252	191	218	154

TABLE 11. Overlying Water Alkalinity Values (mg/L) for Buffalo River Sediments During 10-Day *Chironomus* Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	24	100	110	86	82	96	74	76	120	158	106	80	120	52
10	40	72	82	84	72	74	62	72	106	96	114	88	98	68

TABLE 13. Overlying Water Hardness Values (mg/L) for Buffalo River Sediments During 10-Day *Chironomus* Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	26	88	86	96	116	88	88	84	112	106	106	76	100	64
10	44	76	80	70	68	74	66	70	90	84	100	82	92	76

TABLE 15. Overlying Water Ammonia Values (mg/L) for Buffalo River Sediments During 10-Day *Chironomus* Exposures

Day	West Bankin	11-695-00-R00	11-733-00-L00	11-730-00-R00	11-712-00-L00	11-610-00-C00	11-690-00-C00	11-673-00-L00	11-658-00-L00	11-647-00-C00	11-780-00-C00	11-773-00-R00	11-760-00-R00	11-785-00-R00
0	<1.0	3.1	3.5	2.5	3.6	4.8	3.4	2.1	6.5	7.3	1.4	1.5	<1.0	4.3
10	<1.0	<1.0	1.1	1.3	<1.0	<1.0	<1.0	<1.0	3.5	3.0	2.2	<1.0	2.0	<1.0

15-01-28 87

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # West Bearskin

Rep.	# Organisms			Ashed Pan Wt. (g)	Ashed Pan + Dried Org. Wt. (g)	Ashed Pan + Ashed Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighed							
A	10	0	10	1.25096	1.26523	1.25279	0.01427	0.01244	1.43	1.24
B	9	1	9	1.24840	1.26349	1.25039	0.01509	0.01310	1.68	1.46
C	0*	10	0	1.26094	na	na	na	na	na	na
D	8	2	8	1.25533	1.26978	1.25711	0.01445	0.01267	1.81	1.58
E	9	1	9	1.25846	1.27374	1.26041	0.01528	0.01333	1.70	1.48
Mean	9.0							Mean	1.66	1.44
St.Dev	0.8							St.Dev	0.16	0.14

*Ephemeroptera larvae present. Not included in data analysis.

Hyaella azteca 10-day Survival

Site ID # West Bearskin

Rep.	# Organisms	
	Alive	Dead
A	10	0
B	10	0
C	9	1
D	10	0
E	10	0
Mean	9.8	
St.Dev	0.5	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # 11-695-00-R00

Rep.	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighed							
A	5	5	5	1.24919	1.25507	1.25080	0.00588	0.00427	1.18	0.85
B	8	2	8	1.25130	1.25980	1.25332	0.00850	0.00648	1.06	0.81
C	10	0	10	1.24995	1.25878	1.25212	0.00883	0.00666	0.88	0.67
D	9	1	9	1.25186	1.26359	1.25467	0.01173	0.00892	1.30	0.99
E	7	3	7	1.24821	1.25758	1.25033	0.00937	0.00725	1.34	1.04
Mean	7.8							Mean	1.15	0.87
St.Dev	1.9							St.Dev	0.19	0.15

Hyaella azteca 10-day Survival

Site ID # 11-695-00-R00

Rep.	# Organisms	
	Alive	Dead
A	10	0
B	9	1
C	9	1
D	8	2
E	9	1
Mean	9.0	
St.Dev	0.7	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # 11-733-00-L00

Rep	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighted							
A	10	0	10	1.26214	1.28190	1.26936	0.01976	0.01254	1.98	1.25
B	7	3	5	1.25467	1.26694	1.25698	0.01227	0.00996	2.45	1.99
C	10	0	9	1.24911	1.26618	1.25444	0.01707	0.01174	1.90	1.30
D	9	1	7	1.25714	1.27221	1.26251	0.01507	0.00970	2.15	1.39
E	9	1	9	1.24978	1.26904	1.25449	0.01926	0.01455	2.14	1.62
Mean	9							Mean	2.12	1.51
St.Dev	1.2							St.Dev	0.21	0.30

Hyaella azteca 10-day Survival

Site ID # 11-733-00-L00

Rep	# Organisms	
	Alive	Dead
A	10	0
B	10	0
C	9	1
D	10	0
E	10	0
Mean	9.8	
St.Dev	0.5	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID: 11-730-00-R00

Rep	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Pan + Dried Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighed							
A	10	0	10	1.25921	1.27475	1.26422	0.01554	0.01053	1.55	1.05
B	9	1	9	1.24935	1.26121	1.25266	0.01186	0.00855	1.32	0.95
C	7	3	7	1.25629	1.26947	1.26026	0.01318	0.00921	1.88	1.32
D	8	2	8	1.24192	1.25674	1.24573	0.01482	0.01101	1.85	1.38
E	10	0	10	1.25255	1.26746	1.25640	0.01491	0.01106	1.49	1.11
Mean	8.8							Mean	1.62	1.16
St.Dev	1.3							St.Dev	0.24	0.18

Hyalella azteca 10-day Survival

Site ID: 11-730-00-R00

Rep	# Organisms	
	Alive	Dead
A	10	0
B	9	1
C	10	0
D	10	0
E	10	0
Mean	9.8	
St.Dev	0.5	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # 11-712-00-L00

Rep	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Pan + Dried Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighed							
A	9	1	9	1.25898	1.27531	1.26487	0.01633	0.01044	1.81	1.16
B	8	2	8	1.26555	1.28298	1.27189	0.01743	0.01109	2.18	1.39
C	9	1	9	1.26065	1.27741	1.26601	0.01676	0.01140	1.86	1.27
D	8	2	8	1.25007	1.26452	1.25443	0.01445	0.01009	1.81	1.26
E	10	0	10	1.24646	1.26355	1.25204	0.01709	0.01151	1.71	1.15
Mean	8.8							Mean	1.87	1.25
St.Dev	0.8							St.Dev	0.18	0.10

Hyalella azteca 10-day Survival

Site ID # 11-712-00-L00

Rep	# Organisms	
	Alive	Dead
A	10	0
B	8	2
C	10	0
D	9	1
E	9	1
Mean	9.2	
St.Dev	0.8	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # 11-610-00-C00

Rep	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Above	Dead	Weighed							
A	8	2	8	1.25935	1.27751	1.26546	0.01816	0.01205	2.27	1.51
B	4	6	2	1.25547	1.25987	1.25668	0.00440	0.00319	2.20	1.60
C	10	0	10	1.24769	1.27058	1.25524	0.02289	0.01534	2.29	1.53
D	8	2	8	1.25893	1.27783	1.26436	0.01890	0.01347	2.36	1.68
E	9	1	9	1.25090	1.27000	1.25716	0.01910	0.01284	2.12	1.43
Mean	7.8							Mean	2.25	1.55
St.Dev	2.3							St.Dev	0.09	0.09

Hyaella azteca 10-day Survival

Site ID # 11-610-00-C00

Rep	# Organisms	
	Above	Dead
A	9	1
B	10	0
C	10	0
D	10	0
E	8	2
Mean	9.4	
St.Dev	0.9	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # 11-690-00-C00

Rep.	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Pan + Ashed Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighed							
A	9	1	9	1.24539	1.26394	1.25204	0.01855	0.01190	2.06	1.32
B	10	0	10	1.26032	1.27965	1.26783	0.01933	0.01182	1.93	1.18
C	9	1	9	1.25070	1.26755	1.25638	0.01685	0.01117	1.87	1.24
D	9	1	9	1.26795	1.28722	1.27478	0.01927	0.01244	2.14	1.38
E	9	1	9	1.25726	1.27653	1.26452	0.01927	0.01201	2.14	1.33
Mean	9.2							Mean	2.03	1.29
St.Dev	0.5							St.Dev	0.12	0.08

Hyaella azteca 10-day Survival

Site ID # 11-690-00-C00

Rep.	# Organisms	
	Alive	Dead
A	9	1
B	8	2
C	9	1
D	10	0
E	9	1
Mean	9.0	
St.Dev	0.7	

By 28 x 87

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID: 11-673-00-100

Rep.	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighed							
A	6	4	6	1.25073	1.26620	1.25634	0.01547	0.00986	2.58	1.64
B	10	0	10	1.24477	1.25931	1.24973	0.01454	0.00958	1.45	0.96
C	9	1	9	1.25092	1.26813	1.25754	0.01721	0.01059	1.91	1.18
D	10	0	10	1.25029	1.26824	1.25703	0.01795	0.01121	1.80	1.12
E	5	5	5	1.24859	1.26175	1.25265	0.01316	0.00910	2.63	1.82
Mean	8							Mean	2.07	1.34
St.Dev	2.4							St.Dev	0.51	0.37

Hyaella azteca 10-day Survival

Site ID: 11-673-00-100

Rep.	# Organisms	
	Alive	Dead
A	9	1
B	7	3
C	9	1
D	7	3
E	8	2
Mean	8	
St.Dev	1.0	

**Hyaella* appear smaller than control organisms.

pg 29 of 87

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # 11-658-00-L00

Rep.	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Pan + Dried Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighted							
A	8	2	8	1.25723	1.27664	1.26476	0.01941	0.01188	2.43	1.48
B	9	1	9	1.24843	1.26878	1.25578	0.02035	0.01300	2.26	1.44
C	7	3	7	1.24495	1.26244	1.25204	0.01749	0.01040	2.50	1.49
D	8	2	8	1.25853	1.27800	1.26628	0.01947	0.01172	2.43	1.46
E	10	0	10	1.24099	1.26335	1.25015	0.02236	0.01320	2.24	1.32
Mean	8.4							Mean	2.37	1.44
St.Dev	1.1							St.Dev	0.12	0.07

Hyaella azteca 10-day Survival

Site ID # 11-658-00-L00

Rep.	# Organisms	
	Alive	Dead
A	10	0
B	8	2
C	7	3
D	9	1
E	10	0
Mean	8.8	
St.Dev	1.3	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # 11-647-00-C00

Rep.	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Pan + Ashed Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighed							
A	10	0	10	1.24310	1.26230	1.24889	0.01920	0.01341	1.92	1.34
B	8	2	8	1.25369	1.27050	1.25842	0.01681	0.01208	2.10	1.51
C	9	1	9	1.25419	1.27615	1.26182	0.02196	0.01433	2.44	1.59
D	8	2	8	1.26121	1.28208	1.26884	0.02087	0.01324	2.61	1.66
E	6	4	6	1.25102	1.26946	1.25715	0.01844	0.01231	3.07	2.05
Mean	8.2							Mean	2.43	1.63
St.Dev	1.5							St.Dev	0.45	0.26

Hyaella azteca 10-day Survival

Site ID # 11-647-00-C00

Rep.	# Organisms	
	Alive	Dead
A	10	0
B	8	2
C	10	0
D	9	1
E	9	1
Mean	9.2	
St.Dev	0.8	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # 11-785-00-R00

Rep.	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Pan + Ashed Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighed							
A	7	3	7	1.25164	1.26418	1.25335	0.01254	0.01083	1.79	1.55
B	10	0	10	1.25342	1.26600	1.25537	0.01258	0.01063	1.26	1.06
C	6	4	6	1.24388	1.25330	1.24596	0.00942	0.00734	1.57	1.22
D	7	3	7	1.25682	1.26603	1.25842	0.00921	0.00761	1.32	1.09
E	5	5	5	1.25713	1.26533	1.25862	0.00820	0.00671	1.64	1.34
Mean	7							Mean	1.52	1.25
St.Dev	1.9							St.Dev	0.22	0.20

Hyaella azteca 10-day Survival

Site ID # 11-785-00-R00

Rep.	# Organisms	
	Alive	Dead
A	8	2
B	10	0
C	8	2
D	10	0
E	8	2
Mean	8.8	
St.Dev	1.1	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID #11-780-00-C00

Rep.	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighted							
A	9	1	9	1.25031	1.27122	1.25678	0.02091	0.01444	2.32	1.60
B	10	0	10	1.25203	1.27343	1.25828	0.02140	0.01515	2.14	1.52
C	10	0	10	1.25484	1.27178	1.26051	0.01694	0.01127	1.69	1.13
D	10	0	10	1.26314	1.28872	1.27063	0.02558	0.01809	2.56	1.81
E	9	1	9	1.25366	1.27593	1.26037	0.02227	0.01556	2.47	1.73
Mean	9.6							Mean	2.24	1.56
St.Dev	0.6							St.Dev	0.34	0.26

Hyaella azteca 10-day Survival

Site ID #11-780-00-C00

Rep.	# Organisms	
	Alive	Dead
A	10	0
B	10	0
C	9	1
D	10	0
E	9	1
Mean	9.6	
St.Dev	0.6	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # 11-773-00-R00

Rep.	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighed							
A	7	3	7	1.24663	1.26627	1.25384	0.01964	0.01243	2.81	1.78
B	9	1	9	1.25266	1.27240	1.25931	0.01974	0.01309	2.19	1.45
C	8	2	8	1.24991	1.26720	1.25616	0.01729	0.01104	2.16	1.38
D	10	0	10	1.24833	1.26824	1.25550	0.01991	0.01274	1.99	1.27
E	8	2	8	1.25134	1.27055	1.25809	0.01921	0.01246	2.40	1.56
Mean	8.4							Mean	2.31	1.49
St.Dev	1.1							St.Dev	0.32	0.19

Hyaella azteca 10-day Survival

Site ID # 11-773-00-R00

Rep.	# Organisms	
	Alive	Dead
A	8	2
B	10	0
C	10	0
D	8	2
E	9	1
Mean	9.0	
St.Dev	1.0	

Table 16. *C. tentans* Survival and Growth and *H. azteca* Survival

Chironomus tentans 10-day Survival and Growth Data

Site ID # 11-760-00-R00

Rep	# Organisms			Ashed Pan Wt. (g)	Dried Org. Wt. (g)	Ashed Pan + Dried Org. Wt. (g)	Dried Org. Wt. (g)	Ashed Org. Wt. (g)	Mean Dried Weight (mg)	Mean Ashed Weight (mg)
	Alive	Dead	Weighed							
A	7	3	7	1.25766	1.27847	1.26614	0.02081	0.01233	2.97	1.76
B	10	0	10	1.24530	1.27125	1.25627	0.02595	0.01498	2.59	1.50
C	10	0	10	1.25048	1.27684	1.26126	0.02636	0.01558	2.64	1.56
D	10	0	10	1.25814	1.28055	1.26662	0.02241	0.01393	2.24	1.39
E	10	0	10	1.24481	1.27101	1.25619	0.02620	0.01482	2.62	1.48
Mean	9.4							Mean	2.61	1.54
St.Dev	1.3							St.Dev	0.26	0.14

Hyaella azteca 10-day Survival

Site ID # 11-760-00-R00

Rep	# Organisms	
	Alive	Dead
A	9	1
B	9	1
C	9	1
D	10	0
E	8	2
Mean	9.0	
St.Dev	0.7	