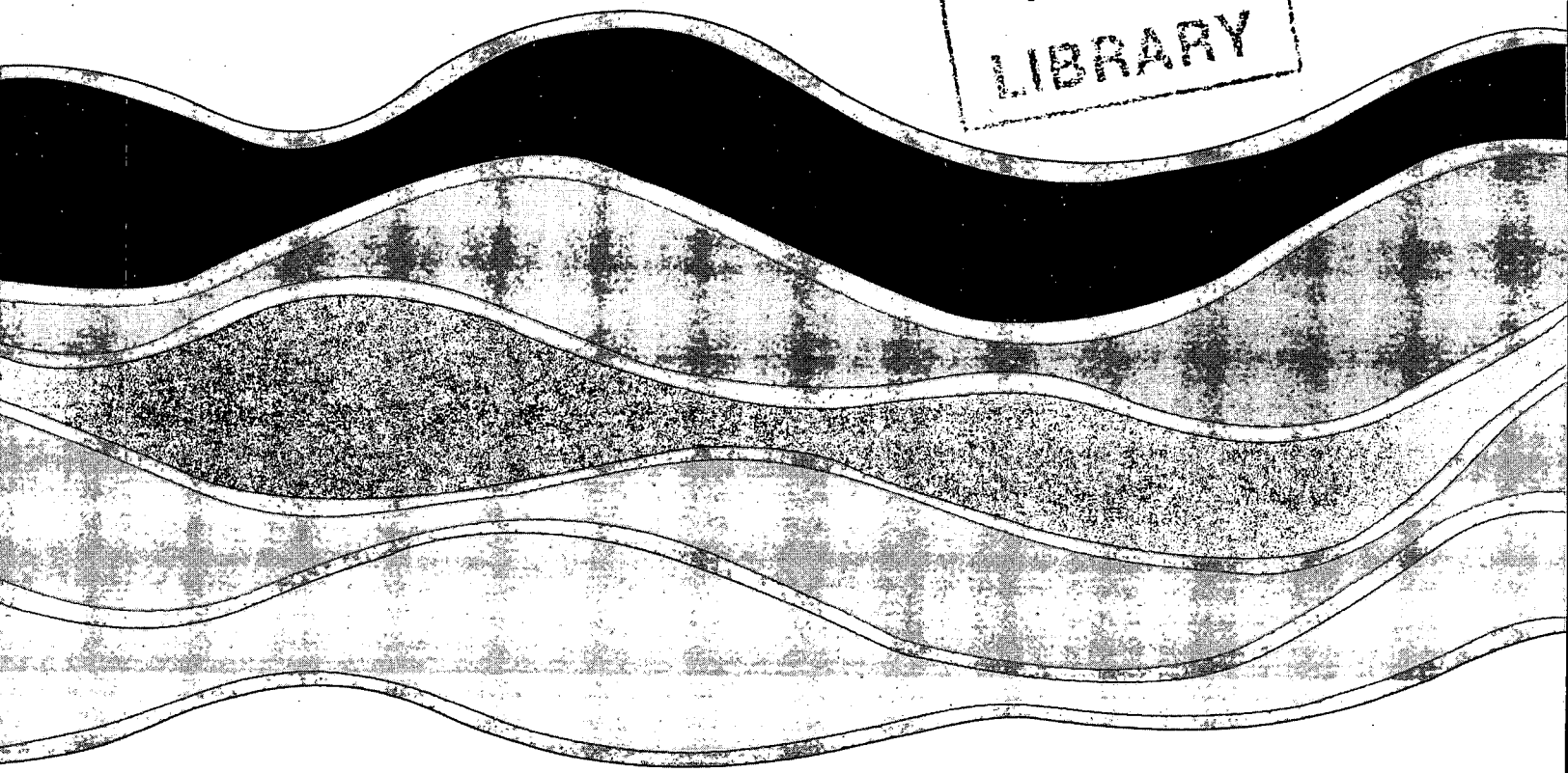
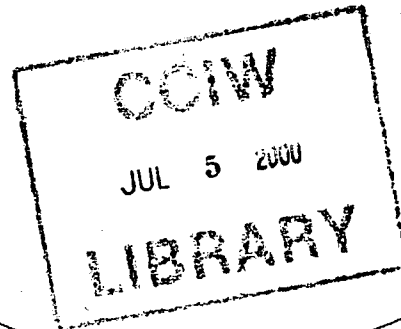
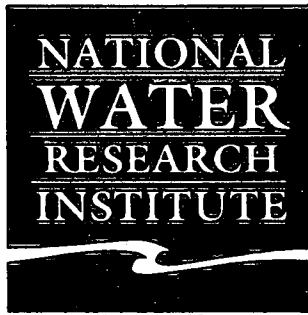


MASTER 98-125



**AN EVALUATION OF THE APPLICABILITY OF THE
TUBIFEX TUBIFEX WHOLE SEDIMENT
REPRODUCTIVE BIOASSAY FOR APPLICATION
IN ASSESSING EFFECTS FROM METAL MINING**

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NWRI Contribution No. 98-125

**An Evaluation of the Applicability of the *Tubifex tubifex* Whole Sediment
Reproductive Bioassay for Application in Assessing Effects from Metal Mining**

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NWRI Contribution No. 98-125

MANAGEMENT PERSPECTIVE

- Title:** An evaluation of the applicability of the *Tubifex tubifex* whole sediment reproductive bioassay for application in assessing effects from metal mining
- Authors:** Danielle Milani, T.B. Reynoldson and P. Hoekstra
- NWRI Publication No:** 98-125
- Citation:** NWRI report
- EC Priority/Issue:** The Aquatic Effects Technology Evaluation Program (AETE) is evaluating methods and approaches to environmental monitoring, for potential application in the EEM for metal mining. As a part of this programme a series of sediment toxicity test are being examined for use in this national programme.
- Current status:** Among the suite of tests being evaluated is the *Tubifex tubifex* 28-day reproduction test. This project is supported by the Canada Centre for Mineral and Energy Technology (CANMET), and involved the assessment of 63 sites from three mining locations. The functional responses of *T. tubifex* (survival and reproduction) were compared to acceptability criteria derived from reference sites sampled in the five Great Lakes over a 3 year period. Three categories of toxicity were developed based on results from 161-212 reference sediments. The 63 sites were classified according to the responses as 1) non toxic, 2) potential toxicity or 3) toxicity. These results showed little evidence of toxicity at these particular sites.
- Next steps:** Data from this test will be compared to other bioassay results to examine their comparative performance and provide recommendations for the most appropriate suite of tests for the metal mining programme.

ABSTRACT

The Aquatic Effects Technology Evaluation Program (AETE) is in the process of evaluating methods and approaches to environmental monitoring, for potential application in the EEM for metal mining. As a part of this programme a series of sediment toxicity tests are being examined for use in this national programme. Among the suite of tests being evaluated is the *Tubifex tubifex* 28-day adult reproduction test. Samples from 63 sites at three mining locations were examined. The functional responses of *T. tubifex* (survival and reproduction) were compared to acceptability criteria derived from reference sites sampled in the five Great Lakes over a 3 year period. Three categories of toxicity were developed based on results from 166-208 reference sediments. The 63 sites were classified according to the responses as 1) non toxic, 2) potentially toxic or 3) toxic. These results showed little evidence of toxicity at these particular sites.

1.0 INTRODUCTION

Toxicity test endpoints most appropriate for assessing ecological impacts are those associated with reproduction and life history (Reynoldson *et al.*, 1991). The tubificid oligochaetes are one of the more widespread benthic groups, making them useful test organisms. The *Tubifex tubifex* 28-day reproduction test was developed at the National Water Research Institute (NWRI) as a component of sediment toxicity assessment (Reynoldson *et al.*, 1991). The test is relatively simple and straightforward, and has been demonstrated to be repeatable and discriminatory (Reynoldson *et al.*, 1991). Although *T. tubifex* does not exhibit discrete age classes, it has the following recognizable life history stages: breeding individuals, cocoons, newly hatched worms, and immature worms, which are used as endpoints in the test (Bonomi and DiCola, 1980; Reynoldson *et al.*, 1991).

As part of the Aquatic Effects Technology Evaluation (AETE) program to evaluate methods and approaches to environmental monitoring, 63 samples from three mines have been assessed for sediment toxicity using the *Tubifex tubifex* 28-day reproduction test. These include the Myra Falls Mine, BC (21 sites); the Dome Mine, ON (21 sites); and the Mattabi Mine, ON (21 sites).

2.0 METHODS

2.1 Culture of Test Organism

Culturing methods for *T. tubifex* are outlined in detail in Reynoldson *et al.*, (1991). Briefly, *T. tubifex* are cultured in 20 cm x 20 cm x 20 cm Plexiglas® aquaria at 23 °C ($\pm 1^\circ\text{C}$). The culture medium is a natural marsh sediment from Long Point (Lake Erie) having a high organic carbon content and appropriate particle size for oligochaetes. This sediment has been used by NWRI, Burlington, ON, as a culture media for the past six years. Approximately 8 cm of sediment is added to a culture aquarium, then filled to the 20-cm mark with culture water (dechlorinated Lake Ontario tap water from the City of Burlington water supply). Once the sediment has settled, approximately 200 full cocoons

are added to the aquarium and the worms cultured to maturity (7-8 weeks). Sexually mature adults (gonads visible) are sieved out and sorted for testing purposes.

2.2 Sediment Sieving and Preparation

Sediment samples are stored in the dark at 4°C until used in toxicity tests. Each sediment sample is completely homogenized, then sieved through a 250-µm sieve (Nytex[®]) to remove indigenous macrofauna (Reynoldson *et al.*, 1994). A 4:1 ratio of culture water:sediment (2 L culture water:500 mL sediment) is used in the sieving process. Culture water is added gradually to the sediment to produce a slurry. This slurry is poured through the sieve, and the residue discarded. The sieved sediment is allowed to settle for a minimum of 24 hours, after which the water is decanted and used as the overlying water. Each 250-mL test chamber receives 100 mL of sieved sediment and 100 mL of overlying water. Food is added to each test chamber (80 mg of crushed Nutrafin[®]) and thoroughly mixed into the sediment-water slurry. The test chambers are then placed in a dark test incubator at 23°C (±1°C) to allow the sediment to settle overnight. Once settled, the samples are aerated for 24 hours before the introduction of the animals. Five replicated beakers are prepared for each test site. In addition, a control sediment (Long Point) is included in each test to ensure that the test responses are within acceptable limits, thereby providing evidence of the health of the test organisms.

2.3 Toxicity Test Procedure

On day 0 (start of test), water chemistry variables (pH, dissolved oxygen (mg/L), temperature (°C) and conductivity (µS/cm)) are measured and recorded for each replicate test chamber. Total ammonia concentration (mg/L) is also determined for each sample by taking a composite water sample from each replicate.

Sexually mature specimens of *T. tubifex* (identifiable gonads) are removed from the culture sediment by adding the sediment to a 500-µm sieve and gently immersing the

sieve into culture water. The worms are washed into petri dishes. These animals are transferred randomly to small plastic dishes containing culture water, until each dish contains four worms. The worms in the dish are added to each replicate beaker.

The test is conducted for 28 days; during this time, all test beakers are aerated, and water loss due to evaporation is supplemented with de-ionized water. Water levels in the test chambers are checked daily and airlines are checked twice daily to ensure proper levels are maintained throughout the test. On day 14 (mid-way point), water chemistry variables are again measured and recorded (pH, dissolved oxygen, temperature, and conductivity).

On day 28, water chemistry variables are measured for the final time, and total ammonia concentration is measured as before. The contents of each beaker are sequentially washed through 500- μm and 250- μm mesh sieves. The contents from the two sieves are washed separately into individual gridded plastic petri dishes for enumeration with a dissecting microscope. Endpoints measured include survival of the original adults, number of full cocoons, number of empty cocoons, number of small offspring (retained on 250- μm mesh), and the number of large offspring (retained on the 500- μm mesh). The results are expressed as four endpoints: mean % survival of adults, the mean number of cocoons per adult, the mean % cocoons hatched (empty cocoons versus total cocoons) and the mean number of offspring per adult. If adult survivorship is less than 100%, the number of cocoons per adult and the number of young per adult is achieved by taking the average of the number of surviving adults and the number of original adults (4). For example, if 3 out of 4 adult worms survived, the number of cocoons or young would be divided by 3.5 to achieve the number per adult.

2.4 QA/QC

The measured endpoints of *T. tubifex* in the laboratory control sediment (Long Point, Lake Erie) are plotted in warning charts (Figures 1, 2 & 3) (Environment Canada, 1990). The number that equals two times the standard deviation above and below the

mean, for each endpoint measured (the upper and lower 95% confidence limits) were plotted. These "warning limits" indicate the normal variability in test responses for *T. tubifex* in control sediment. Each AETE test was compared against these established warning limits. If the test data fell within the limits, they were included in the data set.

Coefficients of Variation (CV) are also determined for the three mine locations for each of the four endpoints to evaluate the precision of the endpoints. The overall mean CV and the range are included at the bottom of each of the 12-endpoint tables (Tables 2 to 13). The CV is used to determine the precision of the test, and a CV of $\leq 20\%$ is generally recommended for laboratory replication.

3.0 RESULTS

The toxicity test results are presented for mean adult survivorship (Tables 2, 3 & 4); mean number of cocoons/adult (Tables 5, 6 & 7); mean number of young/adult (Tables 7, 8 & 9); and mean % cocoons hatched (Tables 11, 12 & 13). Total ammonia concentrations, measured on day 0 and day 28 are presented in Tables 14, 15 & 16. To assess the response of the test endpoints these data have been compared to acceptability criteria derived from reference sites sampled in the Great Lakes over a three year period (Reynoldson *et al.*, 1995; Reynoldson *et al.*, 1997). Three categories of toxicity were developed for four species from sediments in the Great Lakes based on the results from 161-212 reference sediments. The categories are: (1) non-toxic; (2) potential toxicity; and (3) toxicity. The delineations for each of the categories for *T. tubifex* in whole sediment toxicity tests are presented in Table 1. An upper limit is provided in the non-toxic category for reproduction based on twice the standard deviation of the mean. Toxicological effects on sublethal responses are usually considered negative, i.e., growth or reproduction is reduced in comparison to a control. However, in areas of eutrophication or high nutrient impact, sublethality may manifest itself as an increase in biomass or production of young. This effect could have a negative impact on the structure and function of benthic invertebrate communities in aquatic ecosystems.

Therefore, an upper limit for reproduction by *T. tubifex* has been set for the non-toxic category.

Table 1: Limits derived from Great Lakes reference sites for determining toxicity of *T. tubifex* test endpoints

Species	Non toxic (Class 1)	Potentially toxic (Class 2)	Toxic (Class 3)
<i>T. Tubifex</i>			
survival (%)	>88	84 - 87.9	<84
hatch (%)	40 - 78	30.8 - 39.9	<30.8
cocoon/ad. (no.)	7.2 - 12.3	5.9 - 7.1	<5.9
young/ad. (no.)	12.0 - 45.6	3.6 - 11.9	<3.6

The use of two and three times the standard deviation about the mean for each endpoint has been chosen to separate these response categories because it was established to be a more conservative delineation of toxicity than the *Minimum Detectable Differences* (MDD's) determined from the Great Lakes data (Reynoldson *et al.*, 1997).

Adult Survivorship: (Tables 2, 3 & 4)

Adult survival was high in all sites tested (90 - 100%). CV's are low ranging from 0 - 15.2%. All sites are classified as non-toxic.

Cocoons/adult: (Tables 5, 6, & 7)

The number of cocoons produced per adult was sufficient to classify all sites as non-toxic, with the exception of Mattabi Mine sites MMSR2-2 (5.4 cocoons/adult) and MMSR2-3 (7.0 cocoons/adult). These sites are classified as toxic and potentially toxic respectively. Coefficients of variation were $\leq 18.2\%$.

Percent Cocoons Hatched: (Tables 8, 9, & 10)

The percentage of cocoons produced that hatched was sufficient in all sites tested (41.9 - 71.8%), thereby classifying all sites as non-toxic. Coefficients of variation were $\leq 22.6\%$.

Young/adult: (Tables 11, 12 & 13)

The number of young produced per adult was sufficient in all sites to be classified as non-toxic, with the exception of Myra Falls site MN7-S (10.5 young/adult), classifying it as potentially toxic. The range in coefficient of variation was higher for the number of young/adult produced (3.2 – 55.9); however, overall means were $\leq 18.8\%$.

Total Ammonia Levels: (Tables 14, 15 & 16)

Elevated concentrations of total ammonia were recorded on day 0 in Mattabi Mine sites MMSR1-2 (6 mg/L), MMS5-3 (5.5 mg/L) and Dome Mine site D3-1 (5.5 mg/L). By the end of the test, (day 28), these levels were reduced considerably.

4.0 CONCLUSION

These results showed little evidence of toxicity. None of the samples from Dome mines showed evidence of sediment toxicity, and one sample from Myra Falls indicated potential toxicity (Table 11). Only two of 21 sites (Table 7) from the Mattabi Mine were toxic based on cocoon production, MMSR2 (toxic) and MMSR3 (potentially toxic). At both these sites young production was also reduced (Table 13), but was within the criteria established. However, young production is less sensitive than cocoon production because of the greater variability observed in young per individual. It is not possible to interpret these results further in the absence of supporting physical or chemical characterisation of the samples. The elevated total ammonia concentrations observed in Mattabi Mine sites MMSR1-2 (6 mg/L), MMS5-3 (5.5 mg/L) and Dome Mine site D3-1

(5.5 mg/L) did not appear to contribute to toxicity. These sites were classified as non-toxic for all endpoints.

For all test endpoints, the mean CV's were all below the recommended 20%. The CV ranges were greatest for the number of young produced per adult (3.22 - 55.88). This is to be expected, as this endpoint in general tends to be the most variable. For each data set (AETE 1 to 9), the test responses in the laboratory control sediment (Long Point) are within the acceptable limits, thereby accepting all test data.

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Table 2: Adult Survivorship: MYRA FALLS MINE

	SITE	Mean	SD	CV	Classification	
AETE 1	MF1-S	100.00	0.00	0.00	NON TOXIC	
	MF2-S	100.00	0.00	0.00	NON TOXIC	
	MF3-S	100.00	0.00	0.00	NON TOXIC	
	MF4-S	95.00	11.20	11.80	NON TOXIC	
	MF5-S	100.00	0.00	0.00	NON TOXIC	
	MF6-S	100.00	0.00	0.00	NON TOXIC	
	MF7-S	100.00	0.00	0.00	NON TOXIC	
	LAB CONT	100.00	0.00	0.00	NON TOXIC	
AETE 2	MR1-S	100.00	0.00	0.00	NON TOXIC	
	MR2-S	100.00	0.00	0.00	NON TOXIC	
	MR3-S	100.00	0.00	0.00	NON TOXIC	
	MR4-S	100.00	0.00	0.00	NON TOXIC	
	MR5-S	100.00	0.00	0.00	NON TOXIC	
	MR6-S	100.00	0.00	0.00	NON TOXIC	
	MR7-S	95.00	11.20	11.80	NON TOXIC	
	LAB CONT	100.00	0.00	0.00	NON TOXIC	
AETE 3	MN4-S	100.00	0.00	0.00	NON TOXIC	
	MN5-S	100.00	0.00	0.00	NON TOXIC	
	MN6-S	100.00	0.00	0.00	NON TOXIC	
	MN7-S	100.00	0.00	0.00	NON TOXIC	
	MN8-S	100.00	0.00	0.00	NON TOXIC	
	MN9-S	90.00	13.70	15.20	NON TOXIC	
	MN10-S	100.00	0.00	0.00	NON TOXIC	
		LAB CONT	100.00	0.00	0.00	NON TOXIC
		Mean CV	1.62			
		CV Range	0 - 15.20			

Table 3: Adult Survivorship: DOME MINE

	SITE	Mean	SD	CV	Classification
AETE 4	D1B-1	100.00	0.00	0.00	NON TOXIC
	D1B-2	100.00	0.00	0.00	NON TOXIC
	D1B-3	100.00	0.00	0.00	NON TOXIC
	D2-1	100.00	0.00	0.00	NON TOXIC
	D2-2	100.00	0.00	0.00	NON TOXIC
	D2-3	100.00	0.00	0.00	NON TOXIC
	D2-4	100.00	0.00	0.00	NON TOXIC
	LAB CONT	100.00	0.00	0.00	NON TOXIC
AETE 5	D3-1	100.00	0.00	0.00	NON TOXIC
	D3-2	100.00	0.00	0.00	NON TOXIC
	D3-3	100.00	0.00	0.00	NON TOXIC
	D3-4	100.00	0.00	0.00	NON TOXIC
	D3-5	100.00	0.00	0.00	NON TOXIC
	D3-6	100.00	0.00	0.00	NON TOXIC
	D3-7	100.00	0.00	0.00	NON TOXIC
	LAB CONT	100.00	0.00	0.00	NON TOXIC
AETE 6	D4-1	100.00	0.00	0.00	NON TOXIC
	D4-2	100.00	0.00	0.00	NON TOXIC
	D4-3	100.00	0.00	0.00	NON TOXIC
	D4-4	100.00	0.00	0.00	NON TOXIC
	D4-5	100.00	0.00	0.00	NON TOXIC
	D4-6	100.00	0.00	0.00	NON TOXIC
	D4-7	100.00	0.00	0.00	NON TOXIC
	LAB CONT	100.00	0.00	0.00	NON TOXIC
	Mean CV	0.00			
	CV Range	0.00			

Table 4: Adult Survivorship: MATTABI MINE

	SITE	Mean	SD	CV	Classification
AETE 7a	MMSR1-1	100	0.00	0.00	NON TOXIC
	MMSR1-2	100	0.00	0.00	NON TOXIC
	MMSR1-3	100	0.00	0.00	NON TOXIC
	MMSR2-1	95	11.20	11.80	NON TOXIC
	MMSR2-2	90	13.70	15.20	NON TOXIC
	MMSR2-3	90	13.70	15.20	NON TOXIC
		LAB CONT	100	0.00	0.00
AETE 7b	MMS1-1	100	0.00	0.00	NON TOXIC
	MMS1-2	100	0.00	0.00	NON TOXIC
	MMS1-3	100	0.00	0.00	NON TOXIC
	LAB CONT	100	0.00	0.00	NON TOXIC
AETE 8	MMS3-1	95	11.2	11.8	NON TOXIC
	MMS3-2	95	11.2	11.8	NON TOXIC
	MMS3-3	100	0	0	NON TOXIC
	MMS4-1	100	0	0	NON TOXIC
	MMS4-2	93.75	12.5	13.3	NON TOXIC
	MMS4-3	100	0	0	NON TOXIC
	LAB CONT	100	0	0	NON TOXIC
AETE 9	MMS2-1	100	0	0	NON TOXIC
	MMS2-2	100	0	0	NON TOXIC
	MMS2-3	100	0	0	NON TOXIC
	MMS5-1	100	0	0	NON TOXIC
	MMS5-2	100	0	0	NON TOXIC
	MMS5-3	100	0	0	NON TOXIC
	LAB CONT	100	0	0	NON TOXIC
	Mean CV	3.16			
	CV Range	0 - 15.20			

Table 6: Cocoons/Adult: MYRA FALLS MINE

	SITE	Mean	SD	CV	Classification	
AETE 1	MF1-S	9.40	0.58	6.12	NON TOXIC	
	MF2-S	8.98	0.84	9.37	NON TOXIC	
	MF3-S	9.38	0.78	8.29	NON TOXIC	
	MF4-S	8.49	1.39	16.39	NON TOXIC	
	MF5-S	8.65	1.36	15.78	NON TOXIC	
	MF6-S	8.95	1.05	11.75	NON TOXIC	
	MF7-S	8.35	1.26	15.06	NON TOXIC	
	LAB CONT	10.30	0.41	3.99	NON TOXIC	
AETE 2	MR1-S	9.35	0.76	8.15	NON TOXIC	
	MR2-S	9.52	0.89	9.32	NON TOXIC	
	MR3-S	9.45	0.89	9.43	NON TOXIC	
	MR4-S	9.23	1.10	11.91	NON TOXIC	
	MR5-S	9.15	0.38	4.14	NON TOXIC	
	MR6-S	9.95	1.47	14.80	NON TOXIC	
	MR7-S	9.20	0.73	7.96	NON TOXIC	
	LAB CONT	11.35	1.13	9.92	NON TOXIC	
AETE 3	MN4-S	8.60	0.65	7.58	NON TOXIC	
	MN5-S	8.00	0.50	6.25	NON TOXIC	
	MN6-S	8.60	0.96	11.18	NON TOXIC	
	MN7-S	8.25	1.13	13.72	NON TOXIC	
	MN8-S	8.30	0.66	10.30	NON TOXIC	
	MN9-S	8.75	0.70	7.95	NON TOXIC	
	MN10-S	8.41	0.57	6.75	NON TOXIC	
		LAB CONT	10.70	0.37	3.47	NON TOXIC
		Mean CV	9.57			
		CV Range	3.47 - 15.06			

Table 6: Cocoons/Adult: DOME MINE

	SITE	Mean	SD	CV	Classification
AETE 4	D1B-1	11.15	0.45	4.07	NON TOXIC
	D1B-2	10.75	0.53	4.93	NON TOXIC
	D1B-3	10.90	0.68	6.20	NON TOXIC
	D2-1	10.70	0.60	5.58	NON TOXIC
	D2-2	11.12	0.84	7.51	NON TOXIC
	D2-3	10.85	0.83	7.61	NON TOXIC
	D2-4	10.45	0.76	7.26	NON TOXIC
	LAB CONT	10.90	0.98	8.97	NON TOXIC
AETE 5	D3-1	11.25	0.35	3.14	NON TOXIC
	D3-2	10.90	0.55	5.03	NON TOXIC
	D3-3	10.70	0.33	3.05	NON TOXIC
	D3-4	11.00	0.92	8.35	NON TOXIC
	D3-5	10.50	1.84	17.50	NON TOXIC
	D3-6	10.05	0.98	9.70	NON TOXIC
	D3-7	11.25	1.41	12.57	NON TOXIC
	LAB CONT	10.95	0.54	4.95	NON TOXIC
AETE 6	D4-1	11.15	0.58	5.16	NON TOXIC
	D4-2	10.44	0.94	9.04	NON TOXIC
	D4-3	11.63	1.08	9.25	NON TOXIC
	D4-4	11.48	0.91	7.95	NON TOXIC
	D4-5	11.00	0.74	6.69	NON TOXIC
	D4-6	9.96	0.36	3.62	NON TOXIC
	D4-7	11.25	0.46	4.06	NON TOXIC
	LAB CONT	11.50	1.55	13.49	NON TOXIC
	Mean CV	7.32			
	CV Range	3.05 - 17.50			

Table 7: Cocoons/Adult: MATTABI MINE

	SITE	Mean	SD	CV	Classification
AETE 7a	MMSR1-1	9.05	1.35	14.93	NON TOXIC
	MMSR1-2	9.00	1.55	17.24	NON TOXIC
	MMSR1-3	9.25	0.92	9.93	NON TOXIC
	MMSR2-1	8.16	1.11	13.62	NON TOXIC
	MMSR2-2	5.39	0.94	17.53	TOXIC
	MMSR2-3	6.97	1.27	18.23	POT. TOXIC
		LAB CONT	10.80	0.98	9.03
AETE 7b	MMS1-1	11.35	1.07	9.42	NON TOXIC
	MMS1-2	10.94	0.82	7.53	NON TOXIC
	MMS1-3	11.20	0.33	2.91	NON TOXIC
	LAB CONT	11.00	0.73	6.63	NON TOXIC
AETE 8	MMS3-1	9.94	1.27	12.82	NON TOXIC
	MMS3-2	9.14	1.39	15.18	NON TOXIC
	MMS3-3	11.00	0.98	8.95	NON TOXIC
	MMS4-1	9.90	0.42	4.23	NON TOXIC
	MMS4-2	8.30	0.53	6.37	NON TOXIC
	MMS4-3	8.65	0.86	9.93	NON TOXIC
		LAB CONT	11.05	0.51	4.64
AETE 9	MMS2-1	9.50	1.16	12.20	NON TOXIC
	MMS2-2	9.25	0.64	6.89	NON TOXIC
	MMS2-3	9.13	0.52	5.70	NON TOXIC
	MMS5-1	9.20	1.34	14.56	NON TOXIC
	MMS5-2	8.70	1.04	11.92	NON TOXIC
	MMS5-3	8.20	0.91	11.08	NON TOXIC
		LAB CONT	9.80	0.89	9.09
	Mean CV	10.42			
	CV Range	2.91 - 18.23			

Table 8: % Cocoons Hatched: MYRA FALLS MINE

	SITE	Mean	SD	CV	Classification	
AETE 1	MF1-S	65.61	7.40	11.27	NON TOXIC	
	MF2-S	56.25	4.53	8.06	NON TOXIC	
	MF3-S	60.46	5.17	8.55	NON TOXIC	
	MF4-S	64.16	5.56	8.67	NON TOXIC	
	MF5-S	67.74	4.73	6.99	NON TOXIC	
	MF6-S	61.29	3.90	6.36	NON TOXIC	
	MF7-S	57.08	4.35	7.62	NON TOXIC	
	LAB CONT	49.99	3.80	7.59	NON TOXIC	
AETE 2	MR1-S	53.51	4.62	8.63	NON TOXIC	
	MR2-S	59.84	4.05	6.76	NON TOXIC	
	MR3-S	58.16	1.18	2.03	NON TOXIC	
	MR4-S	59.62	4.62	7.74	NON TOXIC	
	MR5-S	60.19	4.15	6.90	NON TOXIC	
	MR6-S	60.78	3.87	6.36	NON TOXIC	
	MR7-S	58.92	2.88	4.88	NON TOXIC	
	LAB CONT	55.65	6.78	12.18	NON TOXIC	
AETE 3	MN4-S	66.71	5.01	7.51	NON TOXIC	
	MN5-S	71.36	5.62	7.87	NON TOXIC	
	MN6-S	65.62	4.65	7.08	NON TOXIC	
	MN7-S	46.32	4.10	8.85	NON TOXIC	
	MN8-S	52.87	5.73	10.83	NON TOXIC	
	MN9-S	64.87	5.44	8.39	NON TOXIC	
	MN10-S	59.18	3.64	6.15	NON TOXIC	
		LAB CONT	56.51	4.97	8.79	NON TOXIC
		Mean CV	7.75			
		CV Range	2.03 - 12.18			

Table 9: % Cocoons Hatched: DOME MINE

	SITE	Mean	SD	CV	Classification	
AETE 4	D1B-1	52.31	5.69	10.87	NON TOXIC	
	D1B-2	53.62	5.49	10.25	NON TOXIC	
	D1B-3	48.46	3.87	7.99	NON TOXIC	
	D2-1	53.20	3.19	6.00	NON TOXIC	
	D2-2	48.50	2.55	5.26	NON TOXIC	
	D2-3	49.34	7.80	15.82	NON TOXIC	
	D2-4	53.43	5.32	9.96	NON TOXIC	
		LAB CONT	52.93	6.47	12.20	NON TOXIC
	AETE 5	D3-1	50.65	3.91	7.72	NON TOXIC
		D3-2	52.67	3.29	6.25	NON TOXIC
		D3-3	57.95	2.56	4.42	NON TOXIC
		D3-4	57.15	3.87	6.78	NON TOXIC
		D3-5	52.89	8.06	15.20	NON TOXIC
D3-6		53.84	2.01	3.73	NON TOXIC	
D3-7		51.13	7.05	13.78	NON TOXIC	
	LAB CONT	48.45	4.87	10.06	NON TOXIC	
AETE 6	D4-1	54.36	4.79	8.82	NON TOXIC	
	D4-2	49.27	5.80	11.77	NON TOXIC	
	D4-3	48.28	7.20	14.92	NON TOXIC	
	D4-4	56.67	7.82	13.80	NON TOXIC	
	D4-5	49.85	11.01	22.08	NON TOXIC	
	D4-6	59.80	2.93	4.91	NON TOXIC	
	D4-7	51.66	2.37	4.60	NON TOXIC	
	LAB CONT	53.96	3.66	6.79	NON TOXIC	
	Mean CV	9.75				
	CV Range	3.73 - 22.08				

Table 10: % Cocoons Hatched: MATTABI MINE

	SITE	Mean	SD	CV	Classification
AETE 7a	MMSR1-1	49.99	4.78	9.55	NON TOXIC
	MMSR1-2	49.42	6.70	13.55	NON TOXIC
	MMSR1-3	46.14	5.44	11.80	NON TOXIC
	MMSR2-1	51.20	9.56	18.67	NON TOXIC
	MMSR2-2	63.75	14.42	22.62	NON TOXIC
	MMSR2-3	50.47	3.81	7.54	NON TOXIC
	LAB CONT	57.49	4.74	8.24	NON TOXIC
AETE 7b	MMS1-1	51.00	4.77	9.35	NON TOXIC
	MMS1-2	44.77	7.12	15.90	NON TOXIC
	MMS1-3	47.31	2.37	5.00	NON TOXIC
	LAB CONT	58.20	4.00	6.87	NON TOXIC
AETE 8	MMS3-1	55.01	6.76	12.30	NON TOXIC
	MMS3-2	51.97	4.98	9.58	NON TOXIC
	MMS3-3	51.31	2.03	3.95	NON TOXIC
	MMS4-1	41.87	3.21	7.67	NON TOXIC
	MMS4-2	48.54	3.48	7.16	NON TOXIC
	MMS4-3	53.28	4.58	8.58	NON TOXIC
	LAB CONT	55.61	2.66	4.78	NON TOXIC
AETE 9	MMS2-1	48.81	4.50	9.22	NON TOXIC
	MMS2-2	49.15	4.58	9.32	NON TOXIC
	MMS2-3	46.54	1.91	4.10	NON TOXIC
	MMS5-1	50.06	6.50	12.97	NON TOXIC
	MMS5-2	53.55	8.78	16.40	NON TOXIC
	MMS5-3	57.70	5.98	10.34	NON TOXIC
	LAB CONT	50.13	5.45	10.87	NON TOXIC
	Mean CV	10.25			
	CV Range	3.95 - 22.62			

Table 11: Young/Adult: MYRA FALLS MINE

	SITE	Mean	SD	CV	Classification	
AETE 1	MF1-S	17.05	5.19	30.45	NON TOXIC	
	MF2-S	16.46	3.42	20.75	NON TOXIC	
	MF3-S	21.38	3.03	14.18	NON TOXIC	
	MF4-S	23.14	4.07	17.61	NON TOXIC	
	MF5-S	17.05	3.85	22.56	NON TOXIC	
	MF6-S	19.90	2.01	10.11	NON TOXIC	
	MF7-S	17.20	2.58	15.01	NON TOXIC	
	LAB CONT	25.80	6.16	23.86	NON TOXIC	
AETE 2	MR1-S	22.95	2.20	9.60	NON TOXIC	
	MR2-S	25.25	1.79	7.11	NON TOXIC	
	MR3-S	23.15	2.14	9.25	NON TOXIC	
	MR4-S	28.21	4.91	17.41	NON TOXIC	
	MR5-S	25.44	1.68	6.59	NON TOXIC	
	MR6-S	29.78	6.47	21.73	NON TOXIC	
	MR7-S	21.11	3.90	18.48	NON TOXIC	
	LAB CONT	28.53	5.63	19.74	NON TOXIC	
AETE 3	MN4-S	18.35	5.52	30.09	NON TOXIC	
	MN5-S	22.35	1.93	8.61	NON TOXIC	
	MN6-S	21.10	0.95	4.48	NON TOXIC	
	MN7-S	10.50	3.21	30.54	POT. TOXIC	
	MN8-S	17.65	1.61	9.10	NON TOXIC	
	MN9-S	23.14	4.92	21.27	NON TOXIC	
	MN10-S	19.67	3.94	20.04	NON TOXIC	
		LAB CONT	27.40	3.91	14.28	NON TOXIC
		Mean CV	16.79			
		CV Range	4.48 - 30.45			

Table 12: Young/Adult: DOME MINE

	SITE	Mean	SD	CV	Classification	
AETE 4	D1B-1	32.88	5.02	15.27	NON TOXIC	
	D1B-2	32.50	5.16	15.88	NON TOXIC	
	D1B-3	34.25	5.34	15.59	NON TOXIC	
	D2-1	37.50	3.74	9.98	NON TOXIC	
	D2-2	25.89	2.36	9.13	NON TOXIC	
	D2-3	30.98	2.68	8.67	NON TOXIC	
	D2-4	32.05	2.46	7.67	NON TOXIC	
		LAB CONT	38.75	7.36	18.99	NON TOXIC
	AETE 5	D3-1	29.25	5.17	17.66	NON TOXIC
		D3-2	29.65	3.79	12.78	NON TOXIC
D3-3		25.35	7.35	29.00	NON TOXIC	
D3-4		37.55	5.06	13.48	NON TOXIC	
D3-5		16.45	1.19	7.24	NON TOXIC	
D3-6		26.20	3.61	13.78	NON TOXIC	
D3-7		27.95	4.52	16.17	NON TOXIC	
	LAB CONT	33.50	3.60	10.74	NON TOXIC	
AETE 6	D4-1	38.45	3.71	9.66	NON TOXIC	
	D4-2	28.81	6.57	22.80	NON TOXIC	
	D4-3	36.00	9.27	25.74	NON TOXIC	
	D4-4	32.70	1.90	5.81	NON TOXIC	
	D4-5	30.19	5.34	17.68	NON TOXIC	
	D4-6	23.46	1.65	7.04	NON TOXIC	
	D4-7	31.81	2.07	6.49	NON TOXIC	
	LAB CONT	40.10	10.54	26.28	NON TOXIC	
	Mean CV	14.31				
	CV Range	6.49 - 29.00				

Table 13: Young/Adult: MATTABIMINE

	SITE	Mean	SD	CV	Classification
AETE 7a	MMSR1-1	25.20	5.33	21.15	NON TOXIC
	MMSR1-2	21.75	5.32	24.45	NON TOXIC
	MMSR1-3	23.65	0.76	3.22	NON TOXIC
	MMSR2-1	20.44	5.22	25.52	NON TOXIC
	MMSR2-2	13.42	4.31	32.14	NON TOXIC
	MMSR2-3	13.85	4.33	31.23	NON TOXIC
	LAB CONT	29.85	4.40	14.75	NON TOXIC
	MMS1-1	18.95	5.38	28.41	NON TOXIC
	MMS1-2	12.22	6.83	55.88	NON TOXIC
	MMS1-3	13.80	1.63	11.84	NON TOXIC
	LAB CONT	34.03	1.43	4.20	NON TOXIC
	MMS3-1	31.66	4.18	13.20	NON TOXIC
	MMS3-2	28.14	5.98	21.30	NON TOXIC
	MMS3-3	33.25	3.82	11.50	NON TOXIC
	MMS4-1	24.75	3.60	14.60	NON TOXIC
	MMS4-2	23.21	2.83	12.20	NON TOXIC
	MMS4-3	32.60	3.44	10.60	NON TOXIC
	LAB CONT	30.15	4.06	13.50	NON TOXIC
	MMS2-1	19.25	4.38	22.74	NON TOXIC
	MMS2-2	24.25	5.10	21.04	NON TOXIC
MMS2-3	16.88	2.57	15.23	NON TOXIC	
MMS5-1	25.30	3.83	15.15	NON TOXIC	
MMS5-2	25.75	2.93	11.36	NON TOXIC	
MMS5-3	19.70	3.55	18.00	NON TOXIC	
LAB CONT	26.05	4.36	16.72	NON TOXIC	
	Mean CV	18.80			
	CV Range	3.22 - 55.88			

Table 14: Total Ammonia (mg/L): MYRA FALLS MINE

	SITE	DAY 0	AY 28
AETE 1	MF1-S	0.03	ND
	MF2-S	ND	0.04
	MF3-S	0.07	ND
	MF4-S	0.04	0.90
	MF5-S	0.03	0.00
	MF6-S	ND	0.05
	MF7-S	0.04	0.04
	LAB CONT	1.00	0.06
AETE 2	MR1-S	0.19	0.05
	MR2-S	0.08	0.05
	MR3-S	0.05	0.05
	MR4-S	0.14	0.05
	MR5-S	0.08	0.06
	MR6-S	0.06	0.05
	MR7-S	0.05	0.05
	LAB CONT	0.08	0.07
AETE 3	MN4-S	0.08	ND
	MN5-S	0.08	ND
	MN6-S	0.07	ND
	MN7-S	0.07	0.02
	MN8-S	0.06	ND
	MN9-S	0.09	ND
	MN10-S	0.06	ND
	LAB CONT	0.28	0.06

ND = NOT DETECTABLE

Table 15: Total Ammonia (mg/L): DOME MINE

	SITE	DAY 0	DAY 28
AETE 4	D1B-1	0.20	0.06
	D1B-2	2.00	0.05
	D1B-3	2.50	0.05
	D2-1	1.60	0.04
	D2-2	1.50	0.05
	D2-3	1.40	0.05
	D2-4	2.40	0.05
	LAB CONT	1.20	0.05
AETE 5	D3-1	5.50	0.20
	D3-2	2.60	0.14
	D3-3	4.10	0.24
	D3-4	4.20	0.16
	D3-5	4.00	0.14
	D3-6	2.90	0.22
	D3-7	4.50	0.21
	LAB CONT	1.50	0.07
AETE 6	D4-1	1.70	0.09
	D4-2	3.50	0.08
	D4-3	2.00	0.09
	D4-4	1.40	0.08
	D4-5	1.60	0.15
	D4-6	2.00	0.08
	D4-7	2.20	0.08
	LAB CONT	1.20	0.07

Table 16: Total Ammonia (mg/L): MATTABI MINE

	SITE	DAY 0	DAY 28
AETE 7a	MMSR1-1	4.00	2.40
	MMSR1-2	6.00	0.30
	MMSR1-3	2.80	0.11
	MMSR2-1	2.30	0.09
	MMSR2-2	0.85	0.08
	MMSR2-3	1.10	0.09
	LAB CONT	0.75	0.13
	AETE 7b	MMS1-1	1.30
MMS1-2		1.25	0.17
MMS1-3		1.40	0.16
AETE 8	LAB CONT	0.70	0.15
	MMS3-1	0.31	0.08
	MMS3-2	0.33	0.10
	MMS3-3	0.45	0.15
	MMS4-1	0.08	0.06
	MMS4-2	0.10	ND
	MMS4-3	0.15	0.05
	LAB CONT	0.51	0.10
AETE 9	MMS2-1	1.75	0.16
	MMS2-2	1.60	0.11
	MMS2-3	1.75	0.10
	MMS5-1	3.10	0.02
	MMS5-2	3.00	ND
	MMS5-3	6.80	0.07
	LAB CONT	4.10	0.05

**Figure 1. Cocoons/Adult Warning Chart for Long Point Control Sediment
T. tubifex 28-day Reproduction Test**

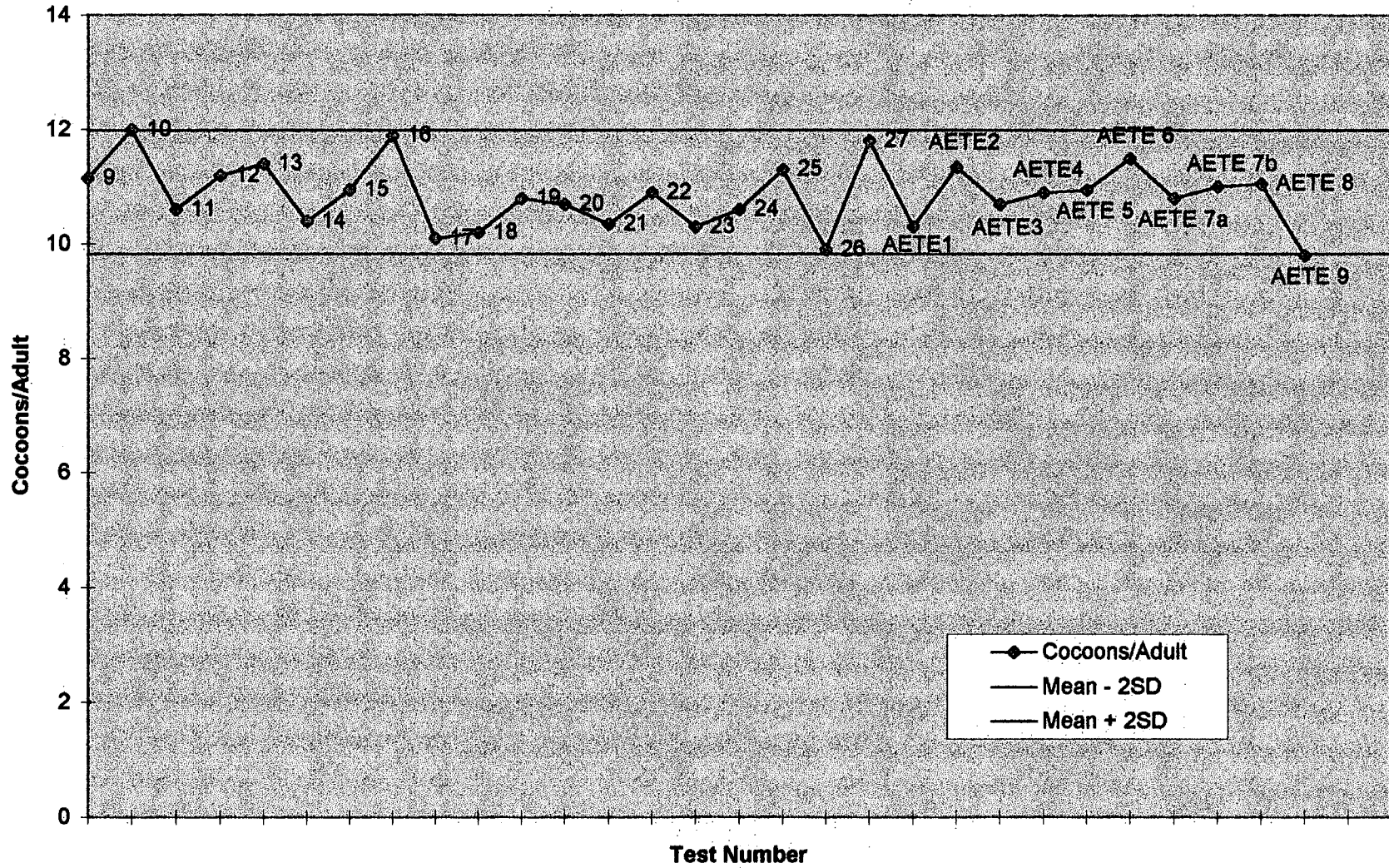


Figure 2. Percent Hatch Warning Chart for Long Point Control Sediment
T. tubifex 28-day Reproduction Test

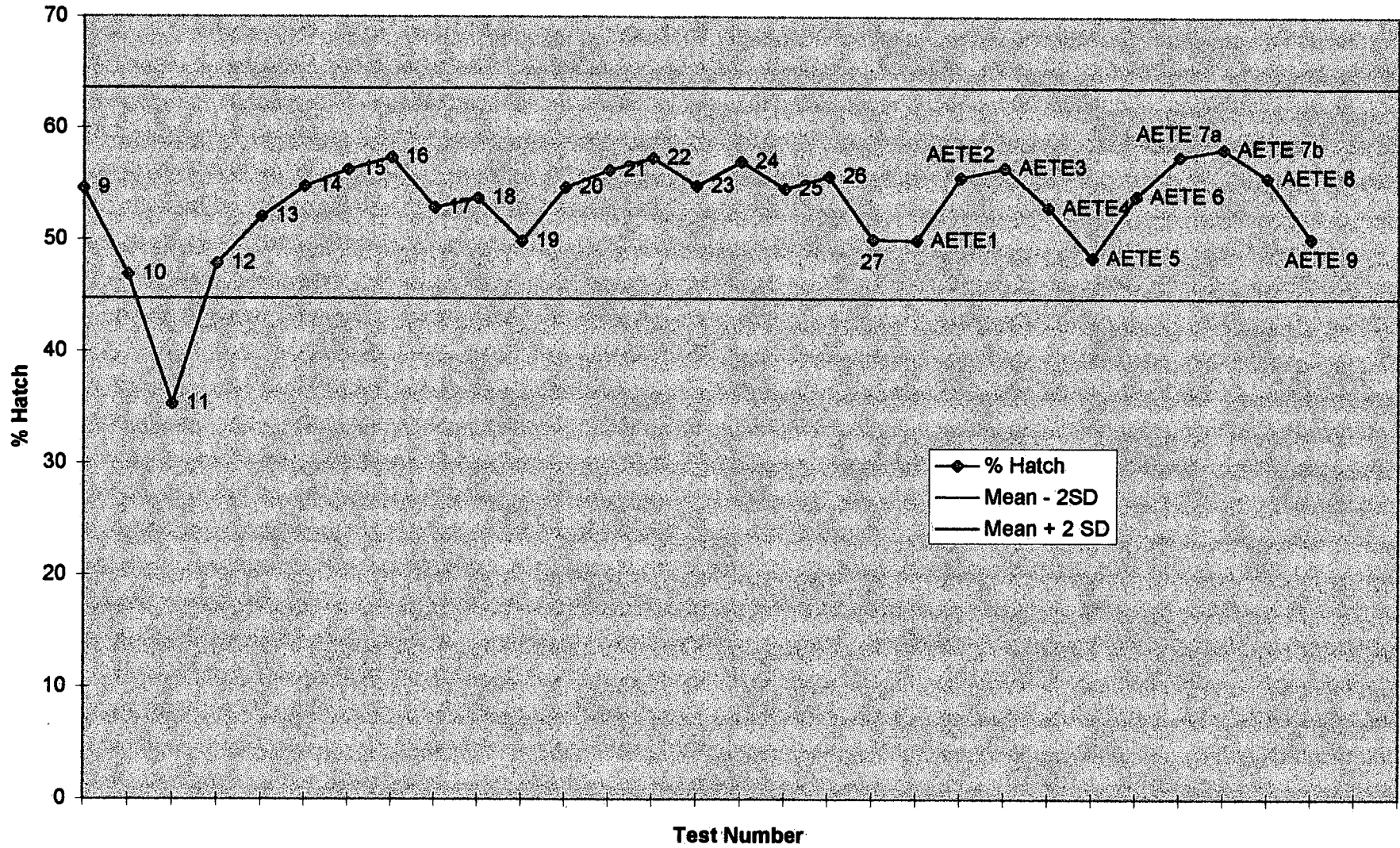
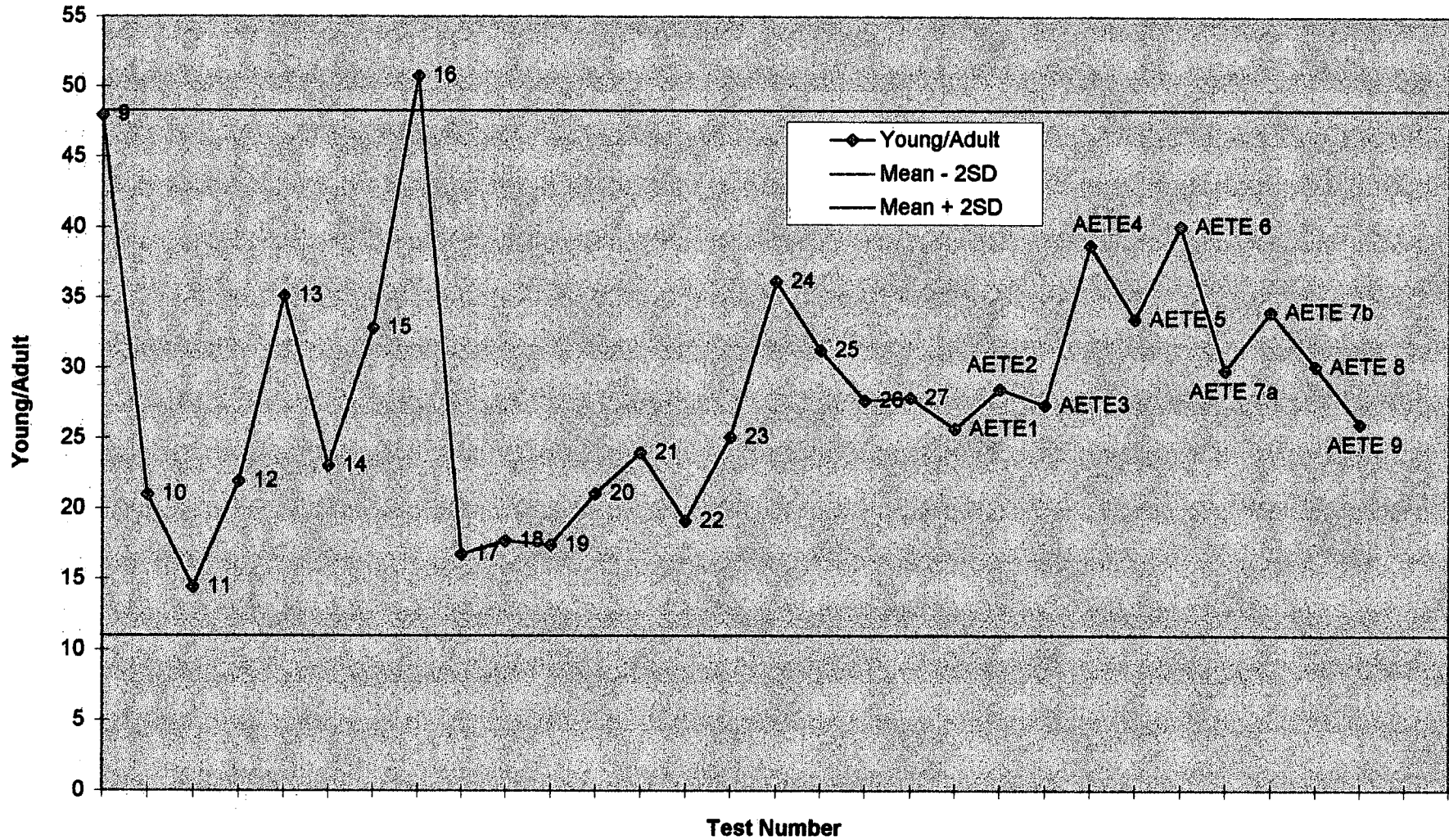


Figure 3. Young/Adult Warning Chart for Long Point Control Sediment
T. tubifex 28-day Reproduction Test



APPENDIX A

Data from 28-day Reproduction test with *Tubifex tubifex* for:

**MYRA FALLS
DOME
MATTABI**

TABLE A1.1 RESULTS OF AETE TEST NO. 1 - MYRA FALLS MINE

AETE 1	Site	Rep	Adult	Mean	SD	CV	Total Cocs	Coc/ad.	Mean	SD	CV	Empty Cocs	% Hatch	Mean	SD	CV	Total Off	Young/ad.	Mean	SD	CV
MF1-S		1	100	100	0	0	37	9.25	9.40	0.58	6.12	28	70.27	65.61	7.40	11.27	56	14.00	17.05	5.19	30.45
		2	100				39	9.75				27	69.23				92	23.00			
		3	100				40	10.00				21	52.50				76	19.00			
		4	100				38	9.50				26	68.42				39	9.75			
		5	100				34	8.50				23	67.65				78	19.50			
MF2-S		1	100	100	0	0	32	8.00	8.98	0.84	9.37	16	50.00	56.25	4.53	8.06	57	14.25	16.46	3.42	20.75
		2	100				34	8.50				20	58.82				55	13.75			
		3	100				36	9.00				21	58.33				62	15.50			
		4	100				32	9.14				17	53.13				58	16.57			
		5	100				41	10.25				25	60.98				89	22.25			
MF3-S		1	100	100	0	0	36	9.00	9.38	0.78	8.29	22	61.11	60.46	5.17	8.55	71	17.75	21.38	3.03	14.18
		2	100				37	9.25				20	54.05				80	20.00			
		3	100				42	10.50				28	66.67				96	24.00			
		4	100																		
		5	100				35	8.75				21	60.00				95	23.75			
MF4-S		1	100	95	11.2	11.8	35	8.75	8.49	1.39	16.39	24	68.57	64.16	5.56	8.67	106	26.50	23.14	4.07	17.61
		2	100				34	8.50				21	61.76				101	25.25			
		3	75				35	10.00				25	71.43				89	25.43			
		4	100				36	9.00				21	58.33				66	16.50			
		5	100				28	8.22				17	60.71				99	22.00			
MF5-S		1	100	100	0	0	42	10.50	8.65	1.36	15.78	27	64.29	67.74	4.73	6.99	70	17.50	17.05	3.85	22.56
		2	100				34	8.50				21	61.76				73	18.25			
		3	100				29	7.25				20	68.97				43	10.75			
		4	100				30	7.50				21	70.00				85	21.25			
		5	100				38	9.50				28	73.68				70	17.50			
MF6-S		1	100	100	0	0	29	7.25	8.95	1.05	11.75	16	55.17	61.29	3.90	6.36	79	19.75	19.90	2.01	10.11
		2	100				35	10.00				23	65.71				77	22.00			
		3	100				38	9.50				23	60.53				79	19.75			
		4	100				35	8.75				22	62.86				67	16.75			
		5	100				37	9.25				23	62.16				85	21.25			
MF7-S		1	100	100	0	0	26	6.50	8.35	1.26	15.06	14	53.85	57.08	4.35	7.62	63	15.75	17.20	2.58	15.01
		2	100				37	9.25				21	56.76				76	19.00			
		3	100				39	9.75				25	64.10				64	16.00			
		4	100				32	8.00				17	53.13				58	14.50			
		5	100				33	8.25				19	57.58				83	20.75			
Long Point		1	100	100	0	0	42	10.50	10.30	0.41	3.99	21	50.00	49.99	3.80	7.59	86	22.00	25.80	6.16	23.86
		2	100				42	10.50				20	47.62				141	35.25			
		3	100				40	10.00				18	45.00				77	19.25			
		4	100				43	10.75				23	53.49				111	27.75			
		5	100				39	9.75				21	53.85				99	24.75			

TABLE A1.2 RESULTS OF AETE TEST NO. 2 - MYRA FALLS MINE

AETE 2	Site	Rep	% Survival	Mean	SD	CV	Total Cocs	Coc/ad.	Mean	SD	CV	Empty Cocs	% Hatch	Mean	SD	CV	Total Off	Young/ad.	Mean	SD	CV
AETE 2	MR1-S	1	100	100	0	0	39	9.75	9.35	0.76	8.15	19	48.72	53.51	4.62	8.63	94	23.50	22.95	2.20	9.80
		2	100				35	8.75				17	48.57				79	19.75			
		3	100				35	8.75				20	57.14				99	24.75			
		4	100				36	9.00				21	58.33				100	25.00			
		5	100				42	10.50				23	54.76				87	21.75			
	MR2-S	1	100	100	0	0	40	10.00	9.52	0.89	9.32	26	65.00	59.84	4.05	6.76	99	24.75	25.25	1.79	7.11
		2	100				36	9.00				20	55.56				101	25.25			
		3	100				35	8.75				20	57.14				101	25.25			
		4	100				38	10.86				24	63.16				98	28.00			
		5	100				36	9.00				21	58.33				92	23.00			
	MR3-S	1	100	100	0	0	37	9.25	9.45	0.89	9.43	22	59.46	58.16	1.18	2.03	84	21.00	23.15	2.14	9.25
		2	100				35	8.75				20	57.14				83	20.75			
		3	100				44	11.00				26	59.09				97	24.25			
		4	100				37	9.25				21	56.76				102	25.50			
		5	100				36	9.00				21	58.33				97	24.25			
	MR4-S	1	100	100	0	0	34	8.50	9.23	1.10	11.91	20	58.62	59.62	4.62	7.74	101	25.25	28.21	4.91	17.41
		2	100				39	11.14				25	64.10				128	36.57			
		3	100				34	8.50				18	52.94				101	25.25			
		4	100				36	9.00				23	63.89				115	28.75			
		5	100				36	9.00				21	58.33				101	25.25			
	MR5-S	1	100	100	0	0	35	8.75	9.15	0.38	4.14	21	60.00	60.19	4.15	6.90	105	26.25	25.44	1.68	6.59
		2	100				39	9.75				22	56.41				92	23.00			
		3	100				36	9.00				22	61.11				107	26.75			
		4	100				36	9.00				24	68.67								
		5	100				37	9.25				21	56.76				103	25.75			
	MR6-S	1	100	100	0	0	41	10.25	9.95	1.47	14.80	25	60.98	60.78	3.87	6.36	111	27.75	29.78	6.47	21.73
		2	100				42	12.00				24	57.14				137	39.14			
		3	100				37	9.25				21	56.76				88	22.00			
		4	100				41	10.25				26	63.41				109	27.25			
		5	100				32	8.00				21	65.63				131	32.75			
	MR7-S	1	100	95	11.2	11.8			9.20	0.73	7.98			58.92	2.88	4.88			21.11	3.80	18.48
		2	100				40	10.00				24	60.00				88	22.00			
		3	100				38	9.00				21	58.33				91	22.75			
		4	100				38	9.50				21	55.26				97	24.25			
		5	75				29	8.29				18	62.07				54	15.43			
	Long Point	1	100	100	0	0	44	11.00	11.35	1.13	9.92	21	47.73	55.85	6.78	12.18	102	25.50	28.53	5.63	19.74
		2	100				53	13.25				32	60.38				116	33.14			
		3	100				44	11.00				27	61.36				112	28.00			
		4	100				41	10.25				20	48.78				109	27.25			
		5	100				45	11.25				27	60.00				115	28.75			

TABLE A1.3 RESULTS OF AETE TEST NO. 3 - MYRA FALLS MINE

AETE 3	Site	Rep	% Survival	Mean	SD	CV	Total Cocs	Coc/ad.	Mean	SD	CV	Empty Cocs	% Hatch	Mean	SD	CV	Total Off	Young/ad.	Mean	SD	CV
AETE 3	MN4-S	1	100	100	0	0	35	8.75	8.60	0.85	7.58	28	74.29	68.71	5.01	7.51	84	21.00	18.35	5.52	30.09
		2	100				35	8.75				24	68.57				98	24.50			
		3	100				31	7.75				19	61.29				68	17.00			
		4	100				38	9.50				25	65.79				78	19.50			
		5	100				33	8.25				21	63.64				39	9.75			
MN5-S	MN5-S	1	100	100	0	0	30	7.50	8.00	0.50	6.25	22	73.33	71.38	5.62	7.87	97	24.25	22.35	1.83	8.61
		2	100				32	8.00				23	71.88				88	22.00			
		3	100				34	8.50				21	61.76				86	21.50			
		4	100				34	8.50				28	76.47				79	19.75			
		5	100				30	7.50				22	73.33				97	24.25			
MN6-S	MN6-S	1	100	100	0	0	37	9.25	8.60	0.96	11.18	25	67.57	65.62	4.65	7.08	88	22.00	21.10	0.95	4.48
		2	100				31	7.75				19	61.29				81	20.25			
		3	100				39	9.75				27	69.23				89	22.25			
		4	100				35	8.75				21	60.00				82	20.50			
		5	100				30	7.50				21	70.00				82	20.50			
MN7-S	MN7-S	1	100	100	0	0	34	8.50	8.25	1.13	13.72	17	50.00	48.32	4.10	8.85	53	13.25	10.50	3.21	30.54
		2	100				25	8.25				10	40.00				20	5.00			
		3	100				35	8.75				17	48.57				43	10.75			
		4	100				35	8.75				17	48.57				48	12.00			
		5	100				36	9.00				18	44.44				48	11.50			
MN8-S	MN8-S	1	100	100	0	0	28	7.00	8.30	0.86	10.30	17	60.71	52.87	5.73	10.83	67	18.75	17.65	1.61	9.10
		2	100				37	9.25				17	45.95				67	18.75			
		3	100				32	8.00				18	56.25				77	19.25			
		4	100				34	8.50				17	50.00				64	16.00			
		5	100				35	8.75				18	51.43				78	19.50			
MN9-S	MN9-S	1	75	80	13.7	15.2	29	8.29	8.75	0.70	7.95	19	65.52	64.87	5.44	8.39	73	20.86	23.14	4.92	21.27
		2	100				35	7.78				21	60.00				109	24.22			
		3	75				33	9.43				24	72.73				101	28.86			
		4	100				37	9.25				22	59.46				103	25.75			
		5	100				36	9.00				24	66.67				84	18.00			
MN10-S	MN10-S	1	100	100	0	0	32	8.00	8.41	0.57	6.75	17	53.13	59.18	3.64	6.15	78	19.50	19.67	3.94	20.04
		2	100				37	9.25				23	62.16				95	23.75			
		3	100				35	7.78				21	60.00				68	15.11			
		4	100				34	8.50				21	61.76				84	23.50			
		5	100				34	8.50				20	58.82				68	16.50			
Long Point	Long Point	1	100	100	0	0	43	10.75	10.70	0.37	3.47	24	55.81	56.51	4.97	8.79	106	26.50	27.40	3.91	14.28
		2	100				43	10.75				22	51.16				109	27.25			
		3	100				45	11.25				28	62.22				112	28.00			
		4	100				41	10.25				25	60.98				108	28.50			
		5	100				42	10.50				22	52.38				115	28.75			

TABLE A1.4 RESULTS OF AETE TEST NO. 4 - DOME MINE

AETE 4	Site	Rep	% Survival	Mean	SD	CV	Total Cocs	Coc/ad.	Mean	SD	CV	Empty Cocs	% Hatch	Mean	SD	CV	Total Off	Young/ad.	Mean	SD	CV	
AETE 4	D1B-1	1	100	100	0	0	45	11.25	11.15	0.45	4.07	24	53.33	52.31	5.69	10.87	107	26.75	32.88	5.02	15.27	
		2	100				47	11.75				26	55.32									
		3	100				42	10.50				18	42.86				152	38.00				
		4	100				45	11.25				26	57.78				143	35.75				
		5	100				44	11.00				23	52.27				124	31.00				
D1B-2	D1B-2	1	100	100	0	0	46	11.50	10.75	0.53	4.93	22	47.83	53.62	5.49	10.25	124	31.00	32.50	5.16	15.88	
		2	100				43	10.75				21	48.84				149	37.25				
		3	100				41	10.25				22	53.66				101	25.25				
		4	100				44	11.00				25	58.82				125	31.25				
		5	100				41	10.25				25	60.98				151	37.75				
D1B-3	D1B-3	1	100	100	0	0	44	11.00	10.90	0.68	6.20	21	47.73	48.46	3.87	7.99	127	31.75	34.25	5.34	15.59	
		2	100				43	10.75				20	46.51				127	31.75				
		3	100				48	12.00				26	54.17				119	29.75				
		4	100				41	10.25				18	43.90				173	43.25				
		5	100				42	10.50				21	50.00				139	34.75				
D2-1	D2-1	1	100	100	0	0	41	10.25	10.70	0.60	5.58	21	51.22	53.20	3.19	6.00	163	40.75	37.50	3.74	9.98	
		2	100				44	11.00				23	52.27				135	33.75				
		3	100				46	11.50				25	54.35				137	34.25				
		4	100				40	10.00				20	50.00				147	36.75				
		5	100				43	10.75				25	58.14				168	42.00				
D2-2	D2-2	1	100	100	0	0	48	12.00	11.12	0.84	7.51	24	50.00	48.50	2.55	5.26	100	25.00	25.89	2.38	9.13	
		2	100				49	10.89				25	51.02				125	27.78				
		3	100				46	10.22				21	45.65				129	28.67				
		4	100				48	12.00				22	45.83				91	22.75				
		5	100				42	10.50				21	50.00				101	25.25				
D2-3	D2-3	1	100	100	0	0	50	11.11	10.85	0.83	7.61	25	50.00	49.34	7.80	15.82	126	28.00	30.98	2.68	8.67	
		2	100				49	10.89				21	42.86				130	28.89				
		3	100				48	12.00				29	60.42				122	30.50				
		4	100				39	9.75				16	41.03				135	33.75				
		5	100				42	10.50				22	52.38				135	33.75				
D2-4	D2-4	1	100	100	0	0	38	9.50	10.45	0.76	7.26	18	47.37	53.43	5.32	9.96	119	29.75	32.05	2.46	7.67	
		2	100				42	10.50				24	57.14				134	33.50				
		3	100				46	11.50				24	52.17				129	32.25				
		4	100				43	10.75				26	60.47				118	29.50				
		5	100				40	10.00				20	50.00				141	35.25				
Long Point	Long Point	1	100	100	0	0	41	10.25	10.90	0.98	8.97	21	51.22	52.93	6.47	12.22	123	30.75	38.75	7.38	16.99	
		2	100				46	11.50				23	50.00				141	35.25				
		3	100				38	9.50				22	57.89				145	36.25				
		4	100				46	11.50				26	60.87				200	50.00				
		5	100				47	11.75				21	44.68				168	41.50				

TABLE A1.5 RESULTS OF AETE TEST NO. 5 - DOME MINE

AETE 5	Site	Rep	% Survival	Mean	SD	CV	Total Cocs	Coc/ad.	Mean	SD	CV	Empty Cocs	% Hatch	Mean	SD	CV	Total Off	Young/ad.	Mean	SD	CV
AETE 5	D3-1	1	100	100	0	0	44	11.00	11.25	0.35	3.14	21	47.73	50.65	3.91	7.72	148	36.50	29.25	5.17	17.68
		2	100				48	11.50				25	54.35				104	26.00			
		3	100				48	11.50				21	45.65				129	32.25			
		4	100				43	10.75				22	51.16				112	28.00			
		5	100				48	11.50				25	54.35				94	23.50			
	D3-2	1	100	100	0	0	43	10.75	10.90	0.55	5.02	21	48.84	52.67	3.29	6.25	122	30.50	29.65	3.79	12.78
		2	100				43	10.75				22	51.16				116	29.00			
		3	100				44	11.00				25	56.82				133	33.25			
		4	100				47	11.75				26	55.32				128	32.00			
		5	100				41	10.25				21	51.22				94	23.50			
	D3-3	1	100	100	0	0	41	10.25	10.70	0.33	3.05	23	56.10	57.95	2.56	4.42	68	17.00	25.35	7.35	28.00
		2	100				43	10.75				24	55.81				137	34.25			
		3	100				44	11.00				25	56.82				112	28.00			
		4	100				44	11.00				26	59.09				116	29.00			
		5	100				42	10.50				26	61.90				74	18.50			
	D3-4	1	100	100	0	0	44	11.00	11.00	0.92	8.35	24	54.55	57.15	3.87	6.78	164	41.00	37.55	5.06	13.48
		2	100				48	11.50				26	56.52				171	42.75			
		3	100				41	10.25				25	60.98				122	30.50			
		4	100				40	10.00				21	52.50				137	34.25			
		5	100				49	12.25				30	61.22				157	39.25			
	D3-5	1	100	100	0	0	42	10.50	10.50	1.84	17.50	24	57.14	52.89	8.06	15.24	73	18.25	16.45	1.19	7.24
		2	100				40	10.00				19	47.50				66	16.50			
		3	100				40	10.00				18	45.00				62	15.50			
		4	100				54	13.50				35	64.81				67	16.75			
		5	100				34	8.50				17	50.00				61	15.25			
D3-6	1	100	100	0	0	43	10.75	10.05	0.97	9.70	22	51.16	53.84	2.01	3.73	95	23.75	26.20	3.61	13.76	
	2	100				43	10.75				23	53.49				94	23.50				
	3	100				43	10.75				23	53.49				94	23.50				
	4	100				35	8.75				19	54.29				118	29.50				
	5	100				37	9.25				21	56.76				123	30.75				
D3-7	1	100	100	0	0	51	12.75	11.25	1.41	12.57	27	52.94	51.13	7.05	13.78	106	26.50	27.95	4.52	16.17	
	2	100				44	11.00				17	38.64				109	27.25				
	3	100				48	12.00				26	54.17				131	32.75				
	4	100				48	11.50				25	54.35				127	31.75				
	5	100				38	9.00				20	55.56				86	21.50				
Long Point	1	100	100	0	0	42	10.50	10.95	0.54	4.95	19	45.24	48.45	4.87	10.06	121	30.25	33.50	3.60	10.74	
	2	100				47	11.75				20	42.55				133	33.25				
	3	100				42	10.50				20	47.62				125	31.25				
	4	100				45	11.25				24	53.33				133	33.25				
	5	100				43	10.75				23	53.49				158	39.50				

TABLE A1.8 RESULTS OF AETE TEST NO. 6 - DOME MINE

AETE 6	Site	Rep	% Survival	Mean	SD	CV	Total Cocs	Coc/ad.	Mean	SD	CV	Empty Cocs	% Hatch	Mean	SD	CV	Total Off	Young/ad.	Mean	SD	CV
AETE 6	D4-1	1	100	100	0	0	48	11.50	11.15	0.58	5.18	25	54.35	54.38	4.79	8.82	159	38.75	38.45	3.71	9.88
		2	100				45	11.25				27	60.00				141	35.25			
		3	100				44	11.00				24	54.55				150	37.50			
		4	100				41	10.25				23	56.10				142	35.50			
		5	100				47	11.75				22	48.81				177	44.25			
	D4-2	1	100	100	0	0	41	10.25	10.44	0.94	9.04	22	53.66	49.27	5.80	11.77	124	31.00	28.81	6.57	22.80
		2	100				43	10.75				23	53.49				100	25.00			
		3	100				37	9.25				18	48.65				148	37.00			
		4	100				46	11.50				19	41.30				89	22.25			
	D4-3	1	100	100	0	0	43	10.75	11.83	1.08	9.25	22	51.18	48.28	7.20	14.92	108	27.00	38.00	9.27	25.74
		2	100				48	11.50				23	50.00				182	45.50			
		3	100				47	11.75				19	40.43				108	27.00			
		4	100				43	10.75				18	41.88				182	45.50			
		5	100				107	13.38				62	57.94				280	35.00			
	D4-4	1	100	100	0	0	81	10.13	11.48	0.91	7.95	38	48.91	58.87	7.82	13.80	268	33.25	32.70	1.90	5.81
		2	100				47	11.75				24	51.08				128	32.00			
		3	100				44	11.00				25	56.82				128	31.50			
		4	100				49	12.25				31	63.27				124	31.00			
		5	100				49	12.25				32	65.31				143	35.75			
	D4-5	1	100	100	0	0			11.00	0.74	6.69			49.85	11.01	22.08			30.19	5.34	17.68
2		100				47	11.75				18	34.04				104	28.00				
3		100				41	10.25				22	53.66				115	28.75				
4		100				46	11.50				24	52.17				152	38.00				
5		100				84	10.50				50	59.52				224	28.00				
D4-6	1	100	100	0	0			9.88	0.38	3.62			59.80	2.83	4.91			23.48	1.65	7.04	
	2	100				83	10.38				51	61.45				185	23.13				
	3	100				39	9.75				22	56.41				88	22.00				
	4																				
	5	100				39	9.75				24	61.54				101	25.25				
D4-7	1	100	100	0	0	43	10.75	11.25	0.48	4.08	21	48.84	51.68	2.37	4.60	122	30.50	31.81	2.07	6.49	
	2	100				44	11.00				24	54.55				119	29.75				
	3	100				48	11.50				24	52.17				131	32.75				
	4	100				47	11.75				24	51.08				137	34.25				
	5																				
Long Point	1	100	100	0	0	39	9.75	11.50	1.55	13.49	22	56.41	53.98	3.66	6.79	108	27.00	40.10	10.54	26.28	
	2	100				48	11.50				25	54.35				132	33.00				
	3	100				50	12.50				24	48.00				203	50.75				
	4	100				41	10.25				22	53.66				157	39.25				
	5	100				54	13.50				31	57.41				202	50.50				

TABLE A1.7a RESULTS OF AETE TEST NO. 7a - MATTABI MINE

AETE 7a	Site	Rep	% Survival	Mean	SD	CV	Total Cocs	Coc/ad.	Mean	SD	CV	Empty Cocs	% Hatch	Mean	SD	CV	Total Off	Young/ad.	Mean	SD	CV
MMSR1-1		1	100	100	0	0	40	10.00	9.05	1.35	14.93	22	55.00	49.99	4.78	9.55	127	31.75	25.20	5.33	21.15
		2	100				40	10.00				22	55.00				107	26.75			
		3	100				27	6.75				13	48.15				68	17.00			
		4	100				38	9.50				18	47.37				98	24.50			
		5	100				36	9.00				16	44.44				104	26.00			
MMSR1-2		1	100	100	0	0	46	11.50	9.00	1.55	17.24	23	50.00	49.42	6.70	13.55	116	29.00	21.75	5.32	24.45
		2	100				36	9.00				21	58.33				67	16.75			
		3	100				35	8.75				14	40.00				65	16.25			
		4	100				29	7.25				15	51.72				94	23.50			
		5	100				34	8.50				16	47.06				93	23.25			
MMSR1-3		1	100	100	0	0	41	10.25	9.25	0.92	9.93	21	51.22	48.14	5.44	11.80	97	24.25	23.85	0.78	3.22
		2	100				31	7.75				12	38.71				90	22.50			
		3	100				38	8.50				19	50.00				93	23.25			
		4	100				38	9.50				16	42.11				97	24.25			
		5	100				37	9.25				18	48.65				96	24.00			
MMSR2-1		1	100	95	11.2	11.8	34	8.50	8.16	1.11	13.82	12	35.29	51.20	9.56	18.87	74	18.50	20.44	5.22	25.52
		2	100				37	9.25				20	54.05				94	23.50			
		3	100				34	8.50				17	50.00				55	13.75			
		4	75				22	6.29				13	59.09				96	27.43			
		5	100				33	8.25				19	57.58				76	19.00			
MMSR2-2		1	100	90	13.7	15.2	15	3.75	5.39	0.94	17.53	13	86.87	63.75	14.42	22.62	60	15.00	13.42	4.31	32.14
		2	75				21	6.00				12	57.14				47	13.43			
		3	100				24	6.00				12	50.00				31	7.75			
		4	75				19	5.43				13	68.42				68	19.43			
		5	100				23	5.75				13	58.52				48	11.50			
MMSR2-3		1	75	90	13.7	15.2	23	6.57	6.97	1.27	18.23	12	52.17	50.47	3.81	7.54	34	9.71	13.85	4.33	31.23
		2	100				31	7.75				14	45.16				57	14.25			
		3	100				29	7.25				14	48.28				40	10.00			
		4	100				20	5.00				11	55.00				60	15.00			
		5	75				29	8.29				15	51.72				71	20.29			
Long Point		1	100	100	0	0	39	9.75	10.80	0.97	9.02	25	64.10	57.49	4.74	8.24	141	35.25	29.85	4.40	14.75
		2	100				46	11.50				24	52.17				96	24.50			
		3	100				45	11.25				26	57.78				106	26.50			
		4	100				39	9.75				21	53.85				121	30.25			
		5	100				47	11.75				28	59.57				131	32.75			

TABLE A1.7b RESULTS OF AETE TEST NO. 7b - MATTABI MINE

Site	Rep	% Survival	Mean	SD	CV	Total Cocs	Coc/ad.	Mean	SD	CV	Empty Cocs	% Hatch	Mean	SD	CV	Total Off	Young/ad.	Mean	SD	CV	
AETE 7b	MMS1-1	1	100	100	0	0	41	10.25	11.35	1.07	9.42	20	48.78	51.00	4.77	9.35	87	21.75	18.95	5.38	28.41
		2	100				41	10.25				21	51.22				98	24.00			
		3	100				50	12.50				24	48.00				91	22.75			
		4	100				48	11.50				22	47.83				54	13.50			
		5	100				49	12.25				29	59.18				51	12.75			
MMS1-2	1	100	100	0	0	48	10.22	10.94	0.82	7.53	20	43.48	44.77	7.12	15.90	24	5.33	12.22	6.83	55.88	
	2	100				40	10.00				15	37.50				47	11.75				
	3	100				47	11.75				25	53.19				58	14.00				
	4	100				44	11.00				17	38.64				29	7.25				
	5	100				47	11.75				24	51.06				91	22.75				
MMS1-3	1	100	100	0	0	44	11.00	11.20	0.33	2.91	19	43.18	47.31	2.37	5.00	49	12.25	13.80	1.63	11.84	
	2	100				48	11.50				22	47.83				48	12.00				
	3	100				45	11.25				22	48.89				58	14.00				
	4	100				43	10.75				21	48.84				61	15.25				
	5	100				48	11.50				22	47.83				62	15.50				
Long Point	1	100	100	0	0	45	11.25	11.00	0.73	6.63	28	57.78	58.20	4.00	6.87	133	33.25	34.03	1.43	4.20	
	2	100				43	10.75				24	55.81				131	32.75				
	3	100				45	10.00				24	53.33				158	34.67				
	4	100				44	11.00				28	63.64				133	33.25				
	5	100				48	12.00				29	60.42				145	36.25				

TABLE A1.8 RESULTS OF AETE TEST NO. 8 - MATTABI MINE

AETE 8	Site	Rep	% Survival	Mean	SD	CV	Total Cocs	Coc/ad.	Mean	SD	CV	Empty Cocs	% Hatch	Mean	SD	CV	Total Off	Young/ad.	Mean	SD	CV
AETE 8	MMS3-1	1	75	95	11.2	11.8	33	9.43	9.94	1.27	12.82	21	63.64	55.01	6.76	12.29	128	36.57	31.66	4.18	13.20
		2	100				37	9.25				22	59.46				131	32.75			
		3	100				48	12.00				28	54.17				116	29.00			
		4	100				41	10.25				19	46.34				104	26.00			
		5	100				35	8.75				18	51.43				136	34.00			
	MMS3-2	1	100	95	11.2	11.8	38	9.50	9.14	1.39	15.18	17	44.74	51.97	4.98	9.58	117	29.25	28.14	5.98	21.27
		2	100				32	8.00				17	53.13				77	19.25			
		3	100				43	10.75				25	58.14				131	32.75			
		4	100				40	10.00				20	50.00				136	34.00			
		5	75				26	7.43				14	53.85				89	25.43			
	MMS3-3	1	100	100	0	0	48	12.00	11.00	0.98	8.95	28	54.17	51.31	2.03	3.95	154	38.50	33.25	3.82	11.48
		2	100				47	11.75				23	48.94				114	28.50			
		3	100				44	11.00				23	52.27				124	31.00			
		4	100				43	10.75				22	51.16				133	33.25			
		5	100				38	9.50				19	50.00				140	35.00			
MMS4-1	1	100	100	0	0	39	9.75	9.90	0.42	4.23	17	43.59	41.67	3.21	7.87	90	22.50	24.75	3.60	14.55	
	2	100				37	9.25				14	37.84				94	23.50				
	3	100				40	10.00				18	45.00				111	27.75				
	4	100				41	10.25				16	39.02				83	20.75				
	5	100				41	10.25				18	43.90				117	29.25				
MMS4-2	1	75	93.75	12.5	13.3	27	7.71	8.30	0.53	6.37	12	44.44	48.54	3.48	7.16	86	24.57	23.21	2.83	12.19	
	2	100				32	8.00				15	46.88				79	19.75				
	3	100				35	8.75				18	51.43				105	26.25				
	4	100				35	8.75				18	51.43				89	22.25				
	5																				
MMS4-3	1	100	100	0	0	38	9.50	8.65	0.86	9.93	21	55.26	53.28	4.56	8.56	147	36.75	32.60	3.44	10.55	
	2	100				34	8.50				17	50.00				128	32.00				
	3	100				33	8.25				16	48.48				110	27.50				
	4	100				38	9.50				20	52.63				129	32.25				
	5	100				30	7.50				18	60.00				138	34.50				
Long Point	1	100	100	0	0	42	10.50	11.05	0.51	4.64	22	52.38	55.61	2.66	4.76	134	33.50	30.15	4.08	13.47	
	2	100				45	11.25				24	53.33				132	33.00				
	3	100				48	11.50				27	58.70				106	28.50				
	4	100				42	10.50				24	57.14				100	25.00				
	5	100				46	11.50				26	56.52				131	32.75				

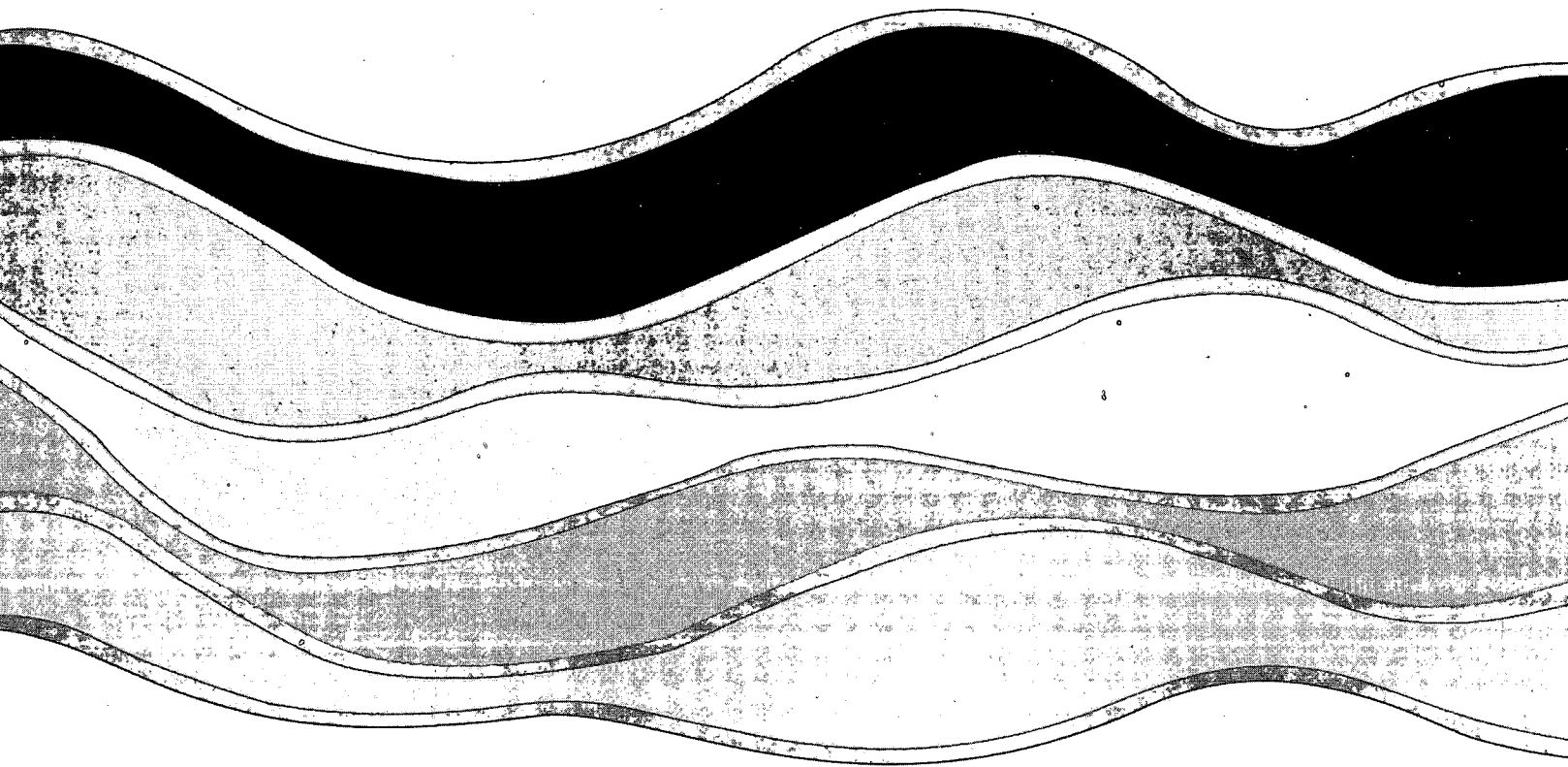
TABLE A1.9 RESULTS OF AETE TEST NO. 9 - MATTABI MINE

AETE 9	Site	Rep	% Survival	Mean	SD	CV	Total Cocs	Coc/ad.	Mean	SD	CV	Empty Cocs	% Hatch	Mean	SD	CV	Total Off	Young/ad.	Mean	SD	CV
MMS2-1		1	100	100	0	0	32	8.00	9.50	1.16	12.20	15	46.88	48.81	4.50	9.22	93	23.25	19.25	4.38	22.74
		2	100				44	11.00				23	52.27				73	18.25			
		3	100				40	10.00				17	42.50				50	12.50			
		4	100				39	9.75				21	53.85				77	19.25			
		5	100				35	8.75				17	48.57				92	23.00			
MMS2-2		1	100	100	0	0	40	10.00	9.25	0.64	6.89	20	50.00	49.15	4.58	9.32	72	18.00	24.25	5.10	21.04
		2	100				35	8.75				16	45.71				94	23.50			
		3	100				39	9.75				21	53.85				129	32.25			
		4	100				34	8.50				18	52.94				95	23.75			
		5	100				37	9.25				16	43.24				95	23.75			
MMS2-3		1	100	100	0	0	39	9.75	9.13	0.52	5.70	18	48.15	48.54	1.91	4.10	58	14.00	16.88	2.57	15.23
		2	100				36	9.00				17	47.22				67	16.75			
		3	100				34	8.50				15	44.12				81	20.25			
		4	100				37	9.25				18	48.65				68	16.50			
		5																			
MMS5-1		1	100	100	0	0	32	8.00	9.20	1.34	14.56	16	50.00	50.06	6.50	12.97	108	26.50	25.30	3.63	15.15
		2	100				42	10.50				17	40.48				94	23.50			
		3	100				35	8.75				17	48.57				114	28.50			
		4	100				43	10.75				25	58.14				114	28.50			
		5	100				32	8.00				17	53.13				78	19.50			
MMS5-2		1	100	100	0	0	32	8.00	8.70	1.04	11.92	13	40.63	53.55	8.78	16.40	120	30.00	25.75	2.93	11.38
		2	100				31	7.75				20	64.52				92	23.00			
		3	100				33	8.25				19	57.58				92	23.00			
		4	100				41	10.25				22	53.68				107	26.75			
		5	100				37	9.25				19	51.35				104	26.00			
MMS5-3		1	100	100	0	0	31	7.75	8.20	0.91	11.08	19	61.29	57.70	5.98	10.34	93	23.25	19.70	3.55	18.00
		2	100				33	8.25				21	63.64				71	17.75			
		3	100				31	7.75				17	54.84				91	22.75			
		4	100				30	7.50				18	60.00				80	20.00			
		5	100				39	9.75				19	48.72				59	14.75			
Long Point		1	100	100	0	0	39	9.75	9.60	0.89	9.09	20	51.26	50.13	5.45	10.87	115	28.75	26.05	4.36	16.72
		2	100				38	9.50				19	50.00				99	24.75			
		3	100				43	10.75				24	55.81				108	27.00			
		4	100				34	8.50				14	41.18				77	19.25			
		5	100				42	10.50				22	52.38				122	30.50			

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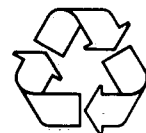


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