EAG LABORATORIES | WE KNOW HOW™

LIFE SCIENCES

HOW DO YOU EVALUATE CULTURE METHODS
TO ENSURE THE HEALTH OF THE TEST ORGANISMS?

APPLICATION NOTE

An Evaluation Of Survival, Growth, Emergence, And Reproduction Of The Midge (*Chironomus Dilutus*) Under Various Culture Conditions

Elizabeth Ostermann, Sediment Team Leader, EAG Laboratories, Easton, Md. USA

INTRODUCTION

Chronic sediment toxicity testing has become a key element of the regulatory process for evaluating the risks of certain contaminants. Current USEPA sediment test guidelines recognize the need for individual laboratories to evaluate culture methods to ensure the health of the test organisms (EPA, 2000). Culture trials with the midge Chironomus dilutus (C. dilutus) were conducted at EAG Laboratories in Easton, MD in 2016 and 2017 to evaluate four key endpoints: survival, growth, emergence and reproduction after exposure to various culture conditions. The four endpoints were chosen based on test acceptability requirements from EPA guidelines for chronic testing of C. dilutus (EPA, 2000). Five different combinations of diet and substrate were compared (Table 1) with two replicate aquaria for each set of conditions. Previous research confirmed that sand is the preferred substrate based on ease of removing larvae for sediment testing (Greer, 1993). The performance endpoints were compared between each set of culture conditions to determine the appropriate conditions to improve the health of the C. dilutus used for testing.

METHODS

The culture trial was initiated using egg masses from the culture at EAG, the egg masses were halved and then held in a glass beaker containing culture water only. The glass beakers containing the egg masses were observed daily until ~490% of hatching had occurred. Next, larvae of approximately the same age (within 24 hours of each other) were indiscriminately distributed among the 10 aquaria. The culture aquaria were set-up in accordance with the five culture conditions (Table 1), with two aquaria for each set of culture conditions and initiated with 200 organisms each.. Throughout the trial, the aquaria were fed once daily, 2.5mL of the appropriate diet.

RESULTS

The results from the culture trial conducted in 2016 demonstrated that the current feeding and substrate regimen of invertebrate slurry and no substrate (Test Group 1) only met two of the four validity criteria. While none of the test groups experienced survival >80%, test group 4 yielded the highest percent hatch (97%), and met 3 of the 4 the validity criteria (growth, emergence, and reproduction). In agreement with EPA culturing procedures, the culture trial has established that sand is the preferred substrate and invertebrate slurry is the preferred food choice for the culturing of C. dilutus. Therefore, EAG Laboratories, Easton has adopted Test Group 4 as the optimal culture conditions for this particular organism. A second culture trial was completed in 2017 with two replicate aquaria using the newly adopted culture conditions (Test Group 4) from the 2016 trial. The results from

Culture Conditions	Aquaria A	Aquaria B
Test Group 1: Diet-Invertebrate Slurry Substrate-None	Terminate on Day 20 post hatch for survival and growth measurements	Terminate after 7 consecutive days of no emergence to measure emergence and reproduction
Test Group 2: Diet-Invertebrate Slurry Substrate-West Bearskin Sediment	Terminate on Day 20 post hatch for survival and growth measurements	Terminate after 7 consecutive days of no emergence to measure emergence and reproduction
Test Group 3: Diet-Tetramin Suspension (56 g/L) Substrate-West Bearskin Sediment	Terminate on Day 20 post hatch for survival and growth measurements	Terminate after 7 consecutive days of no emergence to measure emergence and reproduction
Test Group 4: Diet-Invertebrate Slurry Substrate-Sand	Terminate on Day 20 post hatch for survival and growth measurements	Terminate after 7 consecutive days of no emergence to measure emergence and reproduction
Test Group 5: Diet-Invertebrate Slurry Substrate- Beads	Terminate on Day 20 post hatch for survival and growth measurements	Terminate after 7 consecutive days of no emergence to measure emergence and reproduction

Table 1: The various combinations of culture conditions and the endpoints

An Evaluation Of Survival, Growth, Emergence, And Reproduction Of The Midge (*Chironomus Dilutus*) Under Various Culture Conditions

Culture Conditions	Test Acceptability Requirements									
	>0.48 mg AFDW/Surviving Organism		>80% Larvae/Pupae Survival		>50% Emergence		Mean # of Eggs per Egg Mass >800		>80% Hatchability	
Test Group 1	✓	3.03 mg	✗	17%	✗	25%	✓	1788	✗	65%
Test Group 2	✓	2.90 mg	✗	54%	✓	73%	✓	1672	✓	96%
Test Group 3	✓	1.40 mg	✗	79%	✓	59%	✓	1647	✓	96%
Test Group 4	✓	2.70 mg	✗	46%	✓	52%	✓	1709	✓	97%
Test Group 5	✓	2.75 mg	✗	38%	✗	47%	✓	1916	✓	91%

✓ = Criteria Met
✗ = Criteria Not Met

Table 2: An evaluation of validity criteria results from the 2016 culture trial

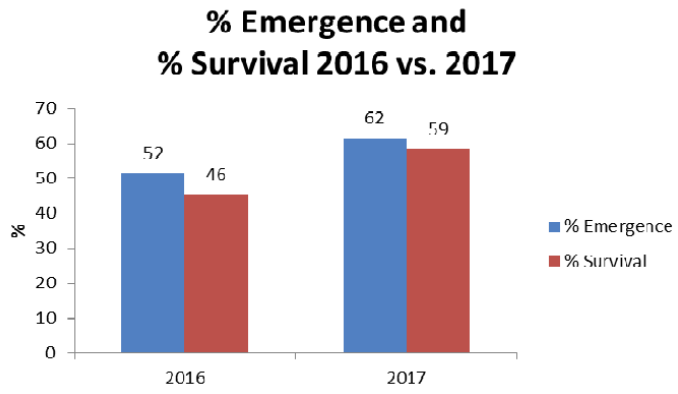

Figure 1: A comparison of % emergence and % survival from Test Group 4 in the 2016 culture trial vs. the 2017 culture trial

2017 still do not meet the >80% survival; however, there was an increase from 46% survival (2016) to 59% survival (2017). In addition, Day 20 growth, percent emergence, and the number of egg masses produced also demonstrated improvement from 2016. There was a slight decline in percent hatch (88.5%) in 2017; however the results from 2017 were based on a larger number of egg masses (78 egg masses in 2017 versus 22 egg masses in 2016) and still exceed the acceptability criterion.

CONCLUSIONS

Prior to conducting the first culture trial, the C. dilutus culture maintained at EAG Laboratories, Easton was fed invertebrate slurry and housed with no substrate. Based on the results of the 2016 trial, it was determined that a diet of invertebrate slurry and a substrate of sand is more likely to produce healthy organisms needed to meet the test acceptability requirements for chronic sediment testing. Therefore, the culture conditions of test group 4 (a diet of invertebrate slurry and sand substrate) continue to be implemented as the culture conditions for C. dilutus. An additional culture trial conducted in 2017 further verified that the newly adopted culture conditions produce healthy organisms and demonstrated an increase in percent emergence and day 20 post hatch survival. Although the trials have yet to yield >80% survival, EAG Laboratories, Easton has conducted 4 successful chronic tests in 2017 using the in-house C. dilutus culture. Further trials are needed to demonstrate that the changes made to the culture procedures continue to improve the health of the organisms, and consistently produce organisms that meet the test acceptability requirements for chronic sediment testing.

ACKNOWLEDGEMENTS

I would like to thank Michele Stence and Rachel Woodward for conducting both culture trials and collecting the raw data. I would also like to thank Susan Thomas for summarizing and compiling the data for both culture trials performed.

REFERENCES

1. Environmental Protection Agency, March 2000. “Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants With Freshwater Invertebrates”. Second Edition. (EPA/600/6-94/024).

2. Greer, I.E. 1993. Standard operating procedures for culture of chironomids (SOP B5.25 dated 02/18/93) and Hyalella azteca (SOP B5.38 dated 09/17/93). USGS, Columbia, MO.