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LIFE SCIENCES

HOW DO YOU ENSURE THE VITALITY OF YOUR TEST ORGANISMS IN SEDIMENT TOXICITY TESTS

APPLICATION NOTE

Control Survival, Growth And Reproduction Of The Marine Amphipod *Leptocheirus Plumulosus* In A Flow-Through Test System

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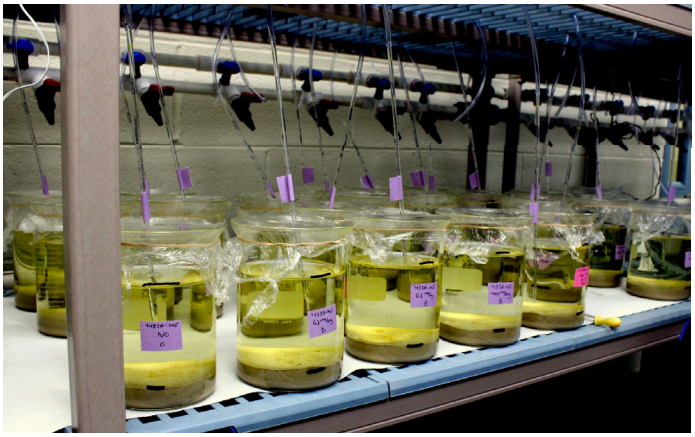


Static-renewal *Leptocheirus* life cycle study testing area

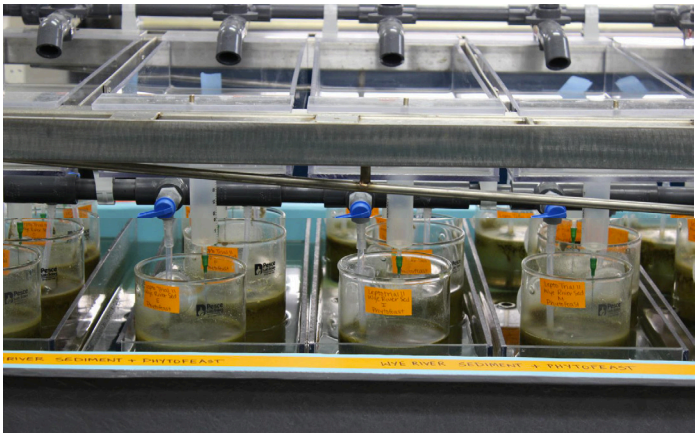
INTRODUCTION

Leptocheirus plumulosus is an estuarine amphipod widely distributed in mesohaline waters along the eastern seaboard of the US from Cape Cod, Massachusetts to northern Florida (1). Amphipods are representative of an important group of aquatic invertebrates, and are used in sediment toxicity tests based upon past history of use in the laboratory and the recommendations of the study guidelines.[2,3] In addition, its relatively short life history makes it ideally suited for evaluating chronic sub-lethal endpoints such as growth and reproduction.[1]

The current USEPA guideline for the *Leptocheirus* life cycle prescribes a staticrenewal test design. The static-renewal design has resulted in minimal to no test organism survival and has difficulty meeting the acceptability requirements for a successful study. Consequently, EAG Laboratories has adapted a flow-through test design similar to that which is being used on chronic sediment tests with freshwater amphipods. The studies described herein were performed based upon procedures in the U.S. Environmental Protection Agency Guidance Document, EPA 600/R-01/020[2] and EPA 600/R-94/025.[3]

METHODS

Static-Renewal *Leptocheirus* Life Cycle Study Design



Flow-through *Leptocheirus* life cycle study testing area

- Organisms <48 hours old or sized between a 0.25-mm and 0.6-mm mesh screen
- Feed- crushed Tetramin® fish food; 20mg per day between day 0 and 13; 40mg per day between day 14-27
- Test chamber 1L glass beaker, 175mL sediment, 750mL overlying water
- Manual renewal of 400mL of water 3x per week to maintain water quality by pouring or siphoning and replacing water by pouring over a baffle as to not disturb the sediment
- Pooled male and female dry weights for growth evaluation

Flow-Through *Leptocheirus* Life Cycle Study Design

- Known aged organisms collected <24 hours old held until 7 to 8 days old
- Algae diet Phyto-Feast® diluted to a density of 1.0X109 cells/mL, ramped up during test. No food on day 0 or Day 28; Days 1-13: 1.0mL per compartment; Days 14-27: 2.0 mL per compartment
- Test compartment 1500-mL glass beakers with a Nitex® mesh-covered hole on one side of the beaker, 2 cm (~240 mL) of sediment, 800 mL of overlying water
- 2 exchanges of overlying water per day; passive water

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exchange through meshcovered hole

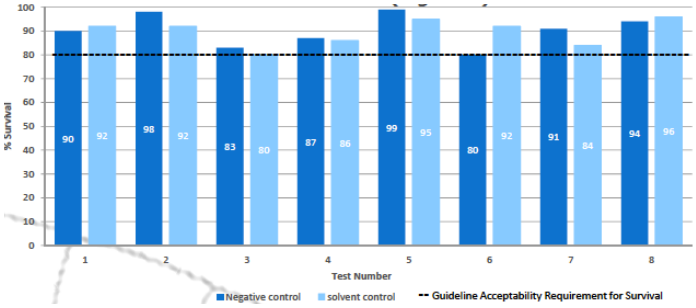
- Dry weights separated by gender for growth evaluation

Test Acceptability Requirements

- B80% survival of *L.plumulosus* in the control(s) at the end of the test.
- Growth and reproduction will be measureable in the control(s).

RESULTS

Figure 1. Control Survival



Eight *L.plumulosus* life cycles studies conducted between 2015 and 2017 by EAG Laboratories met or exceeded the control validity criteria of greater than or equal to 80% survival (Figure 1). Mean negative control survival was 90.3 ± 6.8%; mean solvent control survival was 89.6 ± 5.7%.

Figure 2. Control Growth Rate

Test #	Negative Control					Solvent Control				
	Adult Male Mean Growth Rate (mg/day)	SD	Adult Female Mean Growth Rate (mg/day)	SD	# of reps[2]	Adult Male Mean Growth Rate (mg/day)	SD	Adult Female Mean Growth Rate (mg/day)	SD	# of reps[2]
1	0.090	0.004	0.0601	0.003	5	0.079	0.012	0.054	0.006	5
2	0.085	0.006	0.0542	0.004	5	0.076	0.005	0.055	0.004	5
3	0.059	0.016	0.0237	0.016	5	0.078	0.011	0.047	0.010	5
4	0.068	0.009	0.0524	0.007	5	0.074	0.014	0.058	0.012	5
5	0.064	0.011	0.0431	0.005	5	0.057	0.002	0.042	0.004	5
6	0.073	0.012	0.0467	0.006	5	0.067	0.009	0.043	0.003	5
7	0.091	0.013	0.0774	0.018	5	0.103	0.016	0.069	0.004	5
8	0.091	0.006	0.0630	0.012	5	0.091	0.010	0.056	0.007	5

[1] Calculated as the (mean individual adult dry weight -mean individual neonate dry weight)/28days

[2] Each replicate had 20 organisms

Eight *L.plumulosus* life cycle tests conducted by EAG Laboratories between 2015 and 2017 met and exceeded the control validity criteria of measureable growth (Figure 2). Overall mean growth (mg/day) in the negative controls for males was 0.0774 ± 0.013 and for females was 0.0526 ± 0.016. Overall mean growth in the solvent control for males was 0.0783 ± 0.014 and for females was 0.0529 ± 0.009.

Figure 3. Control Reproduction

Test #	Negative Control			Solvent Control		
	Mean number of neonates/ female	SD	# of reps[1]	Mean number of neonates/ female	SD	#of reps[1]
1	13.3	2.8	5	13.5	2.09	5
2	14.9	3.49	5	11.7	2.25	5
3	7.7	4.44	5	10.7	3.4	5
4	17.4	2.81	5	16	2.76	5
5	9.3	2.51	5	7.7	1.16	5
6	6.9	1.62	5	6	2.41	5
7	12.7	3.28	5	16.6	7.31	5
8	12.5	2.17	5	14.9	2.11	5

[1] Each replicate had 20 organisms

Eight *L.plumulosus* life cycle studies conducted by EAG Laboratories between 2015 and 2017 met and exceeded the validity criteria of measureable reproduction (Figure 3). The overall mean number of young per female for the negative control was 11.8 ± 3.6 neonates per female and for the solvent control was 12.1 ± 3.9 neonates per female.

CONCLUSIONS

The sediment team at EAG has become successful in running *L. plumulosus* life cycle toxicity tests. The amount of water, frequency of water exchanges and the passive exchange through the needles has improved the maintenance in water quality. The feed type and increased rates have aided in healthy growth in the organisms. The age of the organisms at the start of the study has helped improved the survival percentage. The changes made have enhanced the success rate overall of the *L. plumulosus* life cycle test at EAG Laboratories.

Using the flow-through test design we are now more consistently meeting or exceeding the control validity criteria for survival, growth, and reproduction.

ACKNOWLEDGMENTS

Thank you so much! We Know How! Susan Thomas, Andy Nevitt, Hope Gerstler, Nanditha Billa, Liz Ostermann and Hank Krueger

REFERENCES

1. Moore, D. W., Bridges, T. S., Gray, B. R. and Duke, B. M. (1997), Risk of ammonia toxicity during sediment bioassays with the estuarine amphipod Leptocheirus plumulosus. Environmental Toxicology and Chemistry, 16: 1020–1027.

2. U.S. Environmental Protection Agency. 2001. EPA 600/R-01/020: Method for Assessing the Chronic Toxicity of Marine and Estuarine Sediment-associated Contaminants with the Amphipod Leptocheirus plumulosus.

3. U.S. Environmental Protection Agency. 1994. EPA 600/R-94/025: Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods.