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Toxicant Effects on Reproduction and Disruption of the Egg-Length Relationship in Grass Shrimp

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Grass shrimp have been used extensively for acute toxicity tests with pesticides, metals, chlorine, simulated effluents, radiation, and others. Their use in acute toxicity tests has been recommended by the United States Environmental Protection Agency (USEPA) for effluents (PELTIER 1978), hazardous materials (USEPA 1975), premanufacture notification (USEPA 1979), and ocean disposal (TYLER-SCHROEDER 1978b, c). This broad use of grass shrimp in acute toxicity testing has occurred because of its wide distribution and ease of collection and maintenance in the laboratory. Grass shrimp has become an important toxicity test organism because of the abundance of toxicity data on grass shrimp, a substantial physio-ecological data base, and its importance in estuarine food webs.

LITTLE (1968) demonstrated that spawning of Palaemonetes pugio could be induced in the laboratory. However, experimental results applying Little's findings to a life cycle toxicity test were not published until 1979 (TYLER-SCHROEDER). A primary objective in life cycle toxicity tests is an evaluation of reproductive impairment, and Tyler-Schroeder has proposed that reproductive impairment can be evaluated by comparing the number of live young hatched to a theoretical number of eggs produced by stressed females. The theoretical number of eggs produced is estimated from body length - egg number regressions constructed for each test group similar to that found by WOOD (1967) for natural populations. Based on earlier observations, we hypothesize that the relationship between length of a female and the number of eggs she produces is affected by sublethal toxicant stress, and that this relationship need not be linear. The purpose of this research was to test this hypothesis and to determine if other recommended test parameters (e.g., length, weight, days to spawning, etc.) are valid indicators of toxicant stress.

MATERIALS AND METHODS

Adult P. pugio were obtained from Gulf Specimen Company (Panacea, Florida) and held in our laboratory under conditions conducive to spawning and larval development. Although the optimum salinity for juvenile and

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adult P. pugio is 17 ppt salinity (ANDERSON et al. 1974), they tolerate a wide range of salinities (WOOD 1967). The optimum salinity for larval development is 25 ppt at 25° C (FLOYD 1977). These optima approximate field conditions during which fall spawning occurs (WOOD 1967). Our cultures and tests were maintained in artificial seawater of 25 ppt salinity made from Dayno salts diluted with carbon dechlorinated Blacksburg tap water. Test and culture temperature was maintained at 25±1° C. Cultures were held under cool white deluxe fluorescent bulbs and a photoperiod of 16L:8D with an intensity of 50 ft-c at the air-water interface. Grass shrimp were fed Tetramin Fish Food daily, supplemented by freshly hatched brine shrimp nauplii twice a week.

Larval young were obtained by isolating ovigerous females, clipping their first pair of chelae, and suspending them in nylon mesh baskets in 3-L glass jars. Zoea fell through the screen and were collected each morning with a light source and a pipette. They were transferred and reared to the test age in 5-L glass jars. Larvae were fed brine shrimp nauplii ad libitum daily. Grass shrimp were raised under sublethal toxicant stress for 12 wk from 35-day old post-larvae through reproduction.

Sublethal toxicant stress in this experiment were calculated static acute 96-hr LC20 values for 1-day old larvae and adult grass shrimp exposed to an artificial refinery mixture (ARM)(HALL et al. 1978). The 96-hr LC20 values for larvae and adults, respectively, were 0.033 and 0.117 times the ARM formulation. Grass shrimp were raised at these test concentrations, and the test solutions were changed twice a week.

Reproductive tests were begun with forty 21±2-day old post-larval grass shrimp (7-14 mm in length) per test condition; approximately 20 were females. These post-larvae were initially maintained and acclimated to test conditions under a 8L:16D photoperiod and 10-15 ft-c intensity provided by an incandescent 15 w bulb for 2 weeks prior to toxicant exposure. On the 15th day, the post-larvae were exposed to the calculated LC20 values mentioned previously and maintained at the already stated photoperiod and light intensity for an additional 2 weeks. After this interval, photoperiod was increased to 10L:14D, and light intensity was increased to 35-50 ft-c. Thereafter, the photoperiod was increased by 45-min increments every 2 weeks until it became 16L:8D. Light intensity remained at 35-50 ft-c during this time of increasing photoperiod.

During the acclimation and test periods, the animals were observed daily for mortality and spawning. Animals

were fed Tetramin Fish Food daily and brine shrimp nauplii ad libitum twice a week after the test solutions were changed. Additionally, body length of all test organisms was measured every 4 weeks.

After each female spawned, she was measured, weighed, and sacrificed so that accurate egg counts could be obtained before the female could remove attached eggs (LITTLE 1968, TYLER-SCHROEDER 1978a,c, 1979). After the eggs were removed and counted, the relationship between body length and number of eggs for each group was determined using the SAS general linear models program (BARR et al. 1976). Significant differences were determined by ANOVA and Duncan's New Multiple Range test.

RESULTS AND DISCUSSION

No significant differences in survival times between control and test organisms were obtained. Approximately 85% of each group survived the 12 weeks of testing.

Grass shrimp growth, as measured by length, was not significantly affected by ARM. This lack of effect was true for both mixed-sex populations (Table I) and for females when they spawned (Table II). However, at the end of the test, wet and dry weights for animals exposed to 0.033 X ARM were significantly less than the controls. The animals exposed to the higher 0.117 X ARM concentration were intermediate in weight (Table I).

TABLE I

Effects of ARM exposure on growth parameters on mixed sex populations of *P. pugio*. Means connected by the same line are not significantly different.

Parameter	Exposure		
	Control	0.117 X ARM	0.033 X ARM
Mean length (mm)	22.9	21.7	21.5
Mean wet weight (mg)	88.2	78.2	51.5
Mean dry weight (mg)	22.9	18.4	15.2

TABLE II

Effects of ARM exposure on the reproductive capacity of P. pugio. Means connected by the same line are not significantly different.

Parameter	Exposure		
	Control	0.033 X ARM	0.117 X ARM
Number of females spawned	10	5	11
Mean days to spawning	57.1	67.2	57.5
Mean length (mm) at spawning	28.4	27.5	25.4
Mean number of eggs/ female	157.1	104.0	110.0

Ten of approximately 20 females in the control groups spawned. In the exposed groups, 11 females in 0.117 X ARM spawned while only 5 females spawned in 0.033 X ARM. The mean number of days until spawning was not significantly different even though the animals exposed to 0.033 X ARM took approximately 10 days longer to spawn (Table II). The length of females at the time of spawning was not significantly different between groups. Both groups of ARM exposed females deposited significantly fewer eggs than the controls.

The long term exposure of P. pugio to ARM indicates that egg production and body weight were the only parameters where effects could be significantly determined. For the concentrations of the ARM tested, differences in body length and days to spawning were not reliable measures of stress. Since significant effects occurred at 0.033 X ARM and not at 0.117 X ARM, we speculate that a biomodal toxicant effect may have occurred.

In addition to the lower egg production in stressed females, there was a disruption in the pattern of egg production. Linear relationships have been demonstrated for female body length and number of eggs produced in a field population (WOOD 1967). The number of eggs released by control females in our study was linear, and the r^2 was 0.87 (Table III). Egg production was lower in stressed females (Table II), and egg production was erratic and nonlinear. The respective r^2 values for 0.033 and 0.117 X ARM were 0.22 and 0.29 (TABLE III).

TABLE III

Effect of toxicant stress on the relationship between spawned P. pugio body length and number of eggs produced.

Test condition	N	Equation $\hat{y} = bx - a$	r^2	Probability
Control	10	23.7x - 516.19	0.87	0.002
0.033 X ARM	5	6.7x - 81.26	0.22	0.53
0.117 X ARM	11	5.6x - 43.6	0.29	0.21

This disruption of the length-egg relationship by toxicant stress is important because it has been proposed that a linear function could be used to estimate egg production of stressed females. Reproductive impairment is then calculated by comparing the number of hatched young to the estimated number of eggs produced (TYLER-SCHROEDER 1978c, 1979). Our data indicate that length-egg relationships cannot be assumed in life cycle toxicity tests with P. pugio. Further, reproductive impairment measurements as recommended by the USEPA (TYLER-SCHROEDER 1978c) for establishing water quality criteria, effluent standards, or ocean disposal may not be valid. We suggest that more research be conducted on grass shrimp reproductive potential before the USEPA recommended procedure be subsequently required as a method for evaluating waste materials.

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