## Avoidance of Pesticides by Grass Shrimp (Palaemonetes pugio)<sup>1</sup>

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Some fishes can avoid certain pesticides in water. Green sunfish, Lepomis cyanellus, was repelled by chlordane but not lindane (SUMMERFELT and LEWIS 1967); sheepshead minnows, Cyprinodon variegatus, avoided DDT, endrin, Dursban, and 2,4-D (BEE) but did not avoid malathion or Sevin (HANSEN 1969); and mosquitofish, Gambusia affinis, avoided DDT, Dursban, 2,4-D, malathion and Sevin but not endrin (HANSEN et al. 1973).

Crustaceans are usually more sensitive to pesticides, particularly insecticides, than are fishes, but little is known about their ability to avoid pesticide pollution. The purpose of this study was to evaluate the capacity of the euryhaline grass shrimp, Palaemonetes pugio, to avoid DDT, endrin, Dursban, malathion, Sevin and 2,4-D. This shrimp was selected because of its importance in the food web (WOOD 1967) and its abundance in local waters.

## Experimental Procedure

Grass shrimp, 10-40 mm rostrum-telson length, were seined from brackish-water ponds on Sabine Island. They were acclimated for at least 5 days in the laboratory at 20% salinity and 20 C before they were used in experiments.

The avoidance response was tested in a black plastic apparatus designed to allow the shrimp to move from a holding area either into a section which contained water with pesticide or into one which contained water without pesticide (HANSEN et al. 1973). A gate was lowered at the junction between the two sections and the holding area to trap shrimp for counting. When a test was in progress the apparatus was covered with black acrylic plastic to exclude light. Filtered sea water diluted with aerated tap water to 20% salinity and maintained at 20 C entered the upper end of each of the two sections at a rate of 400 ml/minute and flowed to

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the drain in the holding area. Pesticides dissolved in acetone were metered through stopcocks at 0.5 ml/minute into the water entering one of the two sections. The same amount of acetone without pesticide was metered into the water entering the other section. The two upper "Y's" served no specific function in these tests.

Pesticides selected for avoidance testing included five insecticides and one herbicide (Table 1). Concentrations of these chemicals were selected so that one was higher and two or three were lower than the concentration that flowing water bioassays indicated would kill 50 percent of the shrimp in 24 hours (LC50). Concentrations were not checked by chemical analysis.

TABLE 1

Description of chemicals tested and 24-hour LC50's for grass shrimp, Palemonetes pugio.

	Percentage active 24-hour		24-hour	
Pesticide	Туре	ingredient	LC50 (ppm) 1	
DDT	Organochlorine	99	0.0007	
Endrin	Organochlorine	97	0.0015	
Dursban	Organophosphate	99	0.0032	
Malathion	Organophosphate	95	0.032	
Sevin (Carbaryl)	Carbamate	98	0.038	
2,4-D (butoxyethanol	Herbicide	70 (acid	No effect at	
ester)		equivalent)	10 ppm <sup>2</sup>	

Personal communication, Jack I. Lowe, Environmental Protection Agency, Sabine Island, Gulf Breeze, Florida 32561, May 8, 1972.

The avoidance response was tested in two phases:

- 1. The ability of grass shrimp to choose between water that contained a pesticide and water free of pesticide was tested. Response to each concentration of pesticide was tested at least four times; twice with the pesticide entering one section of the apparatus and twice with the pesticide entering the opposite section. For each of the four replications, 50 shrimp were placed in the holding area with the gate lowered. After 30 minutes, the gate was raised to give the shrimp access to both sections. One hour later, the gate was closed and the number of shrimp in each section was recorded. This procedure was repeated when additional data were required to verify the conclusions.
- 2. The capacity of shrimp to discriminate between concentrations of pesticide avoided in the first phase was tested.

<sup>&</sup>lt;sup>2</sup>Static bioassay.

Experimental procedure was the same as in the first series of tests except that the shrimp were given a choice between two concentrations of the same pesticide.

The ability of grass shrimp to avoid pesticides in both phases was evaluated statistically by the chi-square test on the assumption that if there was no response to the pesticides, the shrimp that left the holding area would enter each section with equal frequency. Preliminary tests without pesticides indicated that this assumption was correct. Lack of any preference for the right or left section in avoidance tests (49 vs. 51%) further corroborated this assumption. Avoidance or preference was considered significant if the probability that observed distributions would occur by chance was 0.05 or less. Shrimp remaining in the circular area after a test was completed were not included in the statistical analysis because stationary shrimp may not have been exposed to the two choices and moving shrimp may have been in transit between areas.

## Avoidance

Grass shrimp avoided 1.0 and 10.0 ppm of the butoxyethanol ester of 2,4-D by seeking water free of this herbicide but did not avoid any of the five insecticides (Table 2). The avoidance response of two fishes, sheepshead minnows and mosquitofish, to these same pesticides was tested identically and 2,4-D was the most readily avoided (HANSEN 1969, HANSEN et al. 1973). Fish in TVA reservoirs were apparently repelled by application of 2,4-D at 40-100 pounds per acre (SMITH and ISOM 1967). Concentrations of 2,4-D in reservoir water one hour after application reached 0.16 ppm; slightly less than amounts avoided by shrimp in our tests. Although statistical analysis indicated that grass shrimp preferred 0.0001 ppm of DDT, this was probably not valid because preference was observed in only one of three replications, and shrimp did not respond to greater or lesser concentrations.

Grass shrimp given a choice between two concentrations of 2,4-D selected the lower concentration (Table 3). Up to 78 percent of the shrimp that left the holding area avoided the higher of the two concentrations. Only 2,4-D was tested in this manner because it was the only pesticide that shrimp avoided by seeking water free of toxicant.

TABLE 2

Capacity of grass shrimp to seek water free of pesticides.

N.S. = not significant.  $\chi^2 = P(3.84 = 0.05; 6.63 = 0.01; 10.83 = 0.001$ .

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-	Concentration	Number of	Number of Sh	of Shrimp*	Percentage	
Pesticide	(mdd)	tests	In pesticide	In water	in water	X value
DDT	0.01	7	09	80	57.1	N.S.
	0.001	7	7 97	55	54.4	N.S.
	0.0001	12	218	165	43.1	7.334
	0,00001	7	78	84	48.1	N.S.
Endrin	0.01	7	09	77	56.2	N.S.
	0.001	7	79	· +9	8.44	N.S.
	0.0001	7	9.2	99	46.5	N.S.
Dursban	0.001	† 	63	70	52.6	N.S.
	0.0001	4	92	88	53.6	N.S.
	0.00001	∞	133	105	44.2	N.S.
Malathion	1.0	0	137	117	46.1	N.S.
	0.1	7	62	61	9.67	N.S.
	0.01	7	7.1	77	52.0	N.S.
Sevin	0.1	7	99	61	48.0	N.S.
	0.01	4	62	51	45.1	N.S.
	0.001	<b>∞</b>	129	102	44.2	N.S.
	0.0001	7	57	65	53.3	N.S.
2,4-D	10.1	· · · · · · · · · · · · · · · · · · ·	77	91	67.4	16.363
	1.0	7	51	9/	59.8	4.921
	0,1	4	57	59	50.9	N.S.
*Does not in	*Does not include shrimp in holding	area at end	of test.		·	

TABLE 3

Response of grass shrimp exposed to two<sub>2</sub> different concentrations of the butoxyethanol ester of 2,4-D.  $\chi$  = P(3.84 = 0.05; 6.63 = 0.01; 10.83 = 0.001.

		Number of shrimp*		Percentage	2
Concentrations	(ppm)	In high	In low	in low	X
High	Low	conc.	conc.	Concentration	value
10.0	1.0	43	90	67.7	16.61
10.0	0.1	24	85	78.0	34.14
1.0	0.1	43	67	60.9	5.24

<sup>\*</sup>Does not include shrimp in holding area at end of test.

Our study indicates that grass shrimp are less able to avoid and are more readily affected by pesticides than were the fishes used in earlier experiments (HANSEN 1969, HANSEN et al. 1973). Similarly, the European brown shrimp (Cragon cragon) did not avoid DDT (0.1 ppm), azinphos-methyl (1 ppm), atrazine (10 ppm) and aminotriazole (1,000 ppm) and were more sensitive to these compounds than were fishes (PORTMAN In press). These data suggest that shrimp may be extremely vulnerable to pesticide pollution because they are (1) extremely sensitive to pesticides and (2) generally are unlikely to avoid water polluted by pesticides. Consequently it is important that pesticides destined for use in and near estuaries be tested to determine their toxicity to shrimp and the capacity of shrimp to avoid them.

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