Sensitivity to Pesticides in Three Generations of Sheepshead Minnows[1]

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The increasing use of pesticides to control pests that live in or near the aquatic environment creates a need for more information concerning the effects of these chemicals on fishes. Recent investigations indicate that some pesticides affect reproduction in fishes. For example, mortality of fry of lake trout (*Salvelinus namaycush*) is positively correlated with the amount of DDT present in the eggs (1). Chronic exposure to dieldrin alters the age structure of confined populations of guppies so that proportionately more fry and immature fish appear in the population (2). Sheepshead minnows (*Cyprinodon variegatus*) whose parents had survived toxic concentrations of DDT are more sensitive to DDT and endrin than are offspring of "control" fish whose parents had not been exposed to DDT (3).

The purpose of this study was to determine whether succeeding generations of sheepshead minnows exposed to DDT can develop resistance to DDT, and whether "cross-resistance" to endrin, a related insecticide, can develop.

Methods and Materials

Three lines of fish, each consisting of a parental group and their descendants, are represented in these tests. Fish from which these lines were derived were collected from marsh ditches on Santa Rosa Island, Escambia County, Florida in July 1964, March-April 1965, and July 1965. Salinity in the ditches ranged from 1 to 10 p.p.t. (parts per thousand); water temperature, from 18° to 25° C. Fish were acclimated to laboratory conditions for 24 hours before testing. The fish were subjected to bioassay tests in the laboratory to determine their sensitivity to the pesticides.

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Tests were conducted in acrylic plastic aquaria that contained 20 liters of tap water adjusted to 4 p.p.t. salinity with artificial sea salt. The water was aerated to near saturation and its temperature was maintained at 21 $\pm$ 1° C. Stock solutions of pesticide in acetone were added at the rate of 0.5 ml acetone per liter of water to give the desired concentration of toxicant. Final concentration of DDT ranged from 15 to 40 p.p.b. (parts per billion, $\simeq$ µg/L); that of acetone was 0.05%. From 50 to 100 experimental fish and a similar number of control fish of the same size range were used in each test. After each test, aquaria were washed with detergent and water, and rinsed with a 50% solution of acetone in water.

Since all lines were treated similarly (Figure 1), the treatment is described for only one line.

A sample of wild fish collected the same day was separated into two groups, designated "experimental" and "control". Sixty to 75 fish (50 to 70 mm total length), distributed 5 per aquarium, were exposed for 24 hours to a concentration of DDT that killed 95% or more of the fish (Table 1). Control fish of similar size and number were exposed to acetone-water solution alone. Survivors of the DDT challenge, placed in a brackish-water breeding pond on Santa Rosa Island to reproduce, produced the F_1 generation of the experimental line of fish. Offspring of the control fish, spawned and reared in an adjacent pond, represent the F_1 control generation ($\sim$normal population).

TABLE 1

Survival of wild sheepshead minnows subjected to concentrations of DDT that killed 95% or more of the fish. Survivors provided breeding stock for the three test lines used in this study.

Date	Number tested	Survivors Male	Survivors Female
July 1964	195	2	2
March-April 1965	1050	15	12
July 1965	555	4	5

Breeding ponds were adjacent rectangular excavations 15 m. long, 5 m. wide, and 1.25 m. deep. Sea water was periodically pumped into the ponds to maintain salinity levels from 1 to 10 p.p.t. and depths from 0.5 to 1 m. Environmental conditions were similar

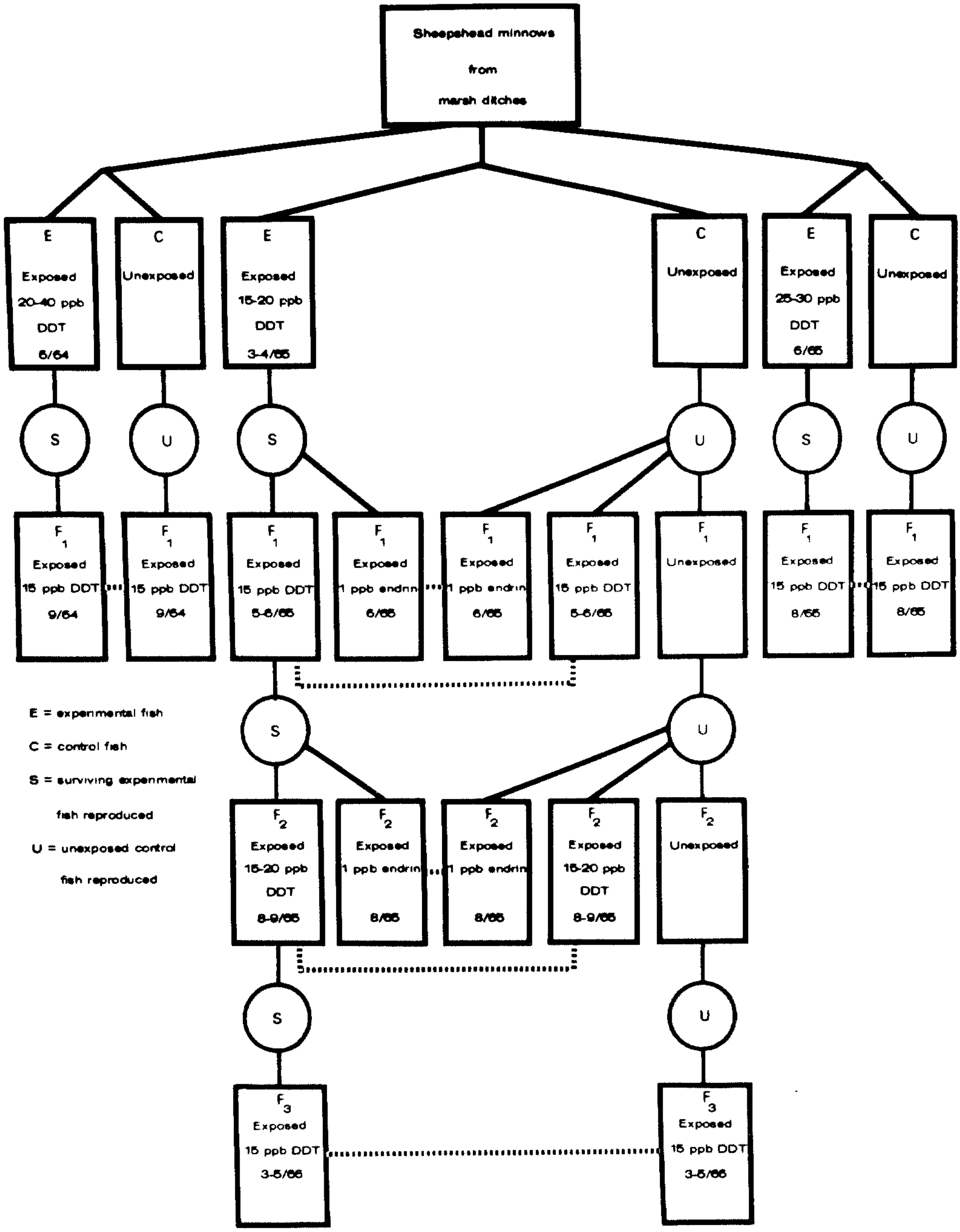

Figure 1. Lineage and treatment of experimental and control fish. Dash lines (---) indicate experimental fish that were compared for sensitivity.

to those of ditches naturally inhabited by this species. Differences among the ponds were negligible.

The F_1 generation of experimental and control fish, in groups of 50 to 100 fish (approximately 30 mm. total length) distributed 10 per aquarium, were challenged for 24 hours with DDT in the laboratory. Surviving experimental fish and unexposed control fish from one line were placed in a second pair of breeding ponds, where each produced an F_2 generation. The other two lines of fish were not reared and tested beyond the F_1 generation.

F_2 fish were similarly challenged with DDT and observed for difference in sensitivity. The surviving F_2 experimental and unexposed F_2 control fish were placed in a third pair of breeding ponds, and each produced an F_3 generation which, in turn, was challenged with DDT in the same way and observed for sensitivity.

Similar experiments with F_1 and F_2 offspring of fish that had survived exposure to DDT were performed to determine whether cross-resistance to endrin, a related chlorinated hydrocarbon insecticide, had developed.

Statistical treatment of data. Chi-square values were calculated to determine whether a real difference existed between sensitivities of experimental fish (observed) and control fish (expected). Differences were considered real at $P= .05$. The data are summarized in Table 2.

Sensitivity of Fish to DDT and Endrin

Original control and experimental fish produced F_1 fish immediately after being placed in ponds. Experimental F_1 fish reached testing size in 1 - 2 months and were compared with controls for sensitivity to 15 p.p.b. DDT. The experimental F_1 fish produced in March-April were tested in May-June 1965 and tended to be less sensitive to DDT and endrin than control fish, but those produced in July 1964 and 1965 and tested in August and September 1964 and 1965 were more sensitive to DDT.

The experimental F_1 fish that survived exposure to DDT in May-June 1965 produced the F_2 generation in July 1965. The experimental F_2 groups, tested in August-September, were more sensitive to DDT and endrin than control fish.

The experimental F_2 fish that survived exposure to 15 and 20 p.p.b. DDT in August-September 1965 produced F_3 fish in February-March 1966. The experimental F_3 fish, tested in March-May 1966, tended

TABLE 2

Sensitivity of three generations of sheepshead minnows to DDT and endrin. Experimental fish were offspring of survivors of exposures to concentrations of DDT that killed 70% or more of the fish in the previous generation. Control fish were offspring of unexposed fish. NS = not significant. χ^2 = P(3.84 = 0.05; 6.63 = 0.01; 10.83 = 0.001). ND = no difference. L = experimental fish were less sensitive than control fish. M = experimental fish were more sensitive than control fish.

Generation	Pesticide	Concentration (p.p.b.)	Control fish: Tested	Control fish: % Killed	Experimental fish: Tested	Experimental fish: Killed, Expected	Experimental fish: Killed, Observed	Chi-square (1 d.f.)	Sensitivity	Date tested (M-D-Y)
F_1	DDT	15	50	88.0	50	44.00	40	NS	ND	5-20-65
"	"		60	96.7	60	58.02	42	134.039	L	5-25-65
"	"		60	98.3	70	68.81	61	52.143	L	5-27-65
"	"		70	92.8	50	46.40	46	NS	ND	6-16-65
"	"		100	76.0	50	38.00	42	NS	ND	6-22-65
"	"		70	82.9	80	66.32	57	7.660	L	6-24-65
"	"		50	46.0	80	36.80	61	29.470	M	8-14-65
"	"		70	40.0	80	32.00	52	20.833	M	8-26-65
"	"		50	56.0	50	28.00	36	5.195	M	9-17-64
F_2	DDT	15	70	42.9	80	34.32	74	80.345	M	8-6-65
"	"		50	34.0	60	20.40	55	88.915	M	8-10-65
"	"		50	38.0	50	19.00	48	71.392	M	8-12-65
"	"		50	18.0	50	9.00	37	106.233	M	8-24-65
"	"	20	60	36.7	60	22.02	49	52.223	M	8-31-65
"	"		50	56.0	50	28.00	37	6.575	M	9-2-65
F_3	DDT	15	60	96.7	60	58.02	56	NS	ND	3-31-66
"	"		60	98.3	60	58.98	53	35.665	L	4-19-66
"	"		60	95.0	60	57.00	51	12.632	L	5-3-66
F_1	endrin	1	70	100	80	80.00	73		L	6-8-65
F_2	"	1	70	5.0	70	3.50	40	400.667	M	8-19-65

to be less sensitive to DDT than controls.

Sensitivity to DDT varied with the time of year in both control and experimental generations. Sensitivity did not decrease consistently in any experimental generation, but all decreases observed occurred during March-June. Sensitivity increased in all experimental fish tested in August-September. The marked decrease in mortality of controls during August-September (18-56% killed at 15 p.p.b.) compared to March-June (76-98.3% killed at 15 p.p.b.) may lead one to suspect an increase in sensitivity of experimental fish due to some difference in treatment of controls. This is unlikely because: handling, rearing, and testing were similar for all fish; the difference was observed in all nine tests of each period; control tests in 1964 and 1966 agree with the same periods in 1965; and it was necessary to use a greater concentration of DDT in July than in March-April to get a similar kill for wild fish from which test strains were derived.

Sheepshead minnows were normally less sensitive to DDT during the late summer period, but the greater mortality of the experimental fish indicated that they were more sensitive than controls. The parents of experimental fish tested during August-September were exposed in May-June and the experimentals were hatched in July. We suggest that lipid metabolism and maturation of ova were greatest when the parent fish were exposed, and that incorporation of DDT via lipids into the ova may be the factor that determined the increased sensitivity. This causal relationship has been suggested for DDT in lake trout (1).

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