

the 1981 fall migration in southwestern Tennessee. Birds were captured using mist nets. The most common warblers were found to be most abundant at different times of the migration season. This may have the effect of reducing competition for food between potentially competing species. Age ratios were used to test the hypothesis that areas within the main migration route of the species contain a lower proportion of hatch year birds than the edges of these routes. Two of four species tested conformed with this hypothesis.

*Some Aspects of the Ecology of the Diamondback Water Snake.*  
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Movements, body temperatures and hibernation sites were monitored for up to a year in 16 female diamondback water snakes, *Nerodia rhombifera*

*rhombifera*. Five males were monitored for up to a month using smaller transmitters.

Home ranges tended to be linear, as snakes seldom left the water's edge in either direction, except for moving to hibernation sites. Estimates were made for the greatest length of each home range, and a mean calculated. Since males were monitored only a month, figures for the sexes are not comparable.

Numerous body temperatures were recorded, and means calculated for active snakes of both sexes. Hibernation temperature data is sparse because of failure of transmitters to operate at low temperatures. Hibernation was usually in crayfish burrows, and it is probable that some individuals were below water level during part of hibernation.

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## EFFECTS OF ALDRIN AND HEPTACHLOR ON SURVIVAL AND FIN REGENERATION IN THE PUGNOSE SHINER (*NOTROPIS ANOGENUS*)

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### ABSTRACT

Caudal fin regeneration of the pugnose shiner (*Notropis anogenus*) was inhibited by constant exposure for 14 days to 8.4  $\mu\text{g/l}$  aldrin or 11.0  $\mu\text{g/l}$  heptachlor. These pesticide concentrations represent 5% of the heptachlor 24-hour  $\text{LC}_{50}$  and 10% of the aldrin 24-hour  $\text{LC}_{50}$  to pugnose shiners. Inhibition of fin regeneration may be due to the depletion of vitamin C, which is needed for fin ray development, brought about by its use as a cofactor in the animal's attempt to detoxify the pesticides by hydroxylation.

### INTRODUCTION

Regeneration of teleost fins will result if a fin is amputated anywhere distal to the basal articulation of the rays. Immediately after amputation, the wound is closed by healing, and a blastema forms from the accumulation of undifferentiated cells derived from the loose interradial and radial tissues and osteoblasts associated with the ray stumps (Goss and Stagg 1957). Regeneration begins at this point and segmentation takes place at regular intervals along the regenerating fin. Actinotrichia develop at the ends of the regenerated rays as they did in the original rays (Goss 1969).

The rate of regeneration is proportional to the amount of fin removed with the rate being faster for fish with the most fin amputated (Tassava and Goss 1966), and younger fish regenerate fins faster than older fish (Comfort and Doljanski 1958). Interruption of the fin's nerve supply retards the rate of regeneration (Goss and Stagg 1957), while increasing the nerve supply increases the rate of regeneration (Weis 1972). Thiouracil (Hopper and Wallace 1970), heavy metals (Weis and Weis 1976, Weis and Weis 1978, Weis and Weis 1980), X-irradiation (Wilkinson et al.

1971), and pesticides (Weis and Weis 1975) have been shown to have an inhibitory effect on fin regeneration in various species of teleosts.

The ability of certain toxins to inhibit the rate of fin regeneration may offer a method to observe sublethal effects of many environmental pollutants on fishes. The objectives of this study were to (1) determine the lethal limits of two organochlorine pesticides, aldrin and heptachlor, and (2) determine the effects of sublethal concentrations of these pesticides on fin regeneration in a freshwater teleost.

### METHODS

Pugnose shiners (*Notropis anogenus*),  $39.8 \pm 2.5$  mm in standard length, were obtained from a local bait shop and acclimated to laboratory conditions for 4 days before use. Prior to acclimation, all fish were treated to tetracycline HCl as a prophylactic measure against bacterial infection.

All tests were carried out in one gallon wide-mouth jars containing 5 fish in 3 l of solution. Tap water (water quality at beginning of tests: total hardness, 60 mg/l as  $\text{CaCO}_3$ ; alkalinity, 50 mg/l as  $\text{CaCO}_3$ ; pH, 7.2;  $20^\circ\text{C}$ ) dechlorinated with sodium thiosulfate was used in all tests. Adequate dissolved oxygen concentrations were maintained by slow aeration of each test vessel.

The pesticides (aldrin=99%, 1,2,3,4,10,10-hexachloro-1,4,4,8,8a-hexahydro-1,4-endoexo-5,8-dimethanonaphthalene; heptachlor=97.4%, 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-endomethanoidene) were introduced to the test water as acetone solutions according to the following procedure. A stock pesticide solution was prepared immediately before use by dissolving 1-3 mg of pesticide in 50 ml of acetone. When transferring the necessary volume of stock solution to the test water, the tip of the transferring pipet was placed below the surface of the water, and the

solution was slowly ejected. The amount of stock solution used never exceeded 2 ml per 3 liters of solution.

Twenty-four hour median lethal concentrations (24-h LC<sub>50</sub>) were determined according to Henderson et al. (1959). The 24-h LC<sub>50</sub> for each pesticide was determined at least twice, and the data for each pesticide were combined.

To determine the effect of the pesticides on fin regeneration, the lower half of the caudal fin was amputated 1 mm from the base with a scalpel. After amputation, all fish were placed in tetracycline HCl solution (15 mg/l) for 24 hours in an effort to control wound infection. At the end of the 24 hours, the fish were transferred to the test vessels. During the tests, fish were fed a ground catfish food to excess daily, and test solutions were changed every other day to maintain proper pesticide concentrations and prevent buildup of toxic metabolites. Control vessels receive an amount of acetone equivalent to that added to the experimental vessels. Two regeneration experiments were conducted. Each experiment consisted of three parts—an aldrin test, a heptachlor test, and a control test. Pesticide concentrations equivalent to 0.05 and 0.10 of the 24-h LC<sub>50</sub> of each pesticide were used in the first and second test, respectively.

Regeneration was determined from some control and experimental fish on day 7 (including the day 1 tetracycline HCl treatment) and day 14. Measurements were made to 0.1 mm by using a miniscale (Laboratory Supply Company) under a dissecting microscope. Dead and moribund fish were not measured. A one-tailed t-test was used to compare each control group to its respective experimental group.

#### RESULTS AND DISCUSSION

The 24-h median lethal concentrations of aldrin and heptachlor to pugnose shiners were determined to be 8.6 µg/l and 219.8 µg/l, respectively (Table 1). These values for aldrin and heptachlor are similar to those reported elsewhere for other species (Henderson et al. 1959).

No significant retardation of regeneration by either pesticide at any concentration was found at the end of 1 week (Table 2). After 2 weeks, heptachlor at 11.0 µg/l and 22.0 µg/l, and aldrin at 8.4 µg/l significantly retarded regeneration. These findings agree with the studies of Weis and Weis (1975), who found that 10 µg/l of the organochlorine pesticide DDT retarded regeneration after 2 weeks but not after 1 week. These results led them to postulate that since the first week after amputation is primarily a time of wound healing and blastema formation, DDT does not affect these processes, but does affect the growth rate once regeneration has started and is probably due to its general toxicity rather than any specific effect.

TABLE 1. Twenty-four hour median lethal concentrations (24h-LC<sub>50</sub>) of aldrin and heptachlor to pugnose shiners. (*Notropis anogenus*).

	ALDRIN	HEPTACHLOR
24-h-LC <sub>50</sub> (µg/liter)	86.6	219.8
95% confidence interval (µg/liter)	85.2-88.0	218.8-220.8
Standard error of the estimate (µg/liter)	1.11	1.01
Slope <sup>a</sup>	-0.00756	-0.00624
y intercept	2.316	2.654
r	-.941	-.997

TABLE 2. Caudal fin regeneration (in mm) and mortality, (after 14 days) of pesticide treated pugnose shiners, *Notropis anogenus*. Values expressed are mean ± S.D. Numbers of fish measured are given in parentheses.

	Experiment 1 (0.05 of respective LC <sub>50</sub> )		Mortality (%)
	Day 7	Day 14	
control	0.38±.13 (5)	1.92±.56 (12)	10
4.2 ppb aldrin	0.42±.34 (21)	2.09±.84 (40)	33
11.0 ppb heptachlor	0.34±.17 (17)	1.35±.90 (49)*	18
	Experiment 2 (0.1 of respective LC <sub>50</sub> )		Mortality (%)
	Day 7	Day 14	
control	0.36±.30 (5)	1.74±.44 (9)	10
8.4 ppb aldrin	0.31±.20 (18)	1.13±.78 (21)*	65
22.0 ppb heptachlor	0.30±.22 (15)	0.77±.51 (15)**	75

\* p≤0.05

\*\* p≤0.01

A possible mechanism by which organochlorine pesticides may inhibit regeneration of fins concerns the effect of the pesticides on the regeneration of the bony fin rays. Recent investigations into the effects of chronic exposure of fish to insecticides have demonstrated that collagen synthesis during bone formation is inhibited (Mehrlé and Mayer 1975a, Mehrlé and Mayer 1975b, Mayer et al. 1978, Hamilton et al. 1981). Inhibition of collagen formation has been attributed to depletion of vitamin C, a necessary cofactor for collagen synthesis. Depletion of vitamin C for collagen synthesis is presumably due to its additional use as a cofactor in the biotransformation of the insecticides by liver enzymes (Mayer et al. 1978). Since fin rays consist of bone (Kemp and Park 1970), their regeneration may be inhibited by the same mechanism.

The results of this study point out a detrimental effect to a common cyprinid of 2 organochlorine pesticides at very low environmental concentrations during a 2 week exposure. Since pesticides can accumulate in fish tissues to levels several hundred times that found in the environment (Edwards 1970), it is conceivable that even trace amounts of pesticides found in many bodies of water may inhibit regeneration of fins in the course of a long term exposure. Results of this study and others (Weis and Weis 1976, Weis and Weis 1975, Wilkerson et al. 1971, Hopper and Wallace 1970) have also suggested the possibility of using fin regeneration as sublethal screening test for some environmental toxins.

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