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Low Concentration Effects of Endosulfan Insecticide on Reproductive Behaviour in the Tropical Cichlid Fish *Sarotherodon mossambicus*

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Endosulfan insecticide is used in the tropics for killing tsetse flies, being applied from aircraft as an aerosol at doses between 6 and 25 g/ha/application. Dose rates such as these in the Okavango Delta of Botswana have caused an average 1% mortality in shallow-water fish populations (Fox & Matthiessen 1982), and produced short-term pathological and haematological changes in survivors (Matthiessen & Roberts 1982; Matthiessen 1981). The only known side-effect with long term implications is that the nests of cichlids (Tilapia rendalli) are about 75% less numerous in sprayed areas, and at least 25% fewer juveniles are recruited (Douthwaite et al. 1981; P.J. Fox in prep.). There is also evidence for reproductive inhibition in trout after an endosulfan spill in Canada (Johnston & Cheverie 1980). Although endosulfan-induced abnormalities in reproductive physiology cannot be ruled out, seasonal gonad development in sprayed T. rendalli is not impaired (Douthwaite et al. 1981), and it has been shown in the laboratory that trout eggs hatch normally even when exposed to 50 mg/l endosulfan (Schoettger 1970). Furthermore, Macek et al. (1976) have shown that if fathead minnows are continuously exposed to low levels of endosulfan, there is no effect on fecundity and hatchability, although above 0.2 µg/l the number of spawnings per female increases.

It seems likely, therefore, that low concentrations of endosulfan interfere with the complex breeding behaviour characteristic of cichlids like T. rendalli. This hypothesis is supported by the observation that T. rendalli experience pathological lesions in brain tissue during endosulfan spraying operations in the breeding season (Matthiessen & Roberts 1982). The purpose of the work described in this paper was to investigate which aspects of cichlid reproductive behaviour are interrupted by endosulfan.

MATERIALS AND METHODS

The experimental animal was Sarotherodon mossambicus (Peters). This African cichlid digs circular nests and is closely related to T. rendalli, but broods its young in the mouth rather than guarding them. Despite this difference, reproductive behaviour is broadly similar in the two species, chromatophore-based body markings being crucial to successful mating (Lanzing & Bower 1974). Most of the

fish for these experiments were kindly supplied as fry by Professor R.J. Roberts of the University of Stirling, the remainder being bred in our laboratory from adults of the same stock. All fry were kept at high density (up to 30 g/l) and reached maturity at 5-15 g. Fish were fed on trout pellets and fresh lettuce.

Apart from an acute toxicity trial with fry carried out in 5 l glass beakers (4 l static water changed daily), all experiments were conducted in rectangular glass tanks containing 34 l of dechlorinated, aerated London tapwater with a through-flow of 8 l/hr (total hardness 291 mg/l ± 5 s.d. as $CaCO_3$; pH 8.2 ± 0.08 s.d.; % air saturation 87.1 ± 6.7 s.d.; temperature 27.6 C ± 0.4 s.d.). All tanks were fitted with loose perspex lids and were visually screened from each other. Lighting was from fluorescent tubes above the tanks on a 12 hr light/12 hr dark cycle.

Endosulfan was semi-continuously applied to the tanks as a water emulsion of Thiodan 35% ec (Hoechst) by means of a fail-safe metering device (modified after McAllister et al. 1972). Endosulfan concentrations in the tanks were checked at intervals by extracting 0.5 or 1.0 l of water with a 'Sep-Pak' silica gel cartridge and analysing by gas-liquid chromatography (Matthiessen et al. 1982).

The acute toxicity of endosulfan for 1-month fry (mean wt 0.1 g ± 0.03 s.d.) and 3-month young adults (5.9 g ± 2.6 s.d.) was measured by exposing groups of 10 fish to different toxicant concentrations and noting the times to 50% mortality. These were log-transformed and plotted against log concentration. Three experimental studies were made of the influence of endosulfan on breeding behaviour, at insecticide concentrations as much as 30 times lower than the 48 hr LC50 for young adults. These concentrations were similar to the mean levels found in Okavango Delta waters soon after aerial spraying (Fox & Matthiessen 1982).

In experiment 1, adult S. mossambicus (5 months old) were allocated randomly to 8 tanks, 4 males and 6 females per tank. After the fish had been observed for 3 weeks, 4 of the tanks were dosed for 4 weeks (0.5 µg/l). Endosulfan-induced mortality was only 2% per week, and the dead were replaced by similarly dosed fish. At 16.00 hrs each day, males showing normal breeding behaviour (defined as a combination of jet-black body colouration and guarding / 'excavation' of a nesting site), and mouth-brooding females, were noted. At the end of the experiment, blood samples were removed from anaesthetised fish by cardiac puncture, and the plasma stored frozen. Ethanol extracts of thawed plasma were analysed for testosterone and 11-keto-testosterone by non-chromatographic radio-immuno-assay techniques that used procedures which were developed by Dr. D.E. Kime and co-workers at the Department of Zoology, University of Sheffield. The anti-sera used were kindly supplied by Dr. Kime, and the (1,2,6,7-3H)testosterone and 11-keto(1,2(N)-3H) testosterone were purchased from Amersham International Ltd. Lastly, the gonads were checked for pathological signs by Professor R. Roberts, Institute of Aquaculture, University of Stirling.

In experiment 2, 10 juvenile fish (3 months old) were randomly allocated to each of 10 tanks, with 4-7 males per group. After acclimation, dosing of 5 tanks was begun (0.6 µg/l endosulfan, although 1.5 µg/l for first 3 days) and continued for 9 weeks. There was 6% mortality per week in the dosed tanks, but dead fish were replaced as in expt. 1. Sexually active males and mouth-brooding females were recorded daily, and gonads were examined after 9 weeks for pathological signs.

Experiment 3 resembled expt. 2 except that attention was focussed on males, and the 4-month old juvenile fish were exposed to either 0.6 or 0.2 µg/l endosulfan (5 tanks each). Mortality was zero except at 0.6 µg/l (1% per week). After 6 weeks exposure, male blood plasma was assayed for 11-ketotestosterone.

RESULTS AND DISCUSSION

The 24 hr LC50 of endosulfan to young adults was 10.4 µg/l, with 95% confidence limits of the log-transformed regression being 3.9 and 31.6 µg/l. The corresponding values for 48 and 96 hrs were 6.7 µg/l (95% limits, 2.3-18.6) and 4.3 µg/l (1.3-11.5). There was no evidence that either sex was preferentially susceptible.

The 24 hr LC50 to fry was 0.5 µg/l (95% confidence limits 0.03-2.0). The corresponding values for 48 and 96 hrs based on extrapolation of the regression curve were 0.2 and 0.06 µg/l, but the reliability of these values is, of course, low. The calculation is based on measured concentrations in the freshly changed solutions (i.e. maximum concentrations), so the true 24 hr LC50 for fry was probably somewhat lower than 0.5 µg/l.

Endosulfan at 0.5 µg/l had no apparent effect on breeding behaviour in young adults (expt. 1), although in this and subsequent experiments, many newly-hatched fry died through exposure to their 24 hr LC50. Fig. 1 (top) shows that male sexual display was apparently unaffected by endosulfan (exact 2x2 contingency table, $p > 0.1$ at all times). Also unaffected was the % of females with a clutch (controls, mean = 40.3% $\pm$ 3.9 s.d.; dosed, mean = 32.2% $\pm$ 8.0 s.d.) except for 2 days (1 week after dosing began) when the proportion of dosed females with clutches dropped significantly to 19% ($p < 0.05$). In addition, the rate of clutch production by control and dosed females was similar (Table 1), as was the mean time that clutches were retained in the mouth (Table 2).

At the end of the exposure period, mean levels of testosterone and 11-ketotestosterone in plasma were not significantly different in the dosed and control groups (Table 3), but it was noted that testosterone levels were significantly depressed in clutch-carrying females, irrespective of treatment (with clutch, mean = 9.2 ng/ml $\pm$6.6 s.d.; without clutch, mean = 21.5 ng/ml $\pm$15.4 s.d.).

Finally, 4 weeks' exposure to 0.5 µg/l endosulfan produced no pathological signs in ovary or testis tissue.

Table 1. Mean rate of clutch production in Expt. 1 fish (clutches/female/week).

		Pre-dose	Post-dose
CONTROL	mean	0.18	0.17
	s.d.	0.14	0.11
	n	24	24
DOSED	mean	0.15	0.18
	s.d.	0.14	0.13
	n	24	24

Table 2. Mean time that egg clutches were retained in the mouth of Expt. 1 fish (days).

		Pre-dose	Post-dose
CONTROL	mean	10.4	9.9
	s.d.	3.0	3.7
	n	26	20
DOSED	mean	10.9	8.6
	s.d.	2.9	2.9
	n	22	22

Table 3. Concentrations of sex hormones in the plasma of Expt. 1 fish (ng/ml plasma) after 4 weeks exposure to 0.5 µg/l endosulfan.

		11-ketotestosterone		testosterone	
		males	females	males	females
CONTROL	mean	70.1	0.9	14.5	18.7
	s.d.	24.8	1.4	2.1	17.0
	n	8	21	8	22
DOSED	mean	60.9	0.5	12.6	18.7
	s.d.	23.4	0.8	3.0	12.5
	n	8	21	8	22

When dosing began in expt. 2 (0.6 µg/l endosulfan), the juvenile fish showed no signs of reproductive activity. After about 12 days, sexual behaviour began in control males (Fig. 1 - middle) but not in the dosed group. This difference was significant ($p < 0.05$) for 15 days, although the mean for dosed fish remained lower than the controls for about 30 days. Throughout this period, dosed males fed poorly, floated with heads higher than tails, and were abnormally sensitive to sudden audial or visual stimuli. Control females started laying eggs after 18 days, while dosed females did not start laying until day 30, but the differences in the proportion of

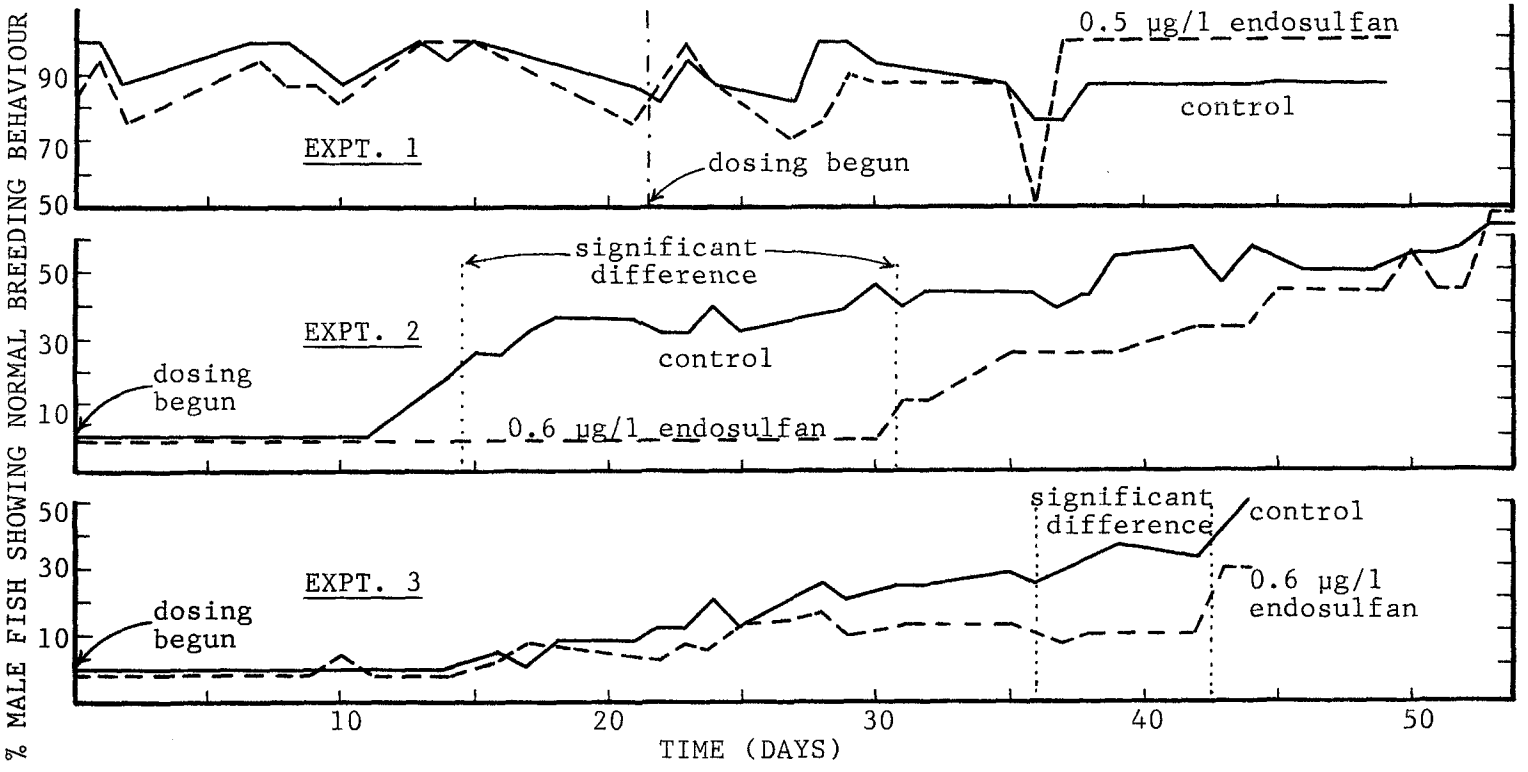

Fig. 1. Percentage of male Sarotherodon mossambicus showing normal reproductive behaviour in experiments 1, 2 and 3. The two regions where control and dosed fish differ significantly (exact 2x2 contingency table, p less than 0.05) are delimited by vertical dotted lines.

clutch-carriers were never statistically significant. However, the rate of clutch production was slightly higher ($p=0.05$) in dosed females, while the length of time that eggs/fry were retained was significantly reduced ($p < 0.05$) in these fish (Table 4).

Table 4. Rate of clutch production (clutches/female/week) and clutch retention time (days) in Expt. 2 fish.

		Clutch production rate	Clutch retention time
CONTROL	mean	0.10	6.4
	s.d.	0.12	4.4
	n	22	12
DOSED	mean	0.20	3.5
	s.d.	0.17	2.7
	n	13	16

The mean clutch retention time of controls was itself low, indicating that some normal females were aborting their clutches, possibly as a result of harassment by males. No pathological signs were seen in gonads at the end of the experiment.

Although mortality of juveniles at 0.6 µg/l endosulfan was lower in expt. 3 (1% per week) than expt. 2 (6% per week), dosed fish were nevertheless hypersensitive to sound and movement and fed poorly. The onset of sexual behaviour in males was retarded at 0.6 µg/l, although the proportions of displaying fish only differed significantly for the week following day 36 (Fig. 1 - bottom). The data for males dosed at 0.2 µg/l (not shown in Fig. 1) never differed significantly from controls, and these fish suffered no other abnormalities. The onset of egg-carrying in dosed females was not retarded at either concentration.

The concentration of 11-ketotestosterone in male fish which had been exposed to endosulfan at 0.6 µg/l was apparently elevated (Table 5), but this was not statistically significant.

The well-known principle that small fish are more susceptible to insecticide poisoning than large ones was observed in this study. Thus, 1 month-old fry were killed by an endosulfan concentration (0.5 µg/l) twenty times lower than the equivalent value for 3-month

Table 5. 11-ketotestosterone concentration (ng/ml) in the plasma of male fish after 44 days exposure to endosulfan in Expt. 3

	Control	Endosulfan 0.2 µg/l	Endosulfan 0.6 µg/l
mean	56.9	64.1	82.7
s.d.	34.0	49.1	49.6
n	17	17	18

adults. Fox & Matthiessen (1982) showed that Botswanan tsetse

control operations resulted in endosulfan concentrations in water of 0.5-4.0 µg/l for up to 10 days after spraying, so it seems likely that the reduction in recruitment of Tilapia rendalli in sprayed areas was at least partly due to very heavy fry mortality.

The absence of an inhibitory effect of 0.5 µg/l endosulfan on breeding in mature S. mossambicus may also be attributable to the influence of size. Another possibility is that endosulfan acts on the physiological processes that trigger breeding behaviour in juveniles and cannot affect the behaviour once it is in progress. However, testosterone, and especially 11-ketotestosterone, which are known to be involved in the control of reproductive development in male fish (Hunt et al. 1982; Kime & Hyder 1983), are abundant in the blood of endosulfan-exposed developing male S. mossambicus. This suggests that the onset of breeding behaviour may be retarded by some other mechanism.

It is well-established that stress in fish, including exposure to pollutants, is characterised by generalised physiological changes including elevation of circulating cortisol and adrenalin (Pickering 1981). Among many other effects, adrenalin causes the pigment granules in skin melanophores to aggregate in the centre of each cell, thereby making the fish go pale (Love 1980). This is sufficient to explain the lack of reproductive colour patterns observed in endosulfan-exposed S. mossambicus, although other processes may also be operating. It has been demonstrated by Pandey et al. (1981) that sub-lethal doses of malathion also cause melanosome aggregation in S. mossambicus, although dichlorvos apparently has the opposite effect in this species (Rath & Misra 1980).

Whatever the mechanism of delayed breeding behaviour in males exposed to 0.5 µg/l endosulfan, the delay was probably the reason for females to abort their presumably unfertilized clutches, while those fry which hatched were immediately exposed to a lethal endosulfan concentration. There was no evidence that egg development was significantly retarded in poisoned females, although this has been observed in fenthion-poisoned Tilapia leucosticta (Kling 1981). No deleterious effects in either sex were observed at 0.2 µg/l endosulfan, so the no-effect threshold lies between 0.2 and 0.5 µg/l.

In the light of the laboratory studies, it is possible that the lack of T. rendalli nests in sprayed areas of the Okavango Delta was also due to a retardation in the development of male breeding displays at a point when many fish were reaching maturity for the first time. Although the present study and other work (Matthiessen 1981) shows that cichlids can acclimate to low levels of endosulfan, it may be that environmental conditions in the Okavango were changing too rapidly to permit successful breeding at a later date.

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