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Effects of Chronic Exposure to Zinc on Reproduction in the Guppy *(Poecilia reticulata)*

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Low levels of zinc (between 10-200 μg/l)are common in fresh water (KEHOE et al. 1944, O'CONNOR et al. 1964). These are often the result of weathering and erosion of ore deposits. The presence of zinc concentrations that are much higher than these background levels (SPRAGUE et al. 1965, KOPP and KRONER 1967, SAUNDERS and SPRAGUE 1967) may cause serious pollution problems that could damage aquatic ecosystems. Weathering of mine refuse heaps; effluents from ore-processing and other zinc industries such as those involved in zinc coating, rubber manufacturing and paper processing are the major sources of zinc pollution.

Much information has been accumulated on the toxicity of zinc to fish (WESTFALL 1945, DOUDOROFF and KATZ 1953, LLOYD 1960, MOUNT 1966, HODSON 1974). In most of the more recent studies (CRANDALL and GOODRIGHT 1962, BRUNGS 1969, SPRAGUE 1970, BENGTSSON 1974a, SPERANZA et al. 1977) the emphasis is on the effects of sublethal levels of zinc and these studies have shown that concentrations which do not kill fish could, during chronic exposure, produce limiting effects that range from decreased growth rates to markedly reduced reproductive potentials.

The present investigation was undertaken to examine the effects of chronic exposure to sublethal levels of zinc on reproduction in the guppy, *Poecilia reticulata*. It differs from previous studies in that it involves a viviparous fish and the effects of zinc on the development of the young, without a direct contact with the eggs, can therefore be examined.

MATERIALS and METHODS

Females used in this study were 5-month old virgins that were 33.3±3.0 cm ($\bar{x}$ ± 1 standard deviation) in standard length (from the tip of the snout to the end of the vertebral column). They were raised in the laboratory. It was necessary to use virgin females because brood size (=number of young in the brood) in the guppy shows a cyclic variation, the value increasing to a maximum and then decreasing to a minimum (ROSENTHAL 1952).

The test system was static and tanks held 4.5 litres of test solution each. Dilution water was distilled water to which

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sodium chloride, potassium bicarbonate and calcium chloride were added in the following respective amounts: 0.03g/l, 0.04g/l and 0.1g/l. Three zinc concentrations, 1.93, 0.97 and 0.48 mg/l zinc (nominal values), were studied. These respectively represent 0.4, 0.2 and 0.1 of the 10-day LC50 that was determined for adult guppies in a previous experiment (Uviovo 1978). Reagent-grade zinc sulphate ($ZnSO_4.7H_2O$) was the source of zinc ions.

To begin the tests, 20 tanks were washed with detergent, rinsed with 70% ethyl alcohol and rinsed twice with distilled water and 4.5 litres of dilution water was added to each. All tanks were then placed in water tables where the temperature was maintained at 25.5°C by YSI thermostatic control units that were connected to heater elements. The light was a 30W cool white fluorescent tube on a 12L/12D photoperiod. The tanks were gently aerated to maintain adequate oxygen levels. One female was placed in each of the tanks and each tank was randomly assigned to one of four groups. The females were then paired with selected males and they remained paired throughout the experiment. The selection of males was on the basis of courtship display. Males showing less than nine displays per minute were not used. Because parent guppies would eat their young, each pair was put into a breeding cage that was suspended in the tank. The cages were made of nylon netting with 3mm appertures.

One day after the fish were paired, the three test concentrations of zinc were established in five tanks for each concentration (1.93, 0.97 and 0.48 μg/l). The remaining five tanks served as controls. To minimize handling the fish, one-half of the test solution in each tank was renewed on Mondays and Thursdays. Zinc determinations were made on acidified 7-day composite samples using a Jarrell-Ash (Fisher) Atomic Absorption Spectrophotometer.

Fish were fed twice daily, from Monday to Friday, on a dry food preparation, Tetramin.

The experiment was run for four months, during which time four broods (representing four parturition periods) were produced. The record kept for each female consisted of the brood size, the date on which the brood was produced (from which the intervals between broods were calculated), and the standard length and condition of young at birth.

Significance was accepted at $P \leq 0.05$.

RESULTS

Measured zinc concentrations were less than the nominal values in all test tanks and the mean values 1.70, 0.88 and 0.36 μg/l zinc were used to characterize the tests.

During the course of the experiments, two female fish, one in 0.36 μg/l zinc and the other in 1.70 μg/l zinc, died in the sixth and seventh weeks respectively. Neither fish was replaced.

Zinc had no effect on the intervals between broods; between group differences at any parturition period were not significant and although the intervals were shortest between pairing and the production of the first brood (Table I), within group differences were also not significant.

TABLE I

Effect of chronic exposure to zinc on the interval (days) between broods over four parturition periods.

Group	1[2]	2[3]	3[3]	4[3]
Control	33.2	35.2	35.6	33.4
0.36 μg/l	32.0	31.0	32.8	35.5
0.88 μg/l	29.2	35.6	34.8	37.8
1.70 μg/l	29.4	35.8	35.8	30.8

1. Mean values.
2. The interval represents the time between pairing and the appearance of the first brood.
3. The interval represents the time between the appearance of young from a female in successive parturition periods 1-2, 2-3, 3-4.

The total number of young produced per female was affected by zinc (Figure 1). Exposure to 0.36, 0.88 and 1.70 μg/l zinc caused a 24, 58 and 50% reduction respectively. The reduction was significant when fish exposed to 0.88 and 1.70 μg/l zinc were compared with either control fish or those exposed to 0.36 μg/l zinc. The effect was not due to a decreased frequency of brood production in fish exposed to the higher zinc concentrations since all fish produced four broods. Rather, it was the result of a marked reduction in the brood sizes of the females exposed to the two higher zinc concentrations.

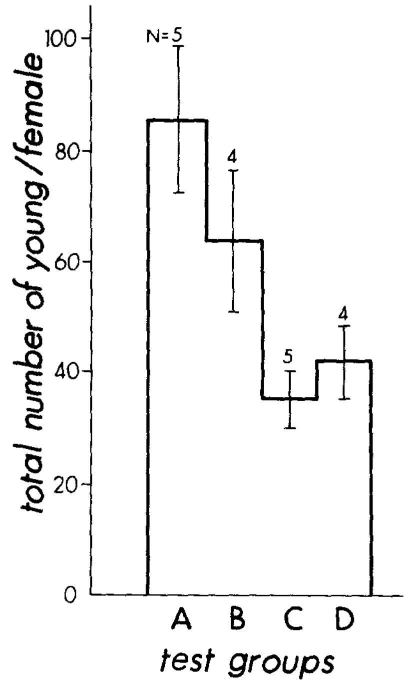

Figure 1. Effect of chronic exposure to zinc on the total number of young produced per female in four parturition periods.
Values are $\bar{x}$±1SE, A = control, B = 0.36 μg/l zinc, C = 0.88 μg/l zinc, D = 1.70 μg/l zinc.

Between group comparisons during each of the four parturition periods show that the differences in brood size were not significant in the first parturition period (Figure 2). In the second parturition period, females in the two higher zinc concentrations produced significantly smaller brood sizes than control females and those exposed to 0.36 μg/l zinc. In each of the third and fourth parturition periods, brood sizes in all three zinc concentrations were not significantly different and only those in the two higher zinc concentrations were significantly different from the control.

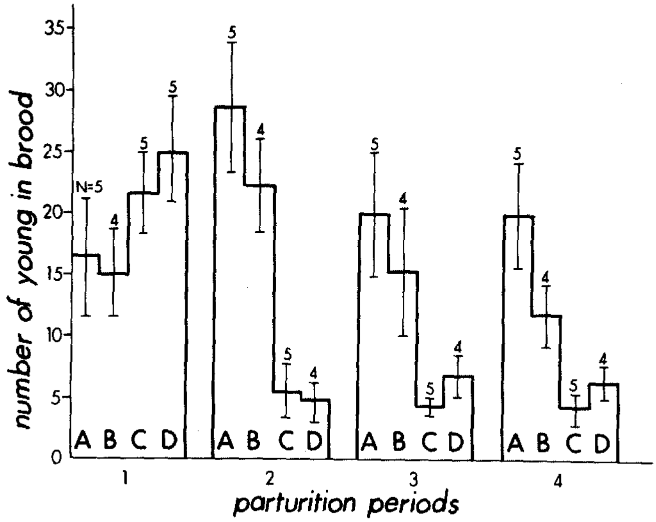

Figure 2. Changes in the brood sizes of female guppies during chronic exposure to zinc during four parturition periods.
Values are $\bar{x}$±1SE, A = control, B = 0.36 μg/l zinc, C = 0.88 μg/l zinc, D = 1.70 μg/l zinc.

Within group comparisons show that after the first parturition period, the brood sizes of females in 0.88 and 1.70 μg/l zinc decreased significantly (Fig.2). Thus during the second, third and fourth parturition periods, brood sizes of these females were only 25, 19, 19 and 18, 27, 25% respectively of the values at the first parturition period. Within group comparisons for control periods and those exposed to 0.36 μg/l zinc were not significant.

Only live young were taken into consideration when recording the brood size. In all zinc concentrations, dead young were produced and these had the following abnormalities: they were less than 4 mm long, had not absorbed the yolk completely and had not uncurled from the embryonic posture. These individuals represented 4, 10 and 4% of the total number of young produced in 0.36, 0.88 and 1.70 μg/l zinc respectively. In 0.88 μg/l zinc, there were three cases in which the fertilized egg had not developed into a distinguishable embryo. Whenever the abnormal young were produced, the brood also contained live and apparently normal individuals. There were no dead or abnormal young produced by control females.

Dead young were also excluded from the determination of standard length of young at birth. Over the four parturition periods, females in 0.88 and 1.70 μg/l zinc produced significantly smaller young than control females and those in

0.36 μg/l zinc (Fig.3). These differences occurred as early as the first broods. Within group comparisons between any parturition periods were not significant.

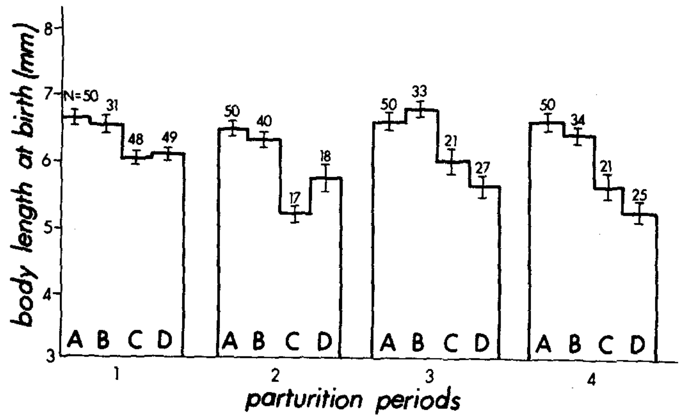

Figure 3. Effect of chronic exposure to zinc on the standard length of young at birth during four parturition periods.
Values are $\bar{x}\pm1SE$, A = control, B = 0.36 μg/l zinc, C = 0.88 μg/l zinc, D = 1.70 μg/l zinc.

DISCUSSION

The present investigation reports that chronic exposure to 0.88 and 1.70 μg/l zinc significantly reduces brood size in the guppy. The brood sizes recorded for test fish are not unusual and can occur under normal conditions (ROSENTHAL and ROSENTHAL 1950, ROSENTHAL 1952). The unusual feature was that most of the fish exposed to these concentrations consistently produced smaller brood sizes.

BRUNGS (1969) observed a significant decrease in fecundity of the fathead minnow, *Pimephales promelas*, during a 10-month exposure to 0.18, 0.34, 0.68 and 1.38 μg/l zinc. Although the two highest concentrations appear comparable to those reported in the present investigation, the former were only 0.07 and 0.14 respectively, of the 96-hour LC50 determined by Brungs. The two studies can not be compared because of differences in the duration of the acute and chronic tests.

A direct relationship between fecundity and the amount of food consumed has been demonstrated in the rainbow trout (SCOTT 1962, BAGENAL 1969), and in the guppy (HESTER 1963). Although the present investigation did not quantify food consumption, information in the literature (CRANDALL and GOODNIGHT 1962, CAIRNS and LOOS 1967, BENGTSSON 1974a) seem to indicate that exposure to sublethal levels of zinc could reduce the amount of food consumed. Also, HILTIBRAN (1971) demonstrated a phosphate 'uncoupling' effect for low levels of zinc in the mitochondria of the bluegill (*Lepomis macrochirus*) liver.

From the above, it can be seen that zinc could decrease energy availability in exposed fish. Chronic exposure to zinc has been shown to cause degenerative changes in fish (CRANDALL and GOODNIGHT 1963, BENGTSSON 1974b). The question of additional tissue repairs therefore arises in exposed fish. Adjustment to the presence of the toxicant could call for the establishment of some kind of a detoxicating mechanism. This might be energy-consuming. If energy transformation is impaired while the energy cost of metabolism and tissue repair rises, it becomes obvious that exposed fish can only budget comparatively smaller amounts of energy for non-maintenance processes including reproduction.

Nutrients required by the developing embryos for maintenance metabolism come from the mother guppy (SCRIMSHAW 1945). If the energy transformation of the mother is impaired, the amount of nutrients made available to the young and the number of young she can therefore successfully support could be reduced. In the present investigation, some abnormal young were produced by exposed females. Apart from being dead, these were smaller, had not uncurled from the embryonic posture and the yolk had not been completely absorbed. Since superfetation does not occur in the guppy (TURNER 1937), it is probable that the toxicant had the effect of slowing down, arresting or even reverting (dedifferentiation) development. As ROSENTHAL and ALDERDICE (1976) have pointed out, arrested development could result "if the available energy budget is sufficient only for maintenance of cells in their current state of organization". One would therefore expect dedifferentiation to occur if the available energy is not sufficient to maintain the tissues at that state of development.

The developing young could accumulate zinc from the mother during oogenesis or later in development, because of the intimate vascular connection between mother and embryo (PURSER 1938, SCRIMSHAW 1945). If the levels are sufficiently high, zinc could exert the same 'uncoupling' effect that HILTIBRAN (1971) has shown in the bluegill.

Decreased energy availability in the developing guppy, resulting from one or more of the factors mentioned above could also account for the decreased size of young at birth in exposed fish.

The present study indicates that the effect of zinc on the size of young at birth is a very sensitive parameter, occurring as early as the first brood. The interval between broods is the least sensitive probably because of the large variation that guppies show in nature (TURNER 1937, YAMAGISHI 1976), with respect to this parameter.

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