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# Studies on Intraperitoneal Toxicity of Lead to Cichlasoma Nigrofasciatum (Guenther) Development

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Reports on toxicity testings often overlook the effect of modifying influences from the test animals and the environment. Effluent discharges and some pollutants act on the gills and death occurs by a combination of chemical and physical injuries rather than by true toxicity ( ELLIS 1937 ). Fishes die from heavy metal poisoning due to mucous precipitation, anoxaemia through respiratory and circulatory failures and interferance with the excretory functions of the gills ( CARPENTER 1927, JONES 1938). Analysis for cobalt and manganese in carps killed by these solutions and the injection of three times the concentrations of cobalt and three hundred times that of manganese into healthy carps did not lead to death ( LLOYD 1965 as was reported by SCHWEIGER ). Epithelial gill lamellae effect the tolerance of fish to heavy metal poisoning and the presence of calcium ions modify lead toxicity ( JONES 1938).

As of now, limited reports are available on the effect of lead ions on the eggs and larvae except works of CRANDALL AND GOODNIGHT (1963) on the viviparous guppies' eggs and larvae, DILLING et al. (1926a, 1926b) on the frogs' eggs and larvae, plaice' eggs and larvae, and DAVIES et al. (1976) on the rainbow trouts, Salmo gairdneri Richardson.

It seems desirable to determine the true toxicity of lead by removing resistances from the environment and the gills by dosing lead i.p to Cichlasoma nigrofasciatum Guenther. Lead was administered in the form of lead(II) acetate trihydrate ( sugar of lead). The influence of injected lead was assessed using laboratory fish production index (LFPI) established by MOUNT AND STEPHAN (1967), and used by BRUNGS (1969). But some modification was made so as to see whether the parent fish passed lead toxicity to their offspring without some metabolic turnover (detoxication).

## MATERIALS AND METHODS

Healthy pairs of Cichlasoma nigrofasciatum of both sexes were used in the control and the experimental group. Wet weights ranged from 6 g to 22 g for the males and from 2 g to 10 g for the females. The concentrations of lead were prepared in non-toxic distilled water and the control were given only distilled water, but they showed no ill-effect so the non-toxicity of distilled water was proved. Concentrations

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of 50, 100, 300, 400, 500, and 600 ppm by weight of lead alone were made and 1 ml per 100 g body weight formed the basis of injection after 24 h acclimatization without food. Feeding was on a mixture of life tubifex interchangeable with dry fish foods three times a day ad lib. The fish ages ranged from 6-9 months as was ascertained from local delivery shops and as far as was concerned they were unmated before.

Breeding and ovulation vessels were glass aquaria 45 x 30 x 30 $cm^3$. They were eight in numbers and one aquarium housed the control. The vessels were equipped with gravelled bottom filters topped with black stones. Artificial aeration was maintained but natural light was cut off while flourescent tubes supplied light regime of 12 h Light and 12 h Darkness from 6 am to 6 pm. Thermostatically controlled heaters regulated temperature at 25 $\pm$ 0.5 $^{o}C$. Aquarium water was (1:1) distilled and tap, later to be replaced with only distilled water.

The eggs spawned on the stones were recovered by rubbing off into dishes filled with water but if the eggs were on the filter or glass bottom, they were retrived by suction siphoning. Hatching of the eggs occurred in glass dishes filled with water and suspended in fish net breeders partially immersed in water of the same temperature as the breeding vessels. Daily inspection and cleaning of the dish together with water renewals reduced the risk of fungal attack and the accumulation of metabolites. The hatchings were allowed in small batches to avoid overcrowding and prompt removal of dead eggs and dead larvae reduced the chances of infection. Spawning response, embryonic mortality, incubation velocity, fry viability and the sizes of the larvae at the time of hatch were studied as they form the most meaningful laboratory index open to observation (LFPI). Light microscopic examinations were made on the fry and chronometric exams were carried out on the heart pulses. Three or more ovulation cycles were studied for each set and the observations were terminated once the fry were one week old.

## RESULTS

### Spawning response

The onset of spawning activity was preceeded by changes in appearance in the females irrespective of the administration of lead. Intensive golden-yellow coloration mingled with greenish operculae and protruded gonadopores signified ovulation readiness. Selection and clearing of the spawning site was the joint action of the pair. Deposition of eggs took about two hours and fertilization took about similar

time. Zebra ciclids show maternal care over their young but the task of looking after the eggs was the sole responsibility of the female. Lead never affected this instinct.

Ovulations occurred in Zebra ciclids at the intervals of two to three weeks if feeding was regular.

TABLE 1

Effect of Lead on the Rhythmicity of Ovarian Cycles

| Cycles | (i.p) ppm Pb/100 g body weight 0 | 50 | 100 | 200 | 300 | 400 |
|---|---|---|---|---|---|---|
| I. | 22 | 27 | 15 | 25 | 22 | |
| 2. | 14 | 28 | 18 | | 27 | |
| 3. | 14 | 37 | 29 | | | |
| | | (5) | (0) | | (62) | (62) |

Figures in parentheses indicate days of termination of the experiment after the last ovulation.

Lead affected ovulation and its frequency. Infrequent ovulations occurred in the subsequent cycles and with the increased doses of lead administered. One 300 ppm female failed to ovulate 62 days after the last ovulation and another 400 ppm female had a lapse of 62 days. Lead is generally mobilised from the tissues to more delicate areas at the height of physiological activities.

Toxicity of Lead

All the doses of lead injected were toxic more especially to the females which experienced ovulation stresses, loss of muscular coordination and balance, irregular swimming bouts, swollen stomachs and finally falling to the bottom followed by death. Higher doses like 500 ppm and above brought about drastic burnt-like scaling followed by tissue eruption. Below 500 ppm partial erosion of tails and fins occurred and one 400 ppm female developed optic disturbance at the left eye which bulged out while another female ( same dose) developed truncal bending and was immovable.

Embryonic mortality

In the Zebra ciclids that out-lived lead toxicity,

spermiogenesis and oogenesis occurred up to 300 ppm lead. Although the frequent regular periodicity of ovulation was affected, the quantities of eggs laid were not affected. Lead affected their qualities. Egg mortality was higher before hatching than at the hatching and the percentage egg mortality calculated from the ovulated eggs was equally high. The percentage larval mortality calculated from the hatched eggs was high. Egg mortality influenced hatching success.

TABLE 2

Effect of Lead on the Percentage Mortality, Hatchability and Larval Survival

| | Cycles | (i.p) ppm Pb/100 g body weight 0 | 50 | 100 | 200 | 300 | 400 | 600 |
|---|---|---|---|---|---|---|---|---|
| Egg mortality | 1. | 2 | 31 | 69 | 72 | 73 | 79 | 56 |
| | 2. | 10 | 48 | 70 | 94 | 61 | | |
| | 3. | 7.5 | 86 | 73 | | 86 | | |
| Larval hatch | 1. | 98 | 69 | 31 | 28 | 27 | 21 | 44 |
| | 2. | 90 | 52 | 30 | 6 | 39 | | |
| | 3. | 92.5 | 14 | 27 | | 14 | | |
| Larval | 1. | 5 | 10 | 24 | 90 | 25 | 40 | 58 |
| mortality | 2. | 10 | 61 | 33 | 58 | 68 | | |
| | 3. | 23 | 96 | 76 | | 64 | | |
| Larval | 1. | 95 | 90 | 76 | 10 | 75 | 60 | 42 |
| survival | 2. | 90 | 39 | 67 | 42 | 32 | | |
| | 3. | 77 | 4 | 24 | | 36 | | |

Increased embryonic mortality occurred in the subsequent ovulations and with the increased concentrations of lead administered, and toxicity climaxed in the eggs and fry.

Incubation velocity and hatching

Incubation occurred between 3-4 days and it was not affected by lead. Successful hatching was determined as the ability of the pro-larvae to free themselves totally or partially from the egg shells and swim about. Lead affected successful hatching since egg mortality was high in the leaded fry when compared with the control.

Fry viability and larval sizes

Lead affected the survival of the fry. The percentage larval survival calculated from the original ovulated eggs in each of the three cycles were:
0 ppm 98.86%, 80.46%, and 71.66%
50 ppm 61.87%, 20.22%, and 0.54%
100 ppm 23.7%, 20.22%, and 6.51%
200 ppm 2.67%, and 2.6%
300 ppm 20.13%, 12.27%, and 5.17%
These figures were not statistically significant at 95% C.I. No major differences in sizes occurred in the fry provided no tail or tail fin cuttings occurred.

Induced abnormalilty in the fry

Lead induced the following to some dead and live fry: deformities in the brain and optic anlagen and trends towards anencephalus, lack of formation of the eye ( anophthalmia ) and some cases of reduced eyes ( microphthalmia ). There were cases where the head piece could not be raised above the yolk. Resorption of yolk was so poor that yolk cells were seen even when covered by interguments. Erosion and atrophy of the tails and fins, lordoscoliosis and body serration occurred. Melonogenesis was interfered with. Cardiac tonicity was so lowered that the leaded fry gave 123 heart pulses per minute while the normal gave 143 per minute. This decreased heart pulsation was apperantly a prelude to heart failure. Blood and its circulation was impaired. In some fry blood was not observed to be circulating even if the heart was beating. Lead inhibits the working of delta-aminolevulinic acid dehydratase, a red blood cell maturation factor.

## DISCUSSION

Relatively nothing is known on i.p effect of lead on fish ovulation in general. However, all writers on lead poisoning call attention to sterility and abortion in both animals and man ( BELL 1924, BELL et al. 1925, COLE AND BACHHUBER 1915, WELLER 1915, DILLING et al. l.c., FERM AND CARPENTER 1967, CHISOLM 1971, HANZLIK AND PRESHO 1923, and KARNOFSKY AND RIDGWAY 1952).
The idea that lead is the active agent in a poor

marine biota derives from LLOYD ( 1965 ), DAVIES et al. (1976), and ARONSON (1971). Apart from the modifying influences to lead toxicity offered by calcium ions and the gills, internal environment modified the toxicity of lead given which would have been lethal to most fishes. The cases of black body, blackning of the tails and mucous precipitation were not obtained when lead was given intraperitoneally. However, the malformations of the head regions were in accord with the theory of CHILD ( 1915) and his school of thought that actively growing areas are sensitive to harmful influences. Accord for this came from HAMMETT AND WALLACE (1928) and KARNOFSKY AND RIDGWAY (1952) who reported hydrocephalus and anterior meningoceles in chick embryos (i.P) injected with lead. CRANDALL AND GOODNIGHT ( 1963) reported granulocytes and lymphoid cells in guppies exposed to lead. DAWSON (1935) reported injury to mature erythrocytes in catfish exposed to chronic lead poisoning. BELL (1924) reported lowering of heart tonicity due to lead.

HALVER (1968) reported that witholding of essential amino acid tryptophan and vitamin C to the diet of coho and sockeye salmons caused lordoscoliosis. Accord came from SHANKS et al. (1962) who reported lordoscoliosis in rainbow trout deprived of tryptophan. Lordoscoliosis disappeared on restoration of tryptophan to diet. Exposure of man to lead increased 2-3 times the requirements in ascorbic acid. Oral administration of lead to guinea pigs reduced their adrenalin vitamin C content 37% as compared with the normal. FATI (1961) observed that lead inhibited tryptophan metabolism.

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