Bull. Environ. Contam. Toxicol. (1989) 43:858–863

Bulletin of Environmental Contamination and Toxicology

Effects of Lead on the Spawning Potential of the Fresh Water Fish, *Anabas Testudineus*

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An accelerated release of heavy metals into the environment particularly due to rapid industrialization and technology poses serious water pollution problem due to toxic properties of these metals. Lead is one such toxic heavy metal which is known to accumulate in tissues of fishes (Reichert et al 1979). This accumulation may have deleterious effects on fish reproduction. The effects of other heavy metals like zinc and mercury on fish reproduction has been reported (Uvivo and Beatty 1979; Ram and Sathyanesan 1983). The studies on the effects of lead on fish reproduction are meagre (Katti and Sathyanesan 1986).

Preliminary experiments in our laboratory have shown that lead nitrate exposure caused reduction in the fecundity of Anabas testudineus. Hence it was proposed to study the spawning potential of the fresh water fish, Anabas testudineus, on exposure to different concentrations of lead nitrate.

MATERIALS AND METHODS

Fresh water fish, Anabas testudineus, were collected from Kolleru Lake (16° 32'N to 16° 47'N Latitude and 81° 5' to 80° 21'E Longitude) in Andhra Pradesh, India, and were acclimated to the laboratory conditions for 2 wk. Anabas is a seasonal breeder and is known to spawn in September and October months. The experiments were started on September 1, 1988, during which period the fish were fully mature with large number of oocytes. Before the start of the experiment, several female fish in the weight range of 40-45g and length 11-12cm were sacrificed and the total number of eggs present in the ovary were counted and gonosomatic index was calculated using the formula:

$$\frac{\text{Wet weight of ovary}}{\text{Wet weight of fish}} \times 100$$

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Anabas was exposed to different concentrations of lead nitrate (BDH, Analar, India) and LC 50 value was determined by Probit method (Finney 1964). The LC 50 value for 96 hr was found to be 60ppm. Mature females of uniform size (40-45g) and length (11-12cm) were exposed to sublethal concentrations of lead nitrate (1.25, 2.5, 5.0 and 20.0mg/L) for a period of 30 d. One set without the addition of lead served as controls. During the experimental period all the fish were fed with a mixture of rice bran and ground nut oil cake on every alternate day. The water in the tanks was renewed every day. At the end of 30 d of experimental period, six fish from each group were sacrificed. It is difficult to determine the number of eggs released during a breeding season in this fish. Therefore, the use of size frequency analysis of oocytes in the ovary, which has been used as indicator of spawning period in fishes as described by Shackley and King (1977) has been applied in the present study. This method allows the classification of oocytes while the count is done. At the same time inclusion of atretic oocytes in the count can be avoided. After determining the fish weight and length, ovary was isolated, ovary weight and ovary length were noted and ovaries were fixed in 5% formalin for 3 d. After 3 d four subsamples were taken and the number of ova were counted. Gonosomatic index was calculated using the above mentioned formula.

The ovary and brain tissues were separated and were dried at 110°C for one hr, followed by ashing at 500°C and digestion in nitric acid. The lead concentration in the tissue samples were determined with atomic absorption spectrophotometer (Perkin Elmer 2380). Detection limits for lead was 0.01 ug/g and recovery was 81% by this method. The drying, ashing and subsequent digestion in nitric acid and determination of lead by atomic absorption spectroscopy in biological tissues has been previously reported by various authors (Borgmann et al 1978). Data are presented as mean of six samples followed by standard error. Significance of the data was calculated using the student-t-test.

RESULTS AND DISCUSSION

Initially at the start of the experiment the average total number of eggs present in the ovary of control fish was 20,140/fish and gonosomatic index was 8.68. At the end of the experimental period, the total number of eggs present in the ovary of control fish was 7,917/fish and gonosomatic index was 4.94. The lead levels in the brain and ovary tissues were < 0.01 ug/g in control tissues. On exposure to lead nitrate the number of eggs were reduced in the ovary of the exposed fish. The ovary was small and regressed and this condition was found to be dose dependent. Gonosomatic index was significantly reduced in exposed fish

(P < 0.001 Table 1).

Lead uptake in the ovary tissue of the exposed fish was very less when compared to brain tissue and was found to increase with increase in lead concentration (Table 2).

The spawning values reached approximately 62% during the 30 d of spawning in control fish, where as in lead exposed fish spawning was 83%, 95%, 97% and 99% at the end of 30 d exposure period.

Number of ova/g body weight, ova/cm body length, ova/g ovary were all reduced on exposure to lead (Table 3).

Reproduction in female teleosts is controlled by the hypothalamo-pituitary-ovarian axis (Peter et al 1986). Localization of different nuclear centers in the brain responsible for release of gonodotropins in the pituitary has been reported for many fishes (DeLeeune et al 1985). In the fish, Clarias batrachus, exposed to lead nitrate for 5 mon, Katti , Sathyanesan (1986) reported degeneration of the nucleus preopticus (NPO) neurons and inhibition of gondal maturation and alteration in reproduction, possibly mediated through the hypothalamo-hypophysial-gondal axis. Accumulation studies indicate concentration of lead in the brain of Anabas testudineus. Such an accumulation might have altered the hypothalamo-hypophysial-gondal function resulting in the altered reproductive potential of the fresh water fish on exposure to lead.

In teleosts, corticosteriods are known to be directly involved in reproduction and high levels of circulating corticosteriods occur at spawning time in fish (Robertson et al 1961; Katz and Eckstein 1974). Exposure of the fish to heavy metals is known to elevate corticosteriods and this effect was found to be dose dependent (Shreck and Lorz 1978). As heavy metals are known to elevate corticosteriods, exposure to lead in the present study might have elevated corticosteriods thereby hastening the spawning activity of the fresh water fish, Anabas testudineus. In the control fish spawning period is known to extend for a period of 2 mon. The ovary is found to contain mature and immature oocytes which are released periodically. In the lead exposed fish, complete spawning occurred within a period of 1 mon. This increased spawning activity may result in the reduced reproductive potential due to the release of immature oocytes. However, such inferences need further work to understand the mode of action of lead on fish reproduction. Since it is only a preliminary

Table 1. Ovary nature and Gonosomatic Index of the fish before and after commencement of spawning on 30 d exposure to varying lead levels.

Concentration of Lead (mg/L)	Nature of the ovary	Gonosomatic Index ($\bar{X}$±SE)	% variation
ND (C_1)	Mature light yellow ovary with large number of oocytes occupying about 3/4 of the body cavity.	8.68 ± 0.10	--
ND (C_2)	Ovary slightly small with relatively few oocytes, occupying nearly half of the body.	4.94 ± 0.08	-43.08
1.25	Ovary small occupying about ¼ of the body cavity.	1.97 ± 0.13	-77.30
2.50	Ovary small with small number of oocytes.	1.12 ± 0.06	-87.09
5.00	Regressed ovary	0.875 ± 0.02	-89.91
20.00	Regressed ovary	0.712 ± 0.02	-91.79

Table 2. Lead levels in the ovary and brain of the fresh water fish after 30 d of exposure to varying lead levels.

Concentration of Lead (mg/L)	Lead concentration (ug/gm dry weight ovary) ($\bar{X}$ ± SE)	Lead concentration (ug/gm dry weight brain) ($\bar{X}$ ± SE)
ND (C_1)	N.D.	N.D.
ND (C_2)	N.D.	N.D.
1.25	3.43 ± 0.82	29.30 ± 0.88
2.50	4.23 ± 0.80	36.81 ± 19.11
5.00	5.66 ± 0.10	117.15 ± 16.82
20.00	8.10 ± 0.20	143.16 ± 9.36

Table 3. Effects of varying lead exposure on the spawning of the fresh water fish.

Concentration of lead (mg/L)	Total no. of eggs in the ovary	No. of ova/g body weight	No. of ova/cm body length	No. of ova/g ovary	% Spawning in 30 d.
ND (C_1)	20,740 ± 164.16	396 ± 12.43	1,571 ± 12.43	4,569 ± 162.56	--
ND (C_2)	7,917 ± 444.15	172 ± 6.81	653 ± 5.31	3,502 ± 201.65	62
1.25	3,519 ± 362.75	77 ± 8.84	272 ± 3.42	4,075 ± 208.66	83
2.50	1,199 ± 14.40	25 ± 3.37	98 ± 2.24	2,927 ± 160.04	94
5.00	603 ± 90.80	13 ± 2.79	52 ± 8.87	1,136 ± 212.69	97
20.00	241 ± 7.56	6 ± 0.31	20 ± 0.62	837 ± 5.10	99

Each value is the mean of six samples followed by standard error.
All values are significantly different at $P < 0.001$ compared with C_1
ND = not detectable, i.e., < 0.01 ug/L or ug/g.
% variation = difference of C_1 and C_2 or lead exposed animals calculated as a percent variation in relation to C_1.
C_1 = controls with lead below detection level and animals before commencement of spawning.
C_2 = controls with lead below detection levels and animals after 30 d of spawning.

observation, and as studies in this direction are still in progress, at this juncture, the observed changes in the reproductive potential are attributed either to changes in pituitary mediated reproductive activity or due to altered levels of corticosteroids in response to lead accumulation.

Acknowledgements: We are thankful to Council of Scientific and Industrial Research (CSIR) New Delhi, India for providing the fellowship.

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Received December 5, 1988; accepted May 3, 1989.