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ARSENIC INDUCED STRUCTURAL CHANGES DURING THE OVARIAN CYCLE OF A FRESHWATER PERCH, *COLISA FASCIATUS* (BLOCH AND SCHNEIDER)

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Jagdamba Prasad Shukla and Kamleshwar Pandey (1984) Arsenic induced structural changes during the ovarian cycle of a freshwater perch, *Colisa fasciatus* (Bloch and Schneider). *Bull. Inst. Zool., Academia Sinica* 23(1): 69-74. Present study deals with the structural changes during the different phases (Preparatory, mature, spawning and post-spawning) of the ovarian cycle of *C. fasciatus* exposed to sublethal concentration (14.00 mg/L for 30 days) of arsenic (III) oxide. Owing to this stress the follicular spaces became prominent and development of the IInd stage of oocyte decreased significantly ($p<0.001$) during the preparatory and mature phases. Besides these, the IIIrd stage of oocytes did not develop while the atretic structure increased numerically and a decrease in the cortical yolk vesicle has been additionally observed during the mature phase. During the spawning phase, apart from the occurrence of atretic structures and stromal haemorrhage, no appreciable change has been observed. However, no apparent change was marked during the post-spawning phase. Possible cause for these structural changes under stress has been discussed. Besides this study general changes of the fishes following arsenic oxide exposure have also been recorded.

Pollutants from the fertilizer factories *viz.* NH_3, NH_2CONH_2 and As_2O_3 in general are detrimental to the survival and growth of the fish fauna (Pandey and Shukla, 1982a, 1982b, 1983 and Pandey *et al.*, 1982). Various organic and inorganic pollutants however adversely affect the sexual maturity and reproductive physiology of the fishes (Crandall and Goodnight, 1963; Mount, 1968; Brungs, 1969; McKim and Benoit, 1972; Sanglong and O'Halloran, 1976; Saxena and Garg, 1978; Farner *et al.*, 1979; Singh and Singh, 1980; Pierson, 1981 and Shukla and Pandey, 1983). Available literatures did not add much to our understanding regarding the deleterious role of industrial pollutants particularly during the reproductive cycling of freshwater teleosts. Consequently there is an urgent need to contribute more informations related to the impact of industrial pollutants on the gonadal cycle of fishes. The present study has been undertaken to record the changes induced by arsenic oxide, a metallic pollutant present in the effluent of various fertilizer factories (Algarsamy *et al.*, 1973; Pandey and Shukla, 1979 and Pandey *et al.*, 1982), on the oogenesis and structure of ovary during its different phases (Preparatory, mature, spawning and post-spawning) in *Colisa fasciatus*, a freshwater teleost. This study on one hand provides certain new informations regarding the ovarian sensitivity to long term arsenic oxide exposure while on the other hand it deals with the physiological imbalances and consequent altered oogenesis during the ovarian cycle of this fish. Besides deleterious effects of sublethal concentration of arsenic oxide on the ovarian

histology of *Colisa fasciatus*. Observations were also made to record behavioural responses of the fishes towards the pollutional media.

MATERIALS AND METHODS

Procurement, acclimatization, measurement of the fishes, duration of the phase and the experiment run were the same as outlined by Shukla and Pandey (1983). 20 healthy female specimens (average length 6.2±1.21 cm and average weight 8.14±1.38 g) were procured periodically throughout this study. Sublethal concentration of arsenic (III) oxide (14.00 mg/L) at which no mortality occurred was detected (Shukla and Pandey, 1983) to observe its effect after 30 days, if any, on the different phases (Preparatory, mature, spawning and post-spawning) during the ovarian cycle of adult *C. fasciatus*. For histological studies small pieces of the ovaries from experimental and control groups were fixed for 24 h in Picro-Mercuro-Formal fixative (Pandey, 1979). Following the routine histological techniques, serial transverse sections (6–8 μ) were cut and stained with Heidenhain Iron Haematoxylin-eosin. Experiments in various phases were conducted at natural photoperiod and ambient temperature of water.

Diameter of the various stages of cocytes (I, II and III) during different phases was measured using oculometer. Student's '*t*' test (Campbell, 1974) was applied to know the level of significance between experimental and control with $p<0.05$ or less.

RESULTS

Under sublethal concentration of arsenic oxide, fishes showed fast movement and increased mucus secretion. *Colisa fasciatus* is since an air breathing fish, it used to gulp air more frequently from the surface of water.

There was no change observed in the histology of ovary during the post-spawning phase, however, apparent alterations took place during the preparatory, mature and spawning under arsenic stress.

During the preparatory phase, follicular spaces were prominent and the development of IInd stage of oocyte decreased significantly ($p<0.001$) (Fig. 2 and Table 1). Though the

TABLE I

Showing diameter of Ist, IInd and IIIrd stages of oocytes in μ during differet phases of ovarian cycle of *C. fasciatus* and those exposed for 30 days under (14.00 mg/L) arsenic (III) oxide

Phase	CONTROL		
	I	II	III
Preparatory	10.22+0.34	25.86±0.64	N*
Mature	10.05±0.31	27.64±0.72	56.18±1.02
Spawning	10.14±0.26	28.23±0.76	57.02±1.12
Post-spawning	10.11+0.26	21.24+0.52	N*
Phase	**EXPERIMENTAL**		
	I	II	III
Preparatory	10.08+0.26*	18.64**	N*
Mature	9.98+0.41*	21.64±0.33**	N*
Spawning	10.16±0.22*	28.98±0.29*	57.06±1.16*
Post-spawning	10.26±0.32*	21.20±0.55*	N*

Each value represents the Mean±S.E. of 30 observations
N* stands for not developed
* stands for not significant ($p>0.05$)
** stands for significant value ($p<0.001$)
Number of the fishes used 20; (10 for Control and 10 for Experimental in each phase)

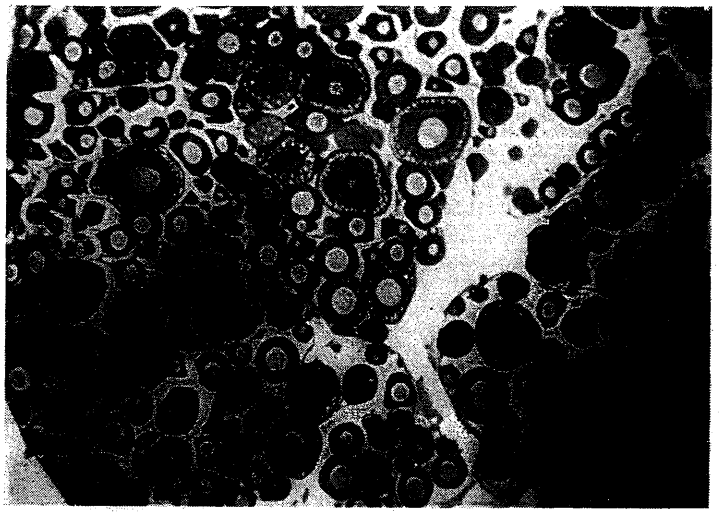

Fig. 1. Showing transverse section of normal ovarian architecture during pre preparatory, mature and spawning phase. ×100

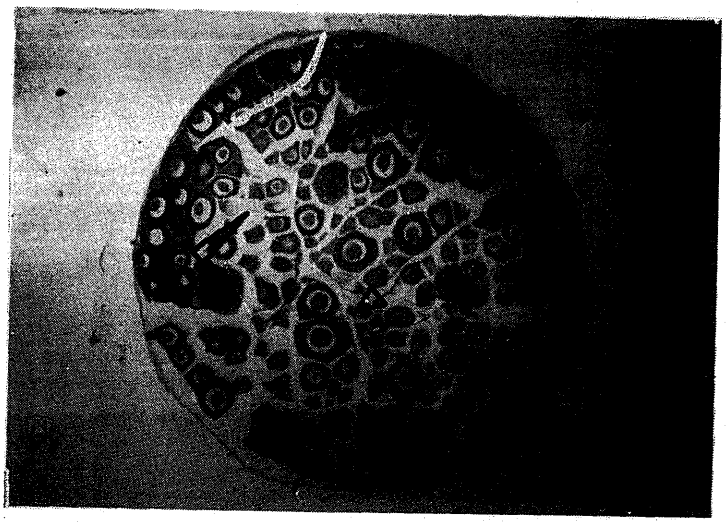

Fig. 2. Prominent follicular spaces (⊳) and decrease in the development of IInd stage of oocyte (▷) during preparatory phase under stress of arsenic oxide exposure. ×100

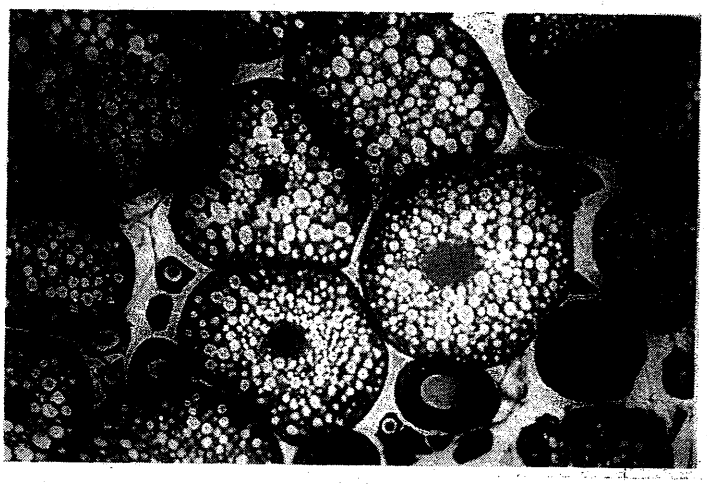

Fig. 3. Showing transverse section of normal ovarian architecture during pre preparatory, mature and spawning phase. ×100

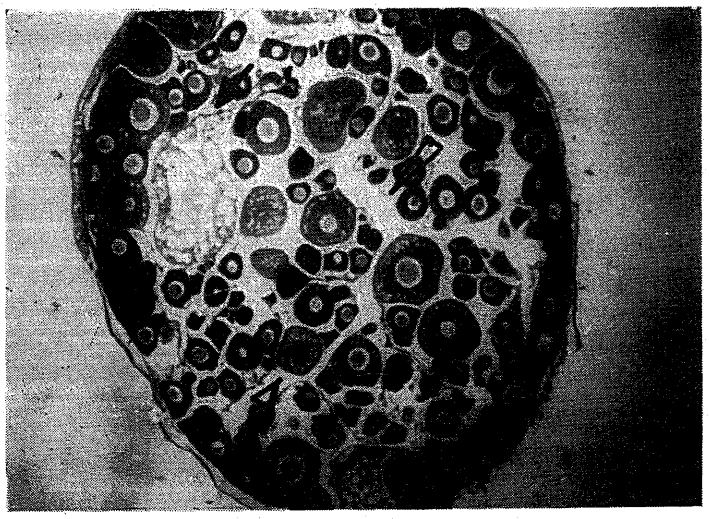

Fig. 4. Increased follicular space (|>) decreased cortical yolk vesicle (▷), prominent atretic structures (—▶) and seized development of IIIrd stage of oocyte during mature phase under stress of arsenic oxide exposure. ×100

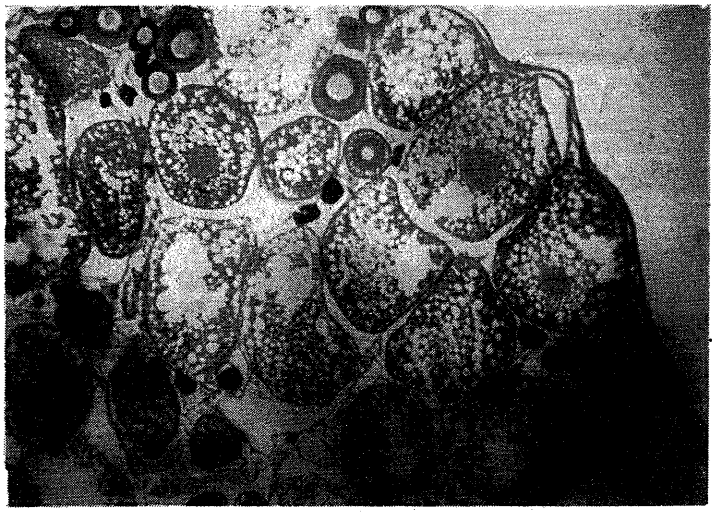

Fig. 5. Showing transverse section of normal ovarian architecture during pre preparatory, mature and spawning phase. ×100

Fig. 6. Increased atretic structures (—▷) and stromal haemorrhage (.>) during spawning phase under stress of arsenic oxide exposure. ×100

development of the Ist stage of oocyte was not affected.

There is an overall more visible effect of the arsenic oxide during the mature phase following increased follicular space and significant reduction ($p<0.001$) in the diameter of IInd stage of oocyte persisted (Table 1). Majority of the oocytes present were either in Ist or IInd stage (Fig. 4) but development of IIIrd stage of oocyte was more or less hampered (Fig. 4). Addition of cortical yolk vesicles is seemingly decreased and deposition of yolk globules was restricted to few oocytes only (Fig. 4). Atretic structures were numerically increased under stress.

Apart from the occurrence of atretic structures and stromal haemorrhage, no appreciable change has been recorded during the spawning phase (Fig. 6).

DISCUSSION

Muirhead-Thomson (1971) has reviewed certain aspects of chemical ecology of fishes and documented the importance of chemical communication in maintaining the behavioural patterns of the fishes. Todd *et al.* (1967) demonstrated that the aquatic fauna especially the fishes are sensitive to chemical signals even at low sublethal concentration and may rely extensively on this sensory input to control their attitudes and behaviour. Flarov (1974) pointed out that with the help of behaviour of the animals, one can assess the toxicological nature of the surrounding. In the present study, fast movement, increased mucus secretion and gulping more atmospheric air may well be attributed to the toxic nature of the water body containing arsenic oxide.

Informations on the effects of chemical pollutants on the gonadal structures and cyclic activity in the fishes are since meagre, the present study has been compared with those of mammals as well.

Various chemical pollutants such as lead and carbon tetra chloride in mammals have been recorded to produce a number of histological alterations viz., formation of atresia marked inhibition of follicles growth and reversal of normal ovarian function (Chaterjee, 1968 and Goyer and Rhyme, 1973).

Ellis (1944) reported severe oedema in the ovary of selinium poisoned catfish, *Ictalurous punctatus*. Saxena and Garg (1978) also reported that carbaryl and fonitrothion treatment arrested ovarian recrudescence to various degrees in *Channa punctatus*. However, carbaryl was more effective and did not allow the appearance of stage IIIrd oocytes and in the number of atretic follicles increased. Our findings including prominent follicular spaces, reduction in the development of IInd and IIIrd stage of oocyte, restricted deposition of yolk globules, increased atretic atructures and stromal haemorrhage are in conformity with the findings of above workers. It may also be inferred that the preparatory and mature phases of the ovarian cycle are more vulnerable and sensitive to chemical pollutants, as revealed from the varying degree of changes in the ovary of *C. fasciatus*. Lower degree of ovarian recrudescence during preparatory and mature phases in the fishes exposed to arsenic oxide may be attributed either to a lower level of gonadotropin secretion or decreased nucleic acids metabolism (Shukla and Pandey, 1983) may be regarded as possible reason for the non-appearance of stage IIIrd oocyte and that a high percentage of atretic follicles result.

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REFERENCES

ALGARSAMY, S. R., B. B. BHALERAO and S. RAJGOPALAN (1973) Treatment of wastes from fertilizer plants. *Ind. J. Env. Hlth.* **15**: 52-71.

BRUNGS, W. A. (1969) Chronic toxicity of zinc to fathead minnow, *Pimephales promelas* (Rafinesque). *Trans. Am. Fish. Soc.* **98**: 272-279.

CAMPBELL, R. C. (1974) *Statistics for biologists.* Cambridge University Press, London. 385 pp.

CHATTERJEE, A. (1968) Effect of carbon tetrachloride on gonadal physiology in female rats. *Acta Anat.* **71**: 82-86.

CRANDALL, C. A. and C. J. GOODNIGHT (1963) The effect of sublethal concentrations of several toxicants to the common guppy, *Lebistes reticulatus*. *Trans. Am. Microscop. Soc.* **82**: 59-73.

ELLIS, M. M. (1944) Industrial wastes and fish life. In *Proc. Ist Ind. Waste. Util. Conf.* Purdue University. 126-134.

FARMER, G. J., D. ASHFIELD and H. S. SAMANT (1969) Effect of zinc on juvenile atlantic salmon, *Salmo salar*: acute toxicity, food intake, growth and accumulation. *Env. Pollut.* **19**: 103-117.

GOYER, R. A. and B. C. RHYME (1973) Pathological effects of lead. *Int. Rev. Exp. Pathol.* **12**: 1-77.

MCKIM, J. M. and D. A. BENOIT (1972) Effects of long term exposure to copper on survival, growth and reproduction of brook trout (*Salvelinus fontinalis*). *J. Fish Res. Bd. Can.* **28**: 655-662.

MOUNT, D. I. (1968) Chronic toxicity of copper to fathead minnow, *Pimephales promelas* (Rafinesque). *Water Res.* **2**: 215-223.

MUIRHEAD-THOMSON, R. C. (1971) *Pesticides and freshwater fauna.* Academic Press, London and New York. 248 pp.

PANDEY, K. (1979) Picro-mercuro-formal: a fixative for histochemical studies. *Micros. Acta.* **82**: 45-46.

PANDEY, K. and J. P. Shukla (1979) Arsenic toxicity in a tropical freshwater fish, *Puntius sophore*. *Nat. Acad. Sci. Let.* **2**: 425-426.

PANDEY, K. and J. P. SHUKLA (1982a) Deleterious effects of arsenic on the growth of fingerlings of a freshwater fish, *Colisa fasciatus* (Bl. & Sch.). *Acta Pharmacol et Toxicol.* **50**: 398-400.

PANDEY, K. and J. P. SHUKLA (1983) Toxicity and long term effects of carbamide on *Colisa fasciatus*. *Acta. Hydrochim et Hydrobiol.* **11**: 145-149.

PANDEY, K. and J. P. SHUKLA (1982b) Hydrobiological parameters as an indicator of water pollution in Chilwa lake, Gorakhpur. *Acta Hydrochim et Hydrobiol.* **10**: 113-116.

PANDEY, K., I. M. TRIPATHI and J. P. SHUKLA (1982) Studies on the toxicity of ammonia to a freshwater fish, *Colisa fasciatus*. *J. Environ. Res.* **3**: 11-12.

PIERSON, K. B. (1981) Effect of zinc exposure on the growth, sexual maturity, reproduction and bioaccumulation of the guppy, *Poecilia reticulata*. *Can. J. Fish. Aquat. Sci.* **38**: 23-31.

SANGLONG, C. B. and M. J. O'HALLORAN (1976) Adverse effects of cadmium on brook trout testis and on *in vitro* testicular androgen synthesis. *Biol. Reprod.* **9**: 394-399.

SAXENA, P. K. and M. GARG (1978) Effect of insecticidal pollution on ovarian recrudescence in the freshwater teleost *Channa punctatus* (Bl.). *Ind. J. Expt. Biol.* **16**: 689-690.

SHUKLA, J. P. and K. PANDEY (1983) Altered nucleic acids metabolism in the ovary exposed under arsenic stress in a freshwater fish, *Colisa fasciatus* (Bl. & Sch.). *Acta Hydrochim et Hydrobiol.* (In press).

SINGH, H. and T. P. SINGH (1980) Effect of two pesticides on total lipid and cholesterol contents of ovary, liver and blood serum during different phases of the annual reproductive cycle in the freshwater teleost *Heteropneustes fossilis* (Bloch) *Env. Pollut.* **23**: 9-17.

TODD, J. H., J. ATEMA and J. E. BARDACH (1967) Chemical communication in social behaviour of a fish, the yellow bullhead (*Ictalurus natalis*). *Science* **158**: 672-673.

克斯蒂鬥魚之卵巢周期因砷引起結構上之變化

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本文記述克斯蒂鬪魚在卵巢發育周期之不同階段中（準備期、成熟期 、 產卵期及產卵後期）因暴露於亞致死濃度之氧化砷（14 mg/L，30天）而引起構造上之變化。魚體因受到此種壓力而使卵泡之空間更爲顯著，使準備及成熟期第二階段卵細胞數減少（$p<0.001$）。除外，在成熟期並額外發現到當萎縮之構造之數量大增及皮質卵黃泡減少之際，第三階段卵細胞並未見有發育之徵象 。在產卵期除發現萎縮之結構均基質出血外，其餘並無顯著之變化。產卵後期則均無顯著之變化。魚體經受壓制下引起結構上改變之可能原因已在本文中討論，並同時記載魚體因暴露於氧化砷而引起一般性之變化。