Chapter 28

Some Aspects of Cadmium Toxicity on the Vitellogenesis in a Hillstream Teleost *Garra mullya* (Sykes)

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Abstract. Effects of sublethal cadmium toxicity on vitellogenesis in a hillstream teleost *Garra mullya* (Sykes) have been described. Results indicated that cadmium produces inhibitory effects on protein synthesis mechanism of liver which is the site of vitellogenin (yolk protein) synthesis as was evident by significant fall in total protein, RNA and DNA in liver as well as reduced hepatosomatic index (HSI) in Cd-treated fish. Reduced protein mobilisation also resulted in fall of serum protein and ovary protein as well as gonosomatic index (GSI) of Cd-exposed fish. Prolonged sublethal treatment of cadmium chloride also resulted in significant reduction of oocyte diameter and total egg count per fish. Data of energy content (calorific value) of liver, ovary and muscle during vitellogenic growth of fish revealed a reduction in energy mobilisation in all three tissues of Cd-treated specimens. Cadmium caused a net reduction of calorific content by 38.5 per cent in liver and 16.5 per cent in ovary. The depletion of calorific content of muscle during vitellogenic growth was reduced by 5.6 per cent in Cd-treated fish when compared to control. Results indicated inhibitory role of cadmium chloride during vitellogenic growth of *Garra mullya*.

Key words : *Cd-toxicity, Garra mullya, liver, ovary, vitellogenesis.*

Introduction

Effects of pollutants, specially, heavy metals, on the aquatic organisms in general and fish in particular, are the area of special interest as most of the pollution causing effluents are discharged into water-bodies like ponds, lakes and rivers. Various genera of fishes found in the rivers and streams form major source of supplementary food for the rural mass who practice capture fisheries in rivers and streams. Heavy metal form several industrial, mining and other sources enormously contribute to the pollution problem in rivers and streams resulting into adverse impact on biota including fishes.

Fish population is generally considered very sensitive to all kinds of environmental changes to which it is exposed as they are exclusively aquatic with external mode of fertilization (most fresh water teleosts). Though certain stages in the life cycle of marine and fresh water fishes are more susceptible to environmental and pollutional stress (Von Westernhagen, 1968, 1970; Von Westernhagen, 1988). Rosenthal and Alderdice 1976; yet the effects on physiological and metabolic processes related with vitellogenic growth of the fish are not less significant (Mount, 1968; Hiltibran, 1971; Bengtesson, 1974; Benoit, 1975; Alderdice *et al.*, 1979 a, b; Rombough and Garside, 1982). The review of wide range of literature available on the effect of different pollutant on fish eggs and larvae (Von Westernhagen, 1988) reveals differences in susceptibility between the early developmental stages like embryos and larvae of cod, *Gadus merhua*, herring, *Clupea harengus*, plaice, *Pleuronectes platessa* (Kuhnhold 1972), Black Sea flounder, (Mazmanidi and Bazhasvili 1975) and several other marine species (Wilson, 1972).

Heavy metal toxicity has been found to cause inhibition of growth in Atlantic salmon (*Salmo salar*) when exposed to low level of toxicity (0.47 µg cd/l) while significant reduction in viable hatch has been noticed at comparatively higher level between 300 and 800 µg cd/l (Rombough and Garside, 1982). Similarly, exposure of mature fish to micro quantities of zinc, cadmium, copper and mercury has been reported to lead to the reduction upto 80 per cent in the eggs produced on some fresh water species. (Von Westernhage, 1988).

The basic requisite for successful reproduction is the production of eggs in sufficient quantities so as to preserve the species. Toxic effects of various heavy metals appear to affect this basic requirement by inhibiting metabolic activities at cellular levels (Nilsson, 1970; Hiltibran, 1971; Sax and Sax, 1974). So far Cd-toxicity is concerned, Banerjee *et al.* (1978) noticed significant changes in haematological parameters and growth as well as Cd-deposition in tissues of air breathing fish *Clarias batrachus and non air breathing fish Tilapia mossumbica*. Inhibition of egg-growth and survival due to cadmium toxicity has been reported in *Salmo gairdneri* (Beattie and Pascoe, 1978); in herring (Von Westernhagen *et al.*, 1974), in flounder (Von Westernhagen and Dethlefsen, 1975) and in garpike eggs (Von Westernhagen *et al.* 1975). But no such report is available on hillstream fish forms.

Egg yolk is the most important material which meets the nutritional requirement of developing embryos in oviparous animals including teleost fishes. In most fish the process of yolk deposition in the oocytes (vitellogenesis) is a seasonal phenomenon. Accordingly, the onset of breeding season in oviparous vertebrates in general and fish in particular is characterised by initiation of biochemical processes related to egg production. The key product in vitellogenesis is a lipophasphoprotein called vitellogenin which is synthesized in liver under the influence of estrogen produced by ovary (Plack *et al.* 1971; Emmersen and Petersen, 1976; Emmersen and Emmersen, 1976; Nath and Sundararaj, 1981 a, b). After its synthesis the vitellogenin is transported by blood to the ovary where it is cleaved into final egg yolk proteins-phosvitin and lipovitellin and deposited in oocytes as yolk granules (Tata, 1976; Ng and Idler, 1983). In accordance with this fundamental process, the initiation of breeding season in fish is marked by variations in the cellular constituents of liver, blood serum, gonad and to some extent muscle which are the results of variations in external (environmental) as well as internal (sex steroid)

factors (Diana and Mackay, 1979 Emmersen and Emmersen, 1976; Medda *et al.* 1980). As the process of vitellogenin synthesis sets in the liver the elevated levels of serum components particularly phosphorous, calcium, protein have been recorded in fish (Follett and Redshaw, 1968; Plack *et al.* 1971; Emmersen and Petersen, 1976; Emmersen and Emmersen, 1976; Nath and Sundararaj, 1981 a, b; Okuzawa *et al.* 1986; Barannikova *et al.* 1989; Quinitio *et al.*1989).

As the production of mature and viable eggs in fish in mediated by complex biochemical and metabolic processes via liver-blood-ovary axis, any change in the external environmental condition, pollutional or other wise, is most likely to act through this axis. A perusal of the available literature, however, indicate an utter paucity of research on the effects of heavy metal toxicity on the biochemical processes related to vitellogenic growth of fresh water fishes. Heavy metals like Zinc, cadmium and copper at low concentration (Cu, 3.7-31.0 µg/l; Cd, 0.6-60.0 µg/l) caused significant decrease in the spawning activity and egg numbers per female (Eaton, 1973). Zinc toxicity has been found in minnow, *Phoxinus phoxinus* (Bengtsson, 1974) and in *Brachydanio rerio* (Speranza *et al.* 1977). Effect of Cd-toxicity on Indian fresh water fishes are limited to few species and only related to histological and hematological aspects (Banerjee *et al.* 1978; Wani and Latey, 1982, 1983).

Further, relatively few works appear to have been devoted to sublethal effects. Notable among them was by Rosenthal and Alderidic (1976). According to Rosenthal and Alderdice (1976), sublethal effects may be defined as "Those responses to environmental changes-histological, morphological, physiological, or ethological – that may be induced in one stage of development but expressed at a later stage of development in terms of reduced survival potential". Thus, the term sublethal for developing fish eggs applies to the different processes and stages of development of vitellogenesis which may result in production of vitellogenesis which may result in production of non-viable egg and/or reduction in the number of viable eggs.

An individual's sublethal response always excludes immediate death, through its life expectancy may be shortened due to impairment of its morphological, histological or physiological set-up (Vonn Westgernhagen, 1988).

The present study was designed specially to study the sublethal effects of cadmium (Cd) toxicity on hill stream teleost (*Garra mullya* (Sykes). This species is a teleost fish belonging to order cypriniformes and it is very common in the rivers and streams of Chotanagpur plateau. It is also greatly relished as food in the surrounding rural areas and dominates the fish catches of local fishermen. Chotanagpur plateau is one of the richest mineral belts in the country having vast deposits of coal, iron, copper, manganese and even uranium. Mining potential of this area has resulted in the concentration of large, moderate and smaller industries in this plateau region which often discharge their effluents containing heavy metals in surrounding rivers and streams.

The objective of the present communication is to provide some information pertaining to the sublethal effects of cadmium on the basic egg producing phenomenon "vitellogenesis" in a hill stream teleost *Garra mullya* from biochemical view point.

TABLE 28.1. *Physico-chemical profile of aquaria-water during the period of experiment*

Parameters	March	April	May	June	M	SD	V	CV	SE	M at 95% CL
Temp°C	23.5	24.7	26.2	25.5	24.975	1.003	1.006	4.016	0.501	24.975 ± 1.594
pH	7.4	7.5	7.2	7.2	7.325	0.129	0.016	1.773	0.064	7.325 ± 0.203
Total hardness (ppm)	66.0	69.0	61.0	72.0	67.000	4.062	16.500	6.062	2.031	67.000 ± 6.462
Total alkalinity (ppm)	105.0	107.0	116.0	112.0	110.000	4.301	18.500	3.910	2.150	110.000 ± 6.841
Dissolved oxygen (ppm)	6.3	6.5	7.2	7.5	6.875	0.491	0.241	7.153	0.245	6.875 ± 0.779
Free carbon dioxide (ppm)	0.5	0.5	1.0	1.0	0.750	0.250	0.062	33.333	0.125	0.750 ± 0.397

M = Mean, SD = Standard Deviation, V = Variance, CV = Co-efficient of variation,
SE = Standard error, M at 95% CI = Mean at 95% confidence level.

Material and Methods

Mature specimens of *Garra mullya* were collected from the Suvarnarekha river at Getalsud, 30 km west of Ranchi town (23°20' latitude and 85°30' longitude) with the help of local fishermen. Fishes were brought to the laboratory and mature specimens of similar weight (40 ± 3g) and length (15.5 ± 1.00 cm) were sorted out. The fishes were kept in aquaria for a week in normal tap water for acclimatization. The 96 hour LC_{50} value of cadmium chloride for the experimental fish was determined by Probit analysis (Finney, 1971; Fisher and Yates, 1974), and was found to be 8.6 mg/l. The water used in the experiment had the Physico-Chemical characteristics as given in Table 28.1.

Experiment plant

Ovary of *Garra mullya* at the collection site enters the preparatory phases in February and spawning begins by mid-July (Khan and Mehrotra, 1991). Ovary remains in the maturing phase during April and eggs become ripe and ready for spawning by June end. As the objective of the present study was to find out the degree of biochemical effect on vitellogenesis, the fishes were kept in Cd-containing water from beginning of March to June end. Care was taken that fishes used for experiment were mature and of similar length and weight. As the LC_{50} value was 8.5 mg/l for cadmium chloride, sublethal dose was considered to be safe at 4.00 mg/l for producing effect

during prolonged period. Experiment was started on 1st March, 1991 and continued till June 30, 1991. About eighty specimens were used each for control and Cd-treatment. Fishes were lodged in four aquaria of similar size, each for control and treated, as keeping them in one aquaria would have caused crowding condition. All the aquaria had sufficient aeration as well as light, and water was changed weekly, Specimens were fed on *Hydrilla* Leaves and some bryophytes and lichens sticked to small stone chips.

Test fishes were lodged in control as well as $CdCl_2$ containing waters on 1st March and dissection for tissue analysis and eggs counts were carried out after 30 days in each month from March to June. Control groups were maintained under identical conditions without $CdCl_2$. Average mortality during the experiment was roughly around 5.00 per cent.

Tissue Analysis

After every 30 days, beginning from March 1, the liver, ovary and muscle of both control and treated fish were dissected out and weight. Gonosomatic index (GSI *i.e.* ovary wt/100 g body Wt) and hepatosomatic index (HSI *i.e.* liver wt/100 g body wt) were determined for each fish. About ten to fifteen fishes were sacrificed each month. Blood was collected by heart puncture. Ovary, liver and muscle samples were kept in fish saline (5.5 g NaCl, 0.14 g KCl and 0.12 g $CaCl_2$ per litre distilled water) until processed for estimation of various biochemical constituents. Few ovaries were also preserved in 10 per cent formaldehyde solution for measuring oocyte diameter and total egg counts.

Extraction of DNA, RNA, and total protein in tissues and protein in blood serum from the homogenates by the method of Munro and Fleck (1966) as modified by Abalain *et al.* (1980). The DNA and RNA contents were measured by determining the optical density at 260 nm on a UV-spectrophotometer with calf thymus DNA and yeast RNA as standards. After extraction, the total protein contents of tissues and blood serum were estimated by the method of Lowery *et al.* (1951) by measuring optical density on spectrophotometer at 620 nm with bovine serum albumin as standard.

The water contents of ovary, liver and muscle were determined by drying for 48 hrs. at 105°C in an electric oven. Ash content was measured by igniting the samples until tissues were completely burnt. Fat content was determined by using the method of Floch *et al.* (1957). Carbohydrate was estimated by difference (Eliassen and Vahl, 1982 a).

Energy Content

Energy Content of tissue were calculated assuming calorific values of 9.5, 5.7 and 4.0 $Kcal^{-1}$ for fat, protein and carbohydrate respectively (Kleiber, 1975).

Oocyte diameter and total egg Count

At least five ovaries were used each month to determine the total number of eggs per fish.

Each preserved ovary was weighed individually and the number of eggs from appropriate weighted sub-samples were counted under a dissecting microscope. The means of the total number of eggs and growing oocytes in the sub-samples were determined to calculate the total number of eggs per ovary (Abidin, 1986).

For the measurement of oocyte diameter, only those eggs were considered in which some degree of yolk deposition was noticed and it was assessed by colour, shape and general appearance, particularly yolk material under microscope (Jacob and Nair, 1983). As first sampling was done during maturing stage (March end), majority of eggs were yolk laden to different extents. Hence the measurement of mean oocyte diameter gave an overall size of the developing oocytes. For measurement of diameter, occular micrometer was used (in × 100). Random samples of 1000 eggs (ova) from each ovary of different regions (anterior, middle and posterior) were taken so as to eliminate sampling error resulting from regional differences.

Statistical analysis of all the data was carried by students 't' test (Bringing and Kintz, 1977).

Results

Sublethal treatment of cadmium chloride to the experimental fish produced a very significant effect during the prolonged treatment. As the objective of the study was to find out the toxic effects on liver-blood-ovary axis in relation to vitellogenesis, biochemical constituents like total protein of liver, ovary and blood serum, liver-RNA, Liver-DNA and gonosomatic index (GSI) as well as hepatosomatic index (HSI) were determined. These parameters provided an overall metabolic state of the fish in relation to the progress of vitellogenic process.

Biochemical effects

Results of the experiment reveal that Cd-treatment begin to produce significant effect in vitellogenin synthesis in liver from the very first month (March) of treatment as is evident from the data of total liver protein, liver-RNA liver-DNA and hepatosomatic index (Fig. 28.1 A-D). These parameters recorded decrease in the liver of treated fish in comparison to control. An analysis of the data by student t-test indicated significant change in liver protein from March itself ($p < 0.005$). The change was more significant in the following months April, May and June ($p < 0.001$). As the RNA-content is prerequisite for protein synthesis and the ratio of RNA to DNA (RNA per unit DNA) is a better index of metabolic activity (Bulow, 1970), the contents of RNA and DNA in liver were determined (Fig. 28.1 C, D). Results indicated highly significant decrease in RNA content of treated fishes during first three months ($p < 0.001$). But the level of significance was low in June ($p < 0.005$) when the protein mobilisation in liver attains decreasing trend due to near completion of vitellogenesis in oocytes. Corresponding change in the liver-DNA was also noticed and this change was also significant being $p < 0.025$ in March and $p < 0.001$ in following months. Variation in the HSI during initial period (March) was statistically non-significant while it was highly significant from April onwards ($p < 0.001$).

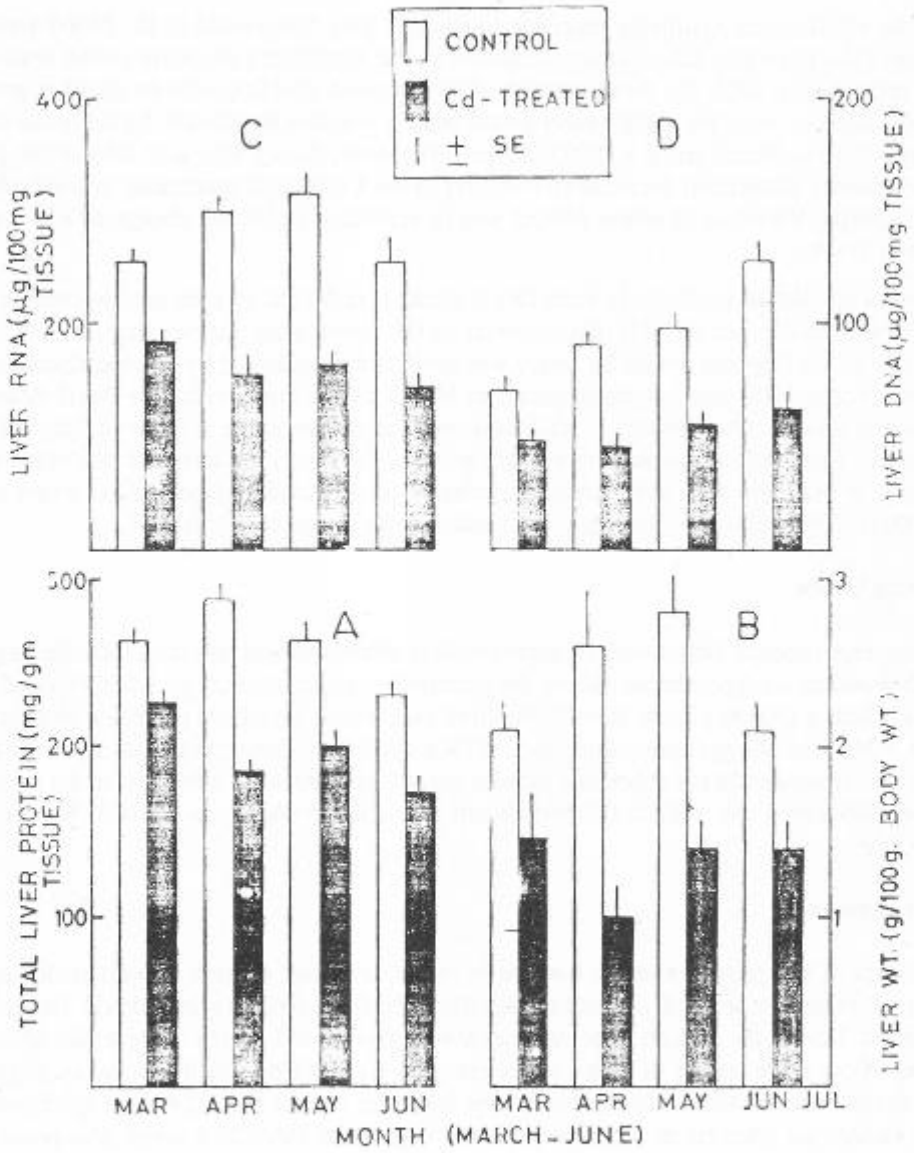

Fig. 28.1. Hepatosomatic index (HSI) and biochemical profile of liver in control as well as Cd-treated specimens between March and June.

A. Total protein content of liver.
Student's t-test values : March = $p < 0.005$;
April-June = $p < 0.001$.

B. HSI
March = Non-significant; April-June = $p < 0.001$.

C. RNA content of Liver
March-May = $p < 0.001$; June = $p < 0.005$.

D. DNA content of Liver
March = $p < 0.025$; April-June = $p < 0.001$.

As the vitellogenin synthesis proceeds in liver, it gets transferred to the blood serum for transportation to ovary. Cd-treatment resulted in the significant decrease in the total blood serum protein (Fig. 28.2, B). As the uptake of liver protein (vitellogenin) by blood is preceded by its synthesis in liver, the variations of serum protein was less significant during initial months being $p < 0.05$ in March and $p < 0.005$ in April. However, during May and June serum protein recorded highly significant decrease ($p < 0.001$) in the Cd-treated specimens in comparison to control groups. Variation of serum protein was in accordance with the change of liver protein, RNA and DNA.

Ovarian up-take of vitellogenin from blood serum is reflected by total protein content of the ovary, gonosomatic index (GSI) and diameter of the developing oocytes (Fig. 28.2 A, C, D). Initial rate of vitellogenin uptake by ovary was slow as was indicated by non-significant change of ovary protein, GSI and oocyte diameter in March and to some extent in April. Maximum turnover of total protein through liver-blood-ovary axis was in the months of May and June when ovary recorded maximum vitellogenic growth. However, the turnover rate significantly decreased in May and June in Cd-treated specimens as was evident from data of ovary protein ($p < 0.001$, 0.005), GSI ($p < 0.0005$, 0.001) and oocytes diameter ($p < 0.005$).

Total Egg Count

As the experimental fishes were of approximately similar weight and size, the total egg count per fish provided an approximate data on the quantitative production of egg when exposed to Cd-toxicity. Data of total egg count in each month of experiment have been presented graphically in Fig. 28.3. Number of eggs during initial period (March) did not change significantly in Cd-treated specimens. However, in the following months there was noticeable reduction in the number of eggs per fish which was statistically significant as $p < 0.005$ (April), $p < 0.001$ (May) and $p < 0.025$ (June).

Energy Content

The data of fat, protein and ash content in ovary, liver and muscle as well as dry and wet weight of these tissues did not differ significantly ($p > 0.05$) in individual fishes under experiment. Hence, the data on these parameter were pooled and energy content was determined in terms of per gram tissues of both control group as well as Cd-treated specimens. Figs. 28.4-28.6 represent the per gram composition of wet tissues in control and Cd-treated specimens. The energy budget per gram tissue during experiment is given in Table 28.2 which also provides the total energy changes of all three tissues (liver, ovary and muscle) during the 4 months alongwith percent changes between March and June. Monthwise data indicate that while liver and ovary were affected by Cadmium Chloride, the muscle remained almost unaffected. However, the net energy changes between April and June indicate a different energy profile. In control group the mobilisation of energy in ovary is maximum (38.5%) between the period of experiments. But liver shows lesser mobilisation (8.2%) and muscle exhibited depletion (18.6%) in terms of

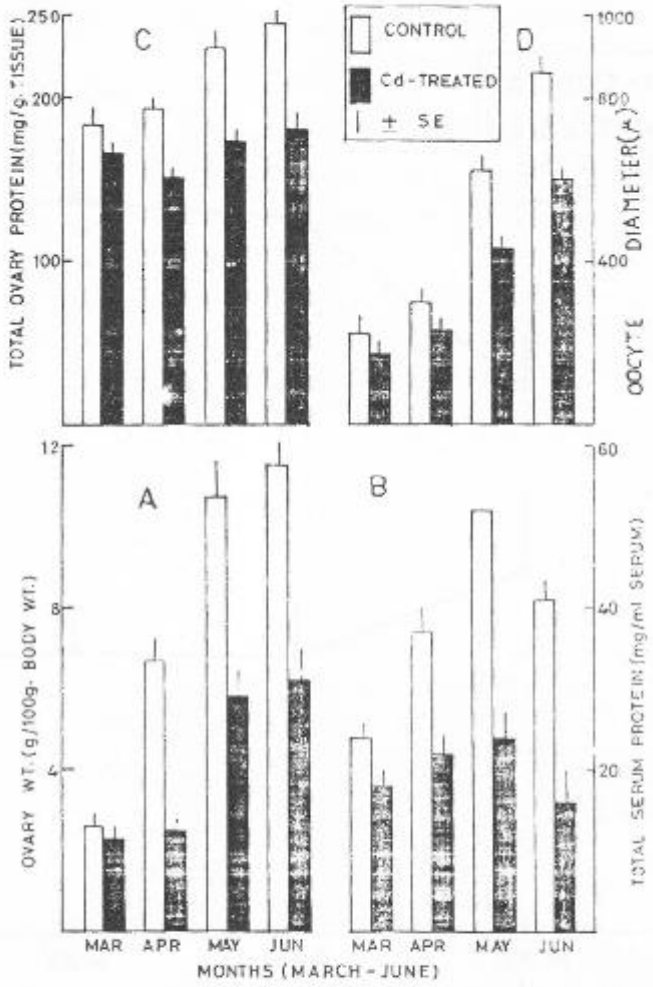

Fig. 28.2. Data of ovary and blood serum in control and Cd-treatd specimen between March and June.

A. Gonosomatic index (GSI)
March = Non-significant; April = $p < 0.001$;
May = $p < 0.005$; June = $p < 0.001$.

B. Total serum protein
March = $p < 0.05$; April = $p < 0.005$;
May-June = $p < 0.001$.

C. Total ovary protein
March = Non-significant; April-May = $p < 0.001$;
June = $p < 0.005$.

D. Oocyte diameter
March-April = Non-significnat; May-June = $p < 0.005$

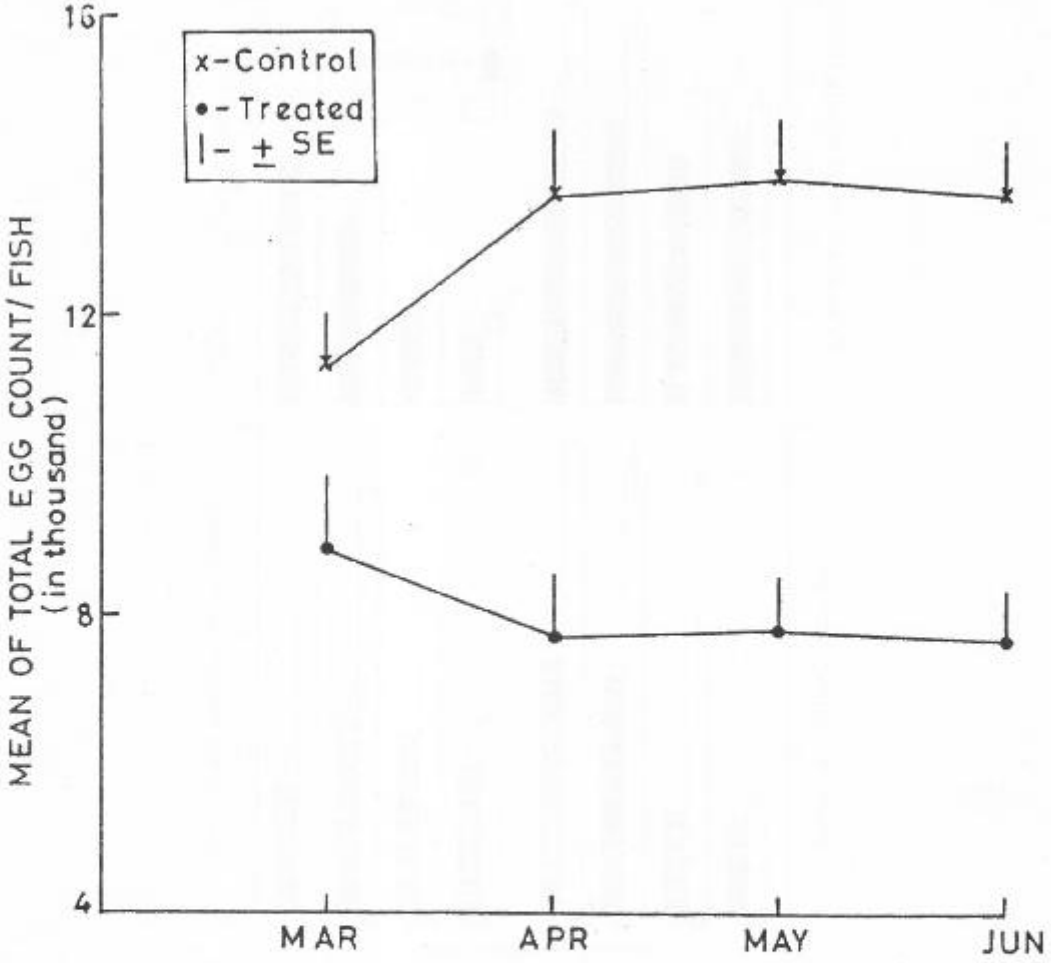

Fig. 28.3. Total egg count per fish of control and Cd-treated specimen between March and June. March = Non-Significant; April = $p < 0.005$; May = $p < 0.001$; June = $p < 0.025$.

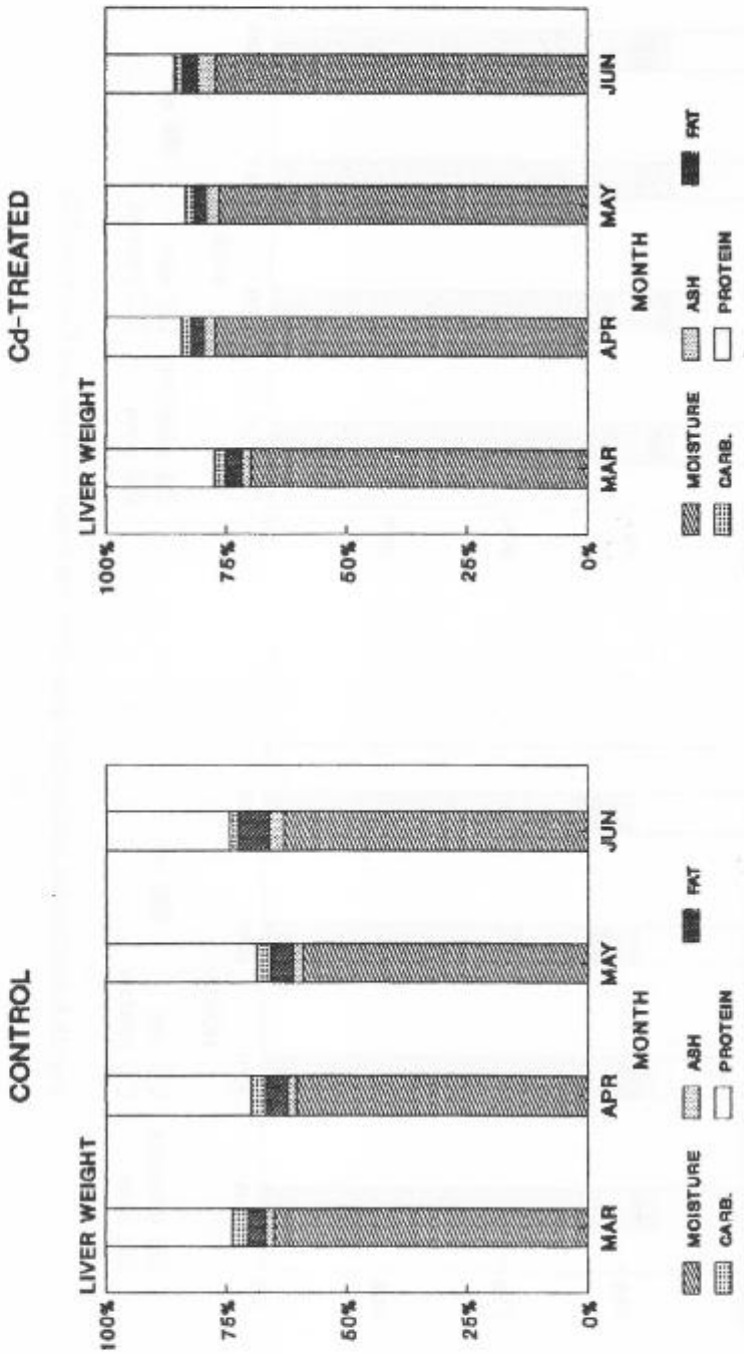

Fig. 28.4. Biochemical profile of per gram liver wet weight in control and Cd-treated fish.

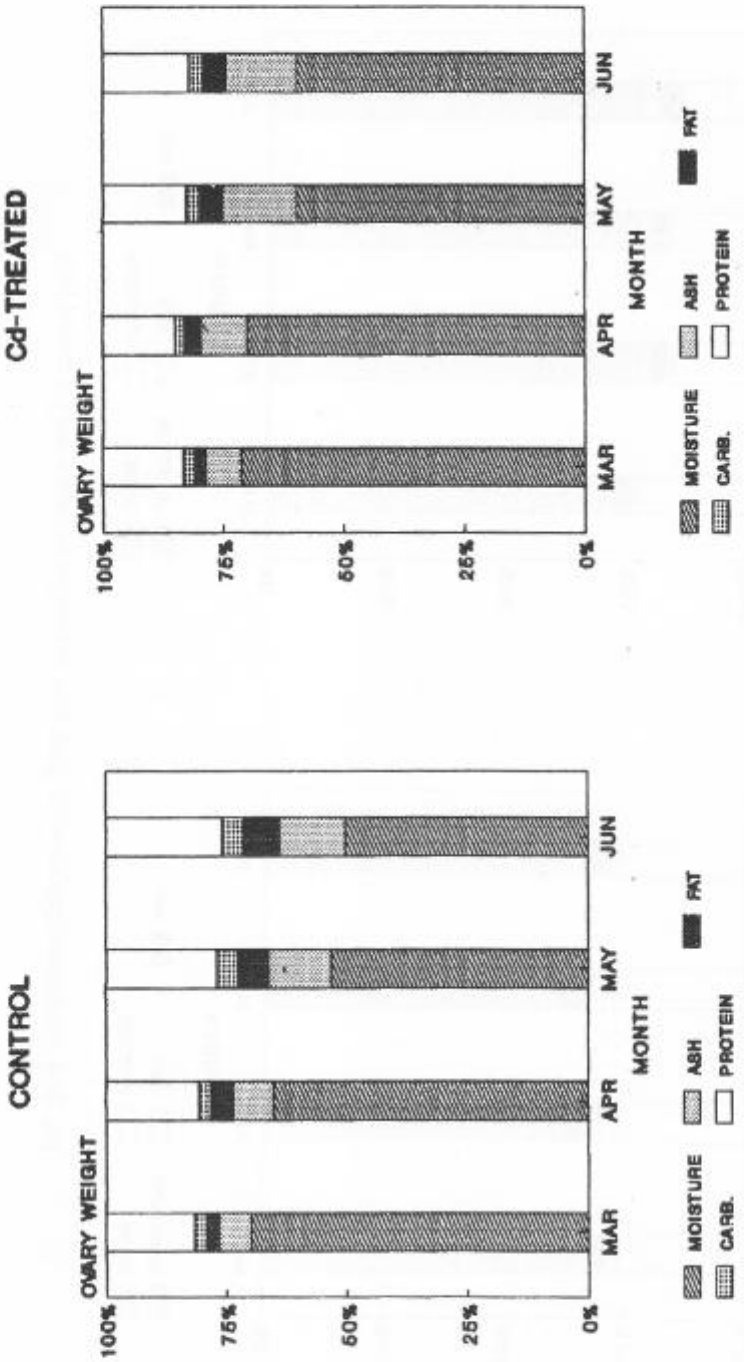

Fig. 28.5. Biochemical profile of per gram ovary wet weight in control and Cd-treated fish.

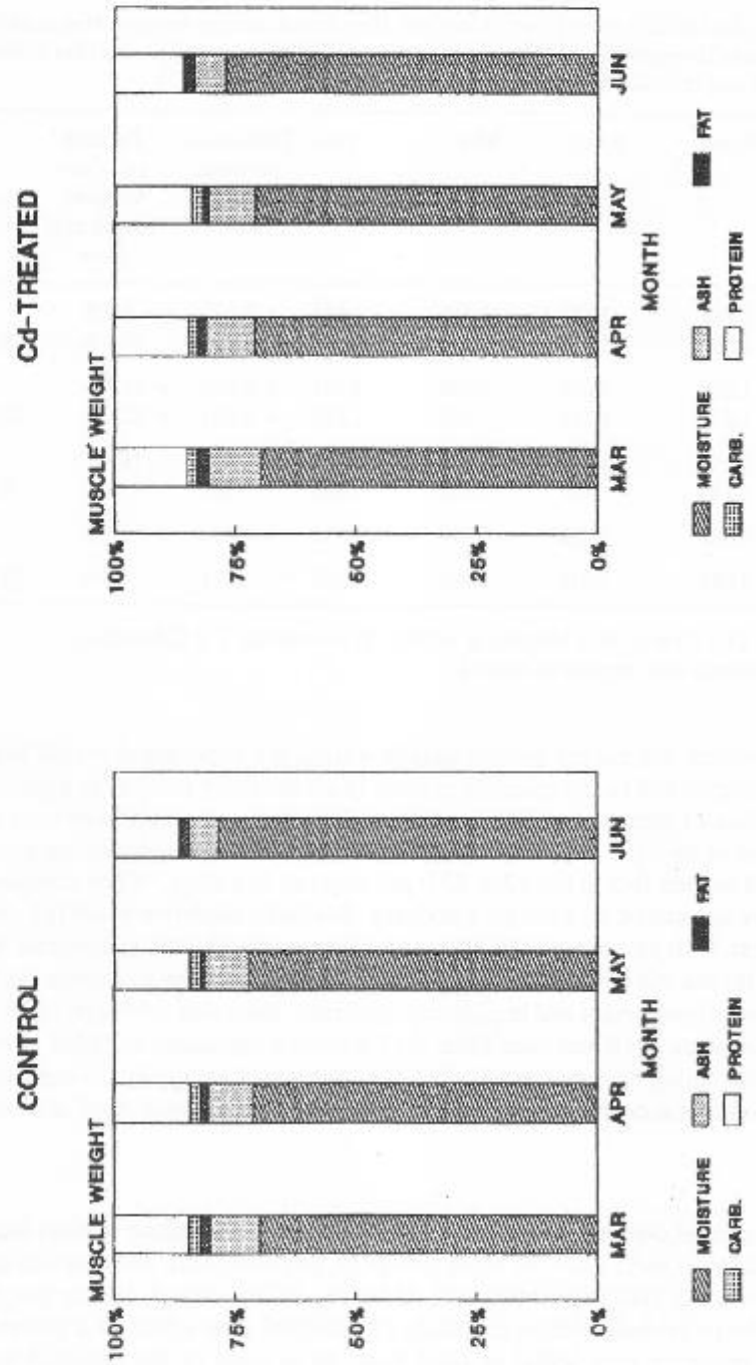

Fig. 28.6. Biochemical profile of per gram muscle wet weight in control and Cd-treated fish.

TABLE 28.2. *Calorific profile ($KCal^{-1}$) of the Liver, Ovary and Muscle and changes between March and June of control and Cd-treated fish. Differences indicate during three month period (April-June) as first analysis was done in March end.*

		March	April	May	June	Difference between March and June	Percent gain/loss between March and June	
Liver	C	1.982	2.237	2.335	2.161	+ 0.179	+ 8.2%	
	T	1.686	1.229	1.253	1.175	- 0.511	- 30.3%	- 38.5%*
Ovary	C	1.381	1.628	2.088	2.247	+ 0.866	+ 38.5%	
	T	1.248	1.246	1.536	1.589	+ 0.351	+ 22.0%	- 16.5%*
Muscle	C	1.159	1.121	1.061	0.944	- 0.215	- 18.6%	
	T	1.149	1.099	1.102	0.998	- 0.151	- 13.1%	- 5.6%*
Total	C	4.522	5.022	5.484	5.352	+ 0.830	+ 15.5%	
(L + O + M)	T	4.083	3.574	3.891	3.762	- 0.321	- 7.8%	- 23.3%*

- = Loss; L = Liver; O = Ovary; M = Muscle; + = Gain; C = Control; T = Cd-treated.
* Percent change in treated with respect to control.

calorific content. However, the energy profile obtained from the experiment reveal that the Cadmium causes significant fall in the calorific content of all the three tissues. In liver of Cd-treated fish the reduction of energy was 30.3 per cent which when compared to control group accounted for a net loss of 38.5 per cent between April and June. Though mobilisation by ovary was also evident in Cd-treated fish, it recorded 22.0 per cent fall in energy. When compared to control it accounted for net loss of 16.5 per cent in ovary. Similarly, depletion in energy content of muscle was evident both in control (18.6%) and Cd-treated (13.1%) specimens which indicated depletion in the muscle of Cd-treated by 5.6 per cent in comparison to control. As far as the total energy content of liver, ovary and muscle are concerned, there was a net gain of 15.5 per cent in control group between April and June while the Cd-treated specimens recorded a net loss of energy by 7.8 per cent during the same period when the total loss of energy by Cd-exposed fish was compared with control it accounted for a net loss of 23.3 per cent between April and June.

Discussion

In natural or experimental condition a sublethal concentration of a pollutant is most likely to produce sublethal effects so as to alter the morphological, physiological, histological and/or ethological condition of the fish (Rosenthal and Alderice, 1976), though it may not cause immediate death of the individual. Hence, the study of sublethal dose effect of a pollutant is comparatively more rationale than lethal or fatal dose, as in most of the polluted natural

environment, sublethal concentration is met which may cause the alteration in the normal survival of organisms over a prolonged period of time. Accordingly, the present study was taken up in terms of sublethal dose response in the experimental fish.

Mortality percentage was very low (5.0%) during the course of experiment. However, as the mortality was noticed in both control and treated groups, it may not be attributed to Cd-toxicity. No visible morphological changes were noticed except that the pigmentation of skin became faint in Cd-treated fishes in comparison to controls. Similar changes in pigmentation have also been reported in Cd-exposed non-air breathing (*Tilapia mossumbica*) and air breathing (*Clarias batrachus*) fishes (Banerjee *et al.* 1978).

The reproductive phenomena in particular reference to vitellogenesis of teleosts is well studied and a generalized plan of activities of vitellogenesis during the reproductive phase of the year of the fish can be presented as follows to discuss our finding in required way.

Reproductive condition largely determines the body composition of the fish (Craik and Harvey, 1984; Strange and Pelton, 1987). Seasonal variation in the cellular constituents is a common observation of almost all fishes (Love, 1970). Cellular constituents of liver, muscle and gonad exhibit cyclical variation correlated with reproductive cycle of the fish, and it has been suggested to be the result of external (environmental) and internal (sex steroid) factors (Diana and Mackey, 1979; Emmersen and Emmersen, 1976; Medda *et al.* 1980; Nath and Sundararaj, 1981 a, b; Okuzawa *et al.* 1986; Quinitio *et al.* 1989).

Lapin and Basaamzhav (1989) also found the environmental conditions as one of the factors for metabolic activities related to the vitellogenesis. Various pollutants including heavy metals, therefore, may have substantial influence on oocyte development, maturation, hatching, growth and survival of different species of teleost fishes (Von Westernhagens, 1988). Cadmium is a non-essential heavy metal whose toxicity effects have been studied by different authors on a variety of fish species (Pickering and Gast, 1972; Hiltibran, 1971; Eaton, 1974; Von Westernhagen *et al.* 1975; Banerjee *et al.* 1978; Beattie and Pascoe, 1978; Alderdice *et al.* 1979 a, b; Wani and Latey, 1982, 1983). Rosenthal and Spiraling (1974) have reported the multifaceted effect on the development and survival of herring due to Cd-toxicity.

Onset of reproductive season is marked by the most remarkable metabolic activity that takes place in the liver where yolk protein (vitellogenin) is synthesized under the influence of estrogen (Ng and Idler, 1983; Mommsen and Walsh, 1988). The key product in the vitellogenesis is a multicomponent lipophosphoprotein called vitellogenin, synthesized in liver, which is transported by blood to ovary where it is cleaved into the final egg yolk protein (phosvitin and lipovitellin) and is incorporated in ovary as yolk granules (Tata, 1976; Nath and Sundararaj, 1981 a, b; Mommsen and Walsh, 1988; Quinitio *et al.* 1989). In normal fish the increased metabolic activity of liver during vitellogeninsynthesis causes proliferation and hypertrophy of liver resulting in the increased HSI as well as DNA, RNA and total peotein (Emmersen and Emmersen, 1976; Medda *et al.* 1980; Quinitio *et al.* 1989). As soon as a substantial amount of vitellogenin is synthesized in liver, it is cleared gradually by blood then incorporated in ovary by pinocytosis under the influence of pituitary gonadotropin (Nath and

Sundararaj, 1981 b). This phenomenon causes gradual rise of serum vitellogenin level (Emmersen and Emmersen, 1976; Nath and Sundararaj, 1981 a, b). Measurement of total protein in blood serum and liver thus provides, an overall indication of vitellogenin level (Emmersen and Emmersen, 1976; Medda *et al.* 1980). The entire process of vitellogenin and incorporation of yolk granules in ovary is thus carried out by hormone mediated liver-blood-ovary axis.

Garra mullya is a seasonal breeder and it enters the preparatory phase of gonadal development by February end (Khan and Mehrotra, 1991) at the site from where the fishes for present study were collected. Maturation of oocytes is completed by June end and spawning begins from mid-July. Hence, whatever impact cadmium may produce on vitellogenesis could be evident from the experiment between the beginning of March and June end. Guraya *et al.* (1975), while comparing the ovarian cycle of *Mystus tengara* in natural and confinded water has reported that the pattern of ovarian activity in confined water followed more or less same course as that in natural waters. Though, in temperate region some species like *Mugil cephallus* and *Mugil capito* exhibit rise in gonosomatic index mainly due to accumulation of athletic oocytes when confined inland locked habitat (Abraham *et al.* 1966), in *Mystus tengara*, a tropic species, the rise in GSI is mainly due to maturation of oocytes (Guraya *et al.* 1975). However, relatively slow growth of oocytes is noted in *Mystus tengara* when maintained in confined water. In the present study, the *Garra mullya* of control groups also exhibited relatively slow growth of oocytes in comparison to natural water (Khan and Mehrotra, 1991) as was evident from the data of total ovary protein, oocyte diameter and GSI (Fig. 28.2 A, C, D).

In control groups, a gradual rise of liver RNA was accompanied by corresponding rise in liver protein as well as HSI. Maximum level of liver protein, however, was recorded in April which indicates that protein mobilisation was at its peak in this month. Levels of RNA and HSI had maximum values in May and in June it decreased substantially (Fig. 28.1 B, C). The blood serum also began to increase from April and was maximum in May. However, the levels of ovary protein and GAS recorded sharp rise from May and were maximum in June (Fig. 28.2 A, C). The entire data of liver, serum and ovary showed well synchronisation of protein mobilisation in liver, its clearance by blood and incorporation in ovary. It is remarkable that large increase in liver weight and serum protein was found earlier than that in ovary. Thus, the highest metabolic activity of liver occurred when ovarian growth phase had advanced, and yolk deposition had started, as was evident from increasing ovarian protein, large oocyte diameter and higher GSI (Fig. 28.2 A, C, D). While ovarian vitellogenic growth was maximum in June, the total protein and RNA of liver as well as HSI and serum protein indicated a declining trend in June (Fig. 28.1 A, B; 28.2 B). These observations agree well with those made by Emmersen and Emmersen (1976) on *Platichthys flesus,* and by Quinitio *et al.* (1989) on *Cottus hangiongensis,* who found that liver weights in females increased during early phase of gonadal ripening, but then declined with progressive maturation of ovary upto the stage at which oocytes were ripe (June, in the present study). Khan and Mehrotra (1991) have also observed similar trends during the annual reproductive cycle of *Garra mullya*. Results indicate that the biochemical changes in liver, serum and ovary of control groups more or less followed the similar

pattern as in natural waters.

Sublethal effects of pollutants on early developmental stages may be caused in two different ways-first by exposure of the parent fish and the second by the exposure of hatched larvae. Although, the complete suppression of ovarian egg development due to sublethal dose of heavy metals may be a rear case (Von Westernhagen, 1988), the inhibition of reproductive process in various stages has been reported due to copper toxicity in fathead minnow, *Pimephales promelas and Phoxinus phoxinus* (Bengtesson, 1974). Zinc, cadmium and copper at low concentration caused progressively decreasing spawning activity in fathead minnow (Eaton, 1973). Zinc, cadmium and copper produced significant effects on egg growth and production even at low concentration (parts per billion) in flagfish *Jordanella floridae* (Spehar *et al.* 1978). Heavy metals are also known to exert detrimental effects on the fertilization rates of spawned eggs (Ojaveer *et al.* 1980; Blaxter, 1977). Studies of the Cd-toxicity on Indian teleosts have revealed the inhibition of enzyme system in liver of *Clarias batrachus* and *Tilapia mossumbica* (Banerjee *et al.* 1978) and sever histological damage of liver and ovary of *Garra mullya* (Wani and Latey, 1982, 1983). Biochemical constituents of liver and ovary during the present investigation and also reported earlier, have also been found to fall significantly in *Garra mullya* (Khan *et al.* 1992 a), though the glycogen level remained unaltered in liver of *Garra mullya* under the influence of cadmium chloride (Khan *et al.* 1992 b). Banerjee *et al.* (1978) have also reported alteration in the haematological parameters in *Clarias batrachus* and *Tilapia mossumbica.*

The data of there present study indicate significant inhibitory influence of cadmium chloride at sublethal dose in the vitellogenin synthesizing system of liver. It is revealed by the noticeable decrease of total protein, RNA and DNA from the very first month of treatment in Cd-exposed fishes. Decreased rate of protein synthesis also resulted in fall of serum protein level which may be due to slower cleavness rate from liver as synthesized protein (vitellogenin) was available to blood in lesser amount in comparison to control group. Hiltibran (1971) noted considerable inhibition of phosphate metabolism in liver of vitellogenin, RNA and DNA, and inhibitory effect on its metabolism may cause retardation of protein synthesis in liver. Though phosphorus content was not estimated in the present study, it is possible that lower protein contents of liver and blood serum may have been caused due to inhibitory effect of liver phosphate metabolism (Hiltibran, 1971) and due to inhibition of liver enzyme system (Banerjee *et al.* 1978).

Ovary protein, GSI and oocyte diameter were found to exhibit non-significant changes during initial period of Cd-treatment in treated fishes. However, a gradual fall was noted in ovary protein, GSI and oocyte diameter with longer exposure time and decrease were statistically significant also. Major source of yolk protein is extra ovarian in the form of vitellogenin from liver though intra ovarian yolk synthesis has also been noticed in most teleosts (Droller and Roth, 1966; Anderson, 1974). Similarly in the present study, the protein, levels were found to exhibit significant fall in all three components – liver, serum and ovary of treated fish. It is indicative of the fact that low synthesis of protein in liver caused slower turn-over rate of yolk protein through liver-blood-ovary axis, resulting in decreased level of ovary protein, GSI

and oocyte diameter which agrees well with the results of Brungs (1969); Hiltibran (1971); Benoit (1975, 1978); Banerjee *et al.* (1978); Wani and Latey (1982, 1983) and Khan *et al.* (1992 a). Indemnification of intra ovarian yolk was not done in the present study. Hence it is difficult to opine conclusively about the extent of influence of the cadmium salt that might have affected to intra ovarian process of yolk synthesis. However, Wani and Latey (1982) recorded severe histological damage of ovary in *Garra mullya* when exposed to cadmium and Powar and Katdare (1983) found drastic fall in GSI of the same species when exposed to sumithion, a commonly used insecticide.

Exposure of mature fish to sublethal levels of certain heavy metals may cause considerable reduction in number of eggs produced; in some cases even 80 per cent reduction has been noticed (review, Von Westernhagen, 1988). Copper administration in aquaria at 18-32 µg/l totally prevented egg deposition in fathead minnow *Pimephales promelas* (Mount, 1968; Mount and Stephan, 1969). However, the perusal of available literature indicates that complete suppression of egg production is not so common. A reduction upto 21 per cent in the number of eggs produced has been noted in *Phoxinus phoxinus* when exposed to 0.13 and 0.2 mg zinc/l (Bengtsson, 1974) and *Brachyrerio danio* also spawned lesser number of eggs when exposed to 5 mg Zn/l for nine days (Speranza *et al.* 1977). Eaton (1973) has also recorded progressive decrease in egg number per females when treated with cadmium and copper. Reduction in egg number due to different heavy metal toxicity at varied concentration have also been found in flag fish *Jordanella floridae* (Spehar *et al.* 1978), in guppy *Poecila reticulata* (Uviovo and Beatty, 1979) and Zebra fish *B. danio* (Kihlstran *et al.* 1971). In the present study, the progressive changes from month to month was recorded for number of eggs in both treated and control groups. A reduction in egg number was noticed from the very first month but it was statistically non-significant thus indicating that the cadmium toxicity at sublethal concentration takes more than a month in *Garra mullya* to produce detrimental effect on the numerical strength of the egg. In the following months the reduction was very significant resulting in a reduction of about 44 per cent in the June – the last month of experiment when oocytes attain maturation. However, an average comparative reduction upto 31 per cent in the diameter of mature oocytes in June of Cd-treated specimens also indicate the possible non-viable nature of eggs produced which may result in either percentage failure of fertilization and/or detrimental effects on post-fertilization development (Blaxter, 1977; Alderdice *et al.* 1979 b; Ojaveer *et al.* 1980). Reduction in the number of eggs also suggests the possible inhibitory action on the germinal epithelial cells of ovary.

There is great paucity of information pertaining to the variation of calorific content in the tissues of fish maintained in heavy metal containing water. However, the biochemical constituents like protein, fat and carbohydrate, producing calorific energy have been found to decrease on mexposure to heavy metals in several species of fish viz. blue gill (Hiltibran, 1971); fathead minnow *Pimephales promelas* (Banerjee *et al.*, 1978); *Clarias batrachus* and *Tilapia mossumbica* (Banerjee *et al.* 1978); flag fish *Jordanella floridae* (Spehar *et al.* 1978) and *Garra mullya* (Khan *et al.* 1992 a, b). The energy data of the present study in Table 28.2 indicate that

cadmium toxicity significantly reduced the energy profile in liver, ovary and muscle. While in liver, the energy content was reduced by 38.5%, in ovary the reduction was 16.1% between April and June. Workers believe that the stored nutrient like protein, fat and carbohydrate in liver and body muscle are transferred to gonad and subsequently utilized as source of energy for metabolism as well as growth and maturation of gonad (Diana and Mackay, 1979; Blay Jr. and Eyeson, 1982; Strange and Pelton, 1987). In the present study maximum energy mobilisation was noticed in ovary of both control as well as treated groups. On the other hand energy depletion was also noticed from liver as well as muscle as the time of experiment advanced. Cadmium, however, was found to greatly influence the mobilisation-deposition process in all three tissues. Energy profile of control group indicates heavy depletion in muscle (18.6%) between April and June. However, the liver of control group mobilised lesser percentage (8.2%) energy indicating that liver too might have contributed substantially in ovarian calorific value where there was a gain of 38.5 per cent between the same period. However, the energy profile of Cd-treated specimens revealed that the depletion of energy content in muscle in this group was lesser (5.5%) with respect to control. Similarly, the liver of Cd-treated group recorded sharp decrease in the calorific content (38.5%) with respect to control between April and June which indicates that cadmium either caused large depletion from liver of riot inhibited energy mobilisation. However, latter possibility seems to be more likely as is evident from the month wise biochemical analysis of liver wet weight (Fig. 28.4). Reduced percentage depletion of energy from muscle and reduced mobilisation in liver of Cd-treated specimens is reflected well by a significant fall by 16.5 per cent in the energy content of ovary of treated fish. Data of total energy content of liver, ovary and muscle exhibited a gain of 15.5 per cent in control group between April and June where as in Cd-treated specimens there was a net loss of 7.8 per cent energy between the same period. Hence, it may be concluded that cadmium chloride inhibited energy mobilisation and depletion process of liver, ovary and muscle of *Garra mullya* which accounted for nearly 23.3 per cent over all loss of total energy in the tissue in question with respect to control group (Table 28.2).

As far as the mechanism of action of cadmium toxicity is concerned, the phenomenon is not fully understood (Nilsson, 1970; Rosenthal and Sperling, 1974). However, it is general agreement that cadmium ions first get deposited in tissues and eggs when exposed to cadmium or its compound (Pickering and Gast, 1972; Beattie and Pascoe, 1978; Benerjee *et al.* 1978; Rombough and Garside, 1982). Nilsson (1970) and Sax and Sax (1974) opine that mechanism of cadmium toxicity is probably due to high affinity of cadmium with sulphydryl and hydroxyl groups and ligands containing nitrogen. Therefore, the binding with such groups in chemical system make the control functions of the organisms vulnerable to cadmium even at low concentration. It is possible, therefore, that cadmium acts at cellular level to inhibit the enzyme system and other related biochemical processes of synthesis and breakdown. The reduced levels of protein, RNA, DNA and energy content of Cd-exposed fished during the period of investigation indicate that cadmium produces inhibitory effects on metabolic processes of liver, ovary and muscle which directly or indirectly contribute to the vitellogenic growth of the ovary.

Acknowledgement

One of the out0shors, E.A. Khan gratefully thank University Grants Commission, New Delhi for providing financial assistance in the form of a Senior Research Fellowship. Authors also thank Prof. P.N. Mehrotra, Head, Zoology Department, Ranchi University for providing Laboratory facilities of the experiment.

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