



Effect of paper mill effluent on the amino acid and protein content in liver, blood and gonads of fresh water fish *Mystus vittatus* (Bloch) during the annual breeding cycle

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Abstract

The effects of effluent from the Rayana paper board industries limited (R.P.B.I.L.E) on some biochemical aspects of the fresh water teleost fish, *Mystus vittatus* were studied under experimental conditions through the course of the annual reproductive cycle of these fishes. A quantitative estimation of the amino acids and protein content was made in liver tissues, blood and gonadal tissues. The content of all these nutrient and building materials was observed to be significantly higher during the spawning phase when compared to the other phases of the annual reproductive cycle. The changes produced in these biochemical parameters on account of exposure of the fishes for 96 hr to 0.4 and 0.8 of LC₅₀ – 96 hr of effluent were also studied during all the three phases of the annual reproductive cycle. All the stress induced biochemical alterations were always found to be greater in case of 0.8 than in case of 0.4 effluent both the sublethal concentrations of the annual reproductive cycle of the fish, the amino acid content of liver, blood and gonads was found to be increased, while the protein content was measured to be decrease in liver, blood and gonads. The present study concludes a stress induced metabolic dysfunction in response to effluent toxicity in the fish.

Keywords: Stress-induced, Spawning phase, Effluent toxicity, Annual reproductive cycle.

Introduction

Biochemical alterations being intimately associated with all physiological disturbances, their study has found wide usage in chemical diagnosis. Biochemical profiles have also been gaining progressive importance in toxicological research. Some of the biochemical changes have already acquired the status of reliable and sensitive indicators of general specific physiological disturbances under environmental stress. Study of biochemical anomalies now occupies an established place in the universally accepted research framework concerning fish toxicology.

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Various kinds of biochemical alterations in the amino acid and protein metabolism of vital tissues have been observed very frequently in fishes under conditions of environmental stress. Numerous studies have shown significant effects of papermill effluents on the fish populations. Physiological and biochemical changes under the effects of pulp and paper mill effluents on blood and tissues have been described especially of *Onchorhynchus kisutch* (McLeay, 1973, 1977; McLeay and Brown, 1974, 1975, 1979; McLeay and Howard, 1977), *Salmo gairdneri* (McLeay, 1977), *Esox lucius* (Oikari, 1977), *Onchorhynchus mykiss* (Oikari *et al.*, 1983, 1985a), Roach, *Rutilus rutilus* L. (Jeney, *et al.*, 1996; Aarno *et al.*, 1998), Perch *Perca fluviatilis* (Forlin *et al.*, 1995; Aarno *et al.*, 1998), Mosquitofish, *Gambusia holbrooki* (Batty and Lim, 1999; Bortone and Cody, 1999; Parks *et al.*, 2001). Thus, the purpose of the present study was to examine the liver, blood and gonads of *Mystus vittatus* for alterations produced under the stress of

effluent. The biochemical effects of 96-h exposure to sublethal effluent concentrations on the free aminoacids and total protein contents of these tissues were observed through the annual reproductive cycle of the test fish.

Materials and Method

The freshwater teleost, *Mystus vittatus*, was selected for the present work. It is an air breathing as well as annual breeder perch and abundantly present in all fresh water bodies of Northern India. The fishes were collected from a local uncontaminated freshwater pond in Basti city area, located approximately at a distance of 4 km from the research centre. They were brought to the research centre in open containers being filled with sufficient quantity of water so that the stress caused by handling and transportation may be minimized. The fishes having an average body length of 7.6 ± 0.18 cm and body weight of 7.3 ± 0.23 gm were selected for the study. The fishes were acclimatized to laboratory conditions for 3 to 4 week at room temperature in the acclimation tanks, filled with dechlorinated tapwater. The whole effluent was collected from a paper mill industry, Magahar, Santkabeer Nagar, Uttar Pradesh (India) for the study of toxicological responses. The effluent samples were collected from three places during the morning shift when the mill was in its normal course of operation. The samples were mixed thoroughly and brought to the research center in sealed polyethylene containers. The chemical characteristics of effluent analyzed according to the procedures recommended by American Public Health Association (2005), within 12 hr of collection, every month (Table 1). The acute toxicity of effluent to the perch was measured in terms of 96 hr, LC_{50} , by using the static bioassay procedures as outlined by USEPA (1989). For each acute toxicity bioassay, a minimum of 8 concentrations of effluent was used and 20 animals were used for each concentration. Controls were also run separately during experimentation, using normal unpolluted and dechlorinated tapwater. No food was provided to either the control or the test fishes during the period of the toxicity experiments. The experiments were conducted every month and 96 hr, LC_{50} values were determined. The effect on the tissue biochemistry of the perch was studied in individuals exposed for a period of 96 hr with two

sublethal concentrations *i.e.* 0.4 (40%) and 0.8 (80%) of the 96 hr - LC_{50} , determined during the mortality studies. Healthy fishes, netted from the acclimation tank, were divided in 3 groups of 50 individuals each. The first group was exposed to 40% R.P.B.I.L.E, and the second group to 80% effluent. The third group of fishes was kept in unpolluted dechlorinated tapwater, and served as control. The experiment was repeated 5 times. After the expiry of the 96 hr exposure period, the fishes from all the 3 groups were taken out for the sampling of their blood and other tissues. Ten fishes were used for each determination. For obtaining blood samples, the caudal vein was severed and blood was drawn into centrifuge tubes containing the anticoagulant mixture of 1mg% sodium fluoride and potassium oxalate. Serum was obtained by centrifuging the blood samples at 3000 to 4000 rpm for 5 min. The total amount of free aminoacid in blood, liver, ovary and testes was measured using the method of Spies (1957). Ten mg of the tissue was homogenized in 1 ml 96% ethanol (10:1 w/v) in a homogenizer for 5 min and centrifuged at 8000 rpm for 20 min. The supernatant was used for aminoacid estimation. The total protein content in the blood, liver, ovary and testes was estimated according to the method of Lowry *et al.*, (1951), using bovine serum albumin as the standard. The homogenates, obtained by mixing 1 mg tissue per ml of 10% TCA in a homogenizer for 5 min, were centrifuged at 6000 rpm for 20 min and the precipitate was used for the estimation of protein. A photo-colorimeter (systonics) was employed for biochemical estimation. The standard deviation ($\pm$ S.D.), and standard error ($\pm$ S.E.), were calculated and tested for significance according to the statistical methods outlined by Snedecor (1961). To test the significance of the differences between the mean experimental and the corresponding mean control values, the Student's t-test was applied as described by Campbell (1974).

Results and Discussion

The concentration of total protein and free aminoacids estimated in the different tissues of control fish was found to be maximum during the spawning phases when compared to the other two phases of the annual reproductive cycle of the fish (Fig. 1 and 2). The spawning phase incorporates a period of growth and proliferation of gonadal



tissues for the formation of gametes. Hence, an accumulation of the building material, *i.e.* protein, would be expected to occur during this phase; a simultaneous increase in the concentration of free aminoacids would also occur since protein synthesis involves a large pool of free aminoacids (Love, 1980). Liver, being the main organ for protein synthesis in spawning fishes (Wallace, 1985; Revathi *et al.*, 2005), its protein and aminoacid contents are most likely to increase during the spawning period of fishes. A similar increase in protein and aminoacid concentration is also most likely to occur within the gonads of spawning fishes, since a greater amount of proteinaceous material and energy is needed for gonadal growth and gamete formation. Infact, active protein synthesis appears to be a common feature of maturing gonads in fishes (Love, 1980), and more of free aminoacids have been recorded in the gonads of spawning fishes like *Flounders* (Sorvachev and Shatunovskii, 1968), *Gadus callarias* (Maslennikova, 1970) and *Mystus vittatus* (Narain and Pandey, 1989), *Channa punctatus*. The increased protein and aminoacid concentration observed in the liver and gonads of spawning *Mystus vittatus* will, thus, be explained. Apparently, a protein reserve is built up, through active protein synthesis, in the liver which is subsequently translocated to other organs, more so the gonads. Building up of proteins and aminoacid reserve and its translocation to spawning gonads has previously been reported in fishes (Love, 1980; Iles, 1984; Potts and Wooten, 1984).

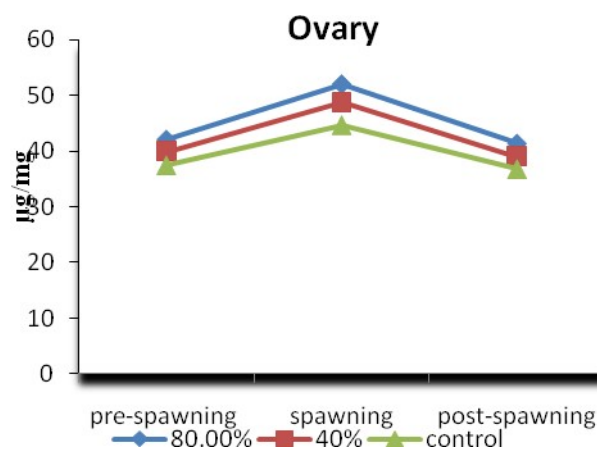
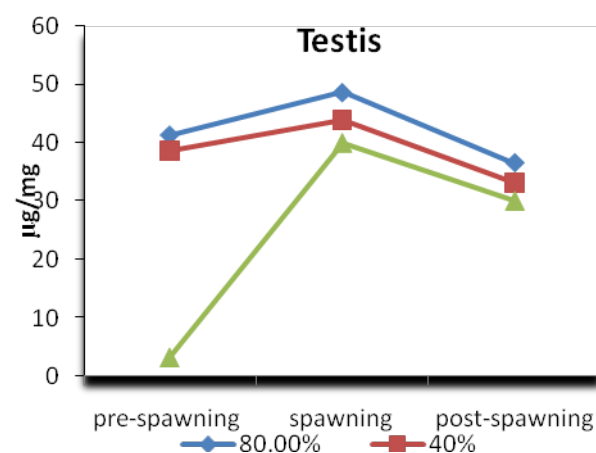
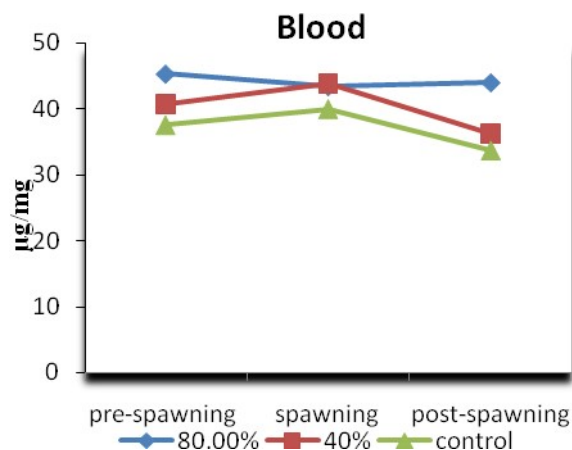
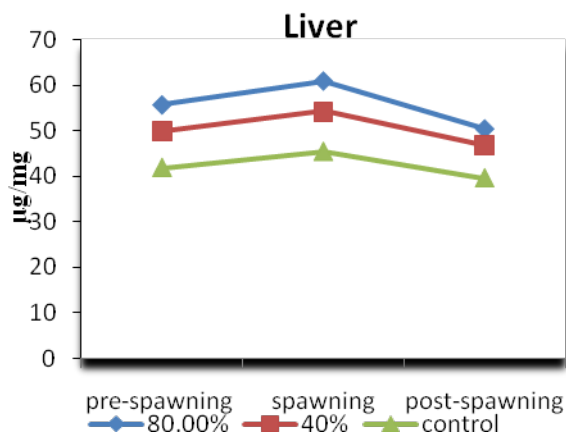


Fig.1: Aminoacids content of control and experimental *M.vittatus* during Pre-Spawning, Spawning and Spawning Phases. All experimental values are significantly different from corresponding control values (<0.05).

Table 1: Physico-chemical characteristics of test effluent (WRPBILE) Magahar, Santkabeer Nagar, (U.P.) India. Data based on samples taken during the morning shift of the normal course of mill operation at 8 A.M.

Characteristics	Variable constituents through the year (mean values)			Yearly Average $\pm$ S.E.
	Jan-Apr	May-Aug	Sept-Dec	
Color	Dark Brownish	Dark Brownish	Dark Brownish	-
Sodium, Na ⁺ (mg/l)	350	320	351	340 $\pm$ 12.2
Cl ⁻ (mg/l)	420	450	425.7	431.9 $\pm$ 11.3
SO ₄ (mg/l)	1.12	5.8	3.5	3.5 $\pm$ 1.7
Nitrate (mg/l)	7.6	7.3	7.2	7.4 $\pm$ 0.1
Total Nitrogen (mg/l)	1.7	6.3	3.8	3.9 $\pm$ 1.6
PO ₄ (mg/l)	0.77	0.52	0.69	0.7 $\pm$ 0.1
pH	7.3	7.3	7.4	7.3 $\pm$ 0.04
Temp (⁰ C)	23.5	28	26.5	26 $\pm$ 1.6
Suspended solid (mg/l)	5021	4643	4256	4640 $\pm$ 270
Dissolved solid (mg/l)	1111	1222	1013	1115 $\pm$ 74
Total solid (mg/l)	6132	5865	5269	5755 $\pm$ 312
BOD (mg/l)	552	538	498	526 $\pm$ 19
COD (mg/l)	2379	2551	2326	2418 $\pm$ 83
Fe (mg/l)	9.6	13.5	10.4	11.2 $\pm$ 1.5
Mg (mg/l)	1.9	1.65	1.28	1.6 $\pm$ 0.2
K (mg/l)	8.6	6.4	4.4	6.5 $\pm$ 1.5
Cu (mg/l)	0.11	0.14	ND	0.1 $\pm$ 0.1
Total Cr (mg/l)	ND	0.077	0.072	0.07 $\pm$ 0.03
Mn (mg/l)	0.34	0.071	0.53	0.31 $\pm$ 0.12
Co (mg/l)	-	0.001	0.0012	0.001 $\pm$ 0.002
Cd (mg/l)	0.025	0.016	0.018	0.02 $\pm$ 0.004
Zn (mg/l)	0.06	0.08	0.106	0.08 $\pm$ 0.021

The genesis of sex proteins is linked to an increase in the liver RNA of developing fishes (Aida *et al.*, 1973; Love, 1980); this further establishes that proteins related to sex are hepatic in origin. Thus, the present observations corroborate their findings. The acute exposure to effluent was found to produce a noticeable change in the protein metabolism of *Mystus vitatus*. The protein content of liver, blood, ovary and testis was reduced significantly and the difference was significant at

<0.05 and 0.001 (Fig. 2), in comparison to control fishes. The reduction in total protein in stressed fishes may be correlated with the increase in aminoacid, pool of the stressed fishes in various tissues examined. The reduction in protein concentration under stressed conditions has also been reported in different tissue of fishes. For example: in the liver of *Channa punctatus* exposed to cythion (Narain and Sathyanesan, 1985), *Oreochromis mossambicus* exposed to endosulfan



(Ganesan *et al.*, 1989), and in *Gambusia offinis* exposed to tannery effluent (Revathi *et al.*, 2005); in the blood of *Catla catla* exposed to mercury (Rai, 1987), and in *Mystus vittatus* exposed to metasystox and sevin (John. 2007); in the ovary of *Brachydanio rerio* exposed to malathion (Ansari and Kumar, 1987), and in *Gambusia offinis* exposed to tannery effluent (Revathi *et al.*, 2005); in both testis and ovary of *C.fasciata* exposed to arsenic (Shukla and Pandey, 1986). The aminoacid concentration in acute sublethal exposure of test effluent to *M. vittatus* was increased significantly and found significant at $p < 0.05$ and 0.001 (Fig.1). The concentration of aminoacids has also been found to increase, under the influence of stressful conditions in the various tissues of fishes. For example, in the liver of *Tilapia mossambica* exposed to heptachlor (Rao *et al.*, 1990); in the ovary of *Brachydanio rerio* (Ansari and Kumar, 1987) exposed to malathion. Pulp and papermill effluents have also been shown to bring about a reduction in protein concentration of the tissues of fishes like *Onchorhynchus kisutch* (McLeay and Brown, 1974, 1979), *Esox lucius* (Oikari, 1977), and *Rutilus rutilus* (Jeney *et al.*, 1996). For the maintenance of physiological balance in stressed fishes, required additional energy, that gluconeogenesis was stimulated as a supplementary physiological response towards this need and that as a result, proteins are broken down and a rich aminoacid pool created for conversion to glucose. This would largely explain the loss in protein and gain in aminoacid concentration observed in the liver of stressed *C. fasciatus*

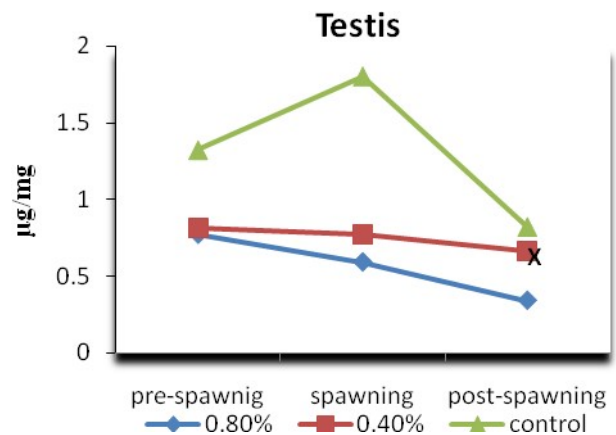
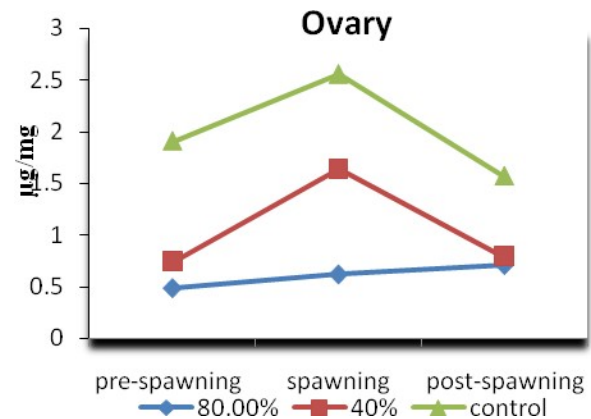
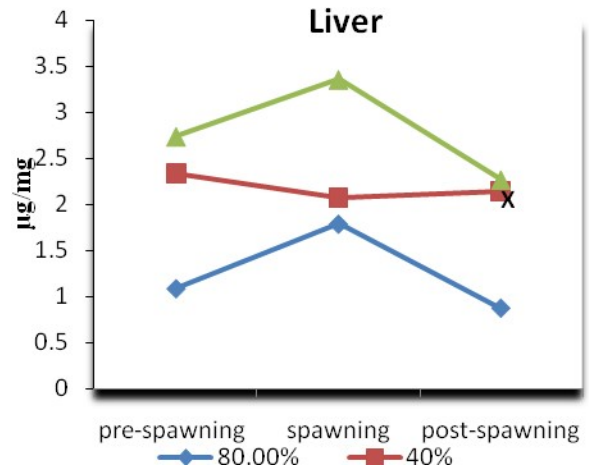
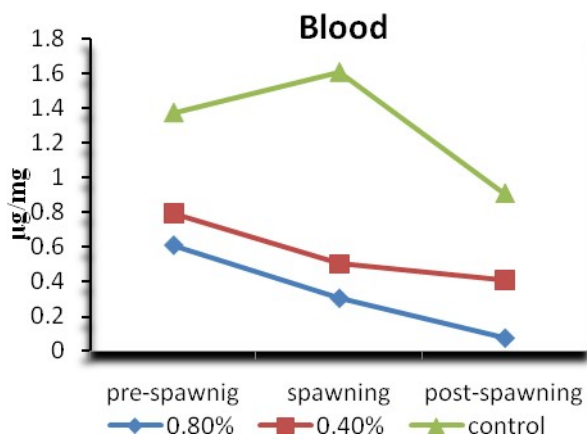


Fig 2: Protein content of the tissues of control and experimental *Mystus vittatus* during pre-spawning spawning and post spawning phases. All experimental values except marked with "X" significantly different from Corresponding control values (<0.05).

because liver is the major gluconeogenetic organ in teleosts (Moon *et al.*, 1985). The protein breakdown observed in the gonads of this stressed fish, may, however, not be explainable on this ground; an alternative explanation would be that the gluconeogenetic breakdown of proteins in the hepatic tissue is supplemented by protein breakdown in other tissue which depresses the protein level and enlarges the aminoacid content of the blood. It has also been reported that gluconeogenesis forms a part of the generalized stress response in vertebrates, whereby a pituitary adrenal excitation tends to promote protein catabolism under the influence of glucocorticoid hormones and that various environmental stress factors are capable of producing similar effects in fishes. Pulp and papermill effluents are also known (McLeay, 1973, 1977; McLeay and Brown, 1974), to elicit such endocrine responses, and increased adrenocorticoid output due to effluent induced stress has been considered an explanation for decreased body protein content of fishes like *Onchorhynchus kisutch* (McLeay and Brown, 1974). So, in *M.vittatus* exposed to papermill effluent, the loss of protein and gain in free aminoacids could also be related to stress-induced gluconeogenetic protein breakdown with a possible involvement of pituitary interrenal hormonal axis. Among other factors contributing to reduction in the tissue protein content of stressed fishes, the histopathological liver damage, if occurring in stressed *M.vittatus*, would be most likely to bring about increased proteolysis leading to lowered protein level in hepatic tissue. Similarly, pancreatic damage would also affect protein metabolism (Matty, 1985).

In view of this, the lowered liver protein and raised aminoacid concentration in the liver tissues of stressed *M. vittatus* may also be related to the damage of insulin secreting Islet cells. Kidney pathology leading to decreased excretory efficiency and liver damage leading to reduced albumin production are also reported (Narain, 1981) causes of hypoproteinemia. In acute conditions, the significant decrease in blood protein in the effluent, stressed fishes reflects the specific effect of the concentrated effluent. Very low blood protein possibly has significance in relation to infectious diseases, kidney damage and nutritional imbalance in fishes (Jeney *et al.*, 1996). The respiratory distress exhibited by the

stressed *Mystus vittatus* and the high BOD and COD values of the tested R.P.B.I.L.E samples point oxygen deficiency; this stressed condition could also be contributing towards the diminution of protein, specially liver and blood, observed in the stressed fishes.

From the present study on *Mystus vittatus*, it can be concluded that the tested effluent with higher concentrations of toxic constituents cause serious disturbances to vital biochemical and physiological functions, hence the fishes suffer from a general stress syndrome in short-term exposure to effluents, which can affect the health status, survival and most importantly the reproductive capacity of the fishes. The findings also revealed that the biochemical parameters like protein and aminoacid metabolism can be used as sensitive and reliable indicators of toxic effects on natural populations of fishes exposed to papermill effluent.

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