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Liver glycogen content in *Clarias batrachus* exposed to sublethal and semilethal dose of organochlorine and organophosphate pesticides with reference to season and sex

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Abstract : Organochlorine pesticide (Aldrin) and Organophosphate pesticide (Cythion) that are used by farmers in field move with run off water and erosion from treated area to nearby waterbodies . Bio-magnification of these pesticides in waterbodies adversely affect the physiology of fishes and other fauna. Aldrin and Cythion pesticide intoxications adversely affect the liver glycogencontent in both the sexes of the test fish. The value of liver glycogen is found high in male of all the three experimental conditions during all the three annual reproductive periods, prebreeding, breeding and postbreeding periods. Liver glycogen is found high in control condition followed by semilethal and sublethal conditions during annual reproductive cycle. When seasonal variations are taken into account, high liver glycogen content is found during breeding period followed by prebreeding and postbreeding periods.

Keywords : *Clarias batrachus,* Cythion, Aldrin, Liver glycogen

INTRODUCTION

Quality of water is the important determinants of the quality of human environment. Management of water resources and control of water pollution are equally important for the developing societies. Unfortunately situation has worsened by the increased use of agrochemicals. In our country pesticides have come to occupy a major place with agricultural achievements. Researches carried out have unanimously called the situation alarming and have echoed for the ban of these toxicants.. In developing countries like India haphazard use of these pesticides in fields affect the health of fish vis-à-vis man and other animals that use fish as their one of the food source. Pesticides affect the normal physiology of fish and result in number of disorders in vital systems which are important for the proper functioning of the body. Pesticides adversely affect the haematological and biochemical constituents of the fish. Keeping in view, in the present paper influence of sublethal (LC_0) and semilethal (LC_{50}) dose of Aldrin (organochlorine pesticide) and Cythion (organophosphate pesticide) on the liver glycogen of *Clarias batrachus* with reference to season and sex has been discussed.

MATERIALS AND METHODS

Clarias batrachus of appximately equal weight and size were procured from local fish market. Six aquaria of considerable sizes were selected for experiment. In each aquarium about ten fishes were kept and left to acclimatize. Sublethal (LC_0) and semilethal (LC_{50}) concentration of Aldrin (organochlorine pesticide) (Table 1) and Cythion (organophosphate pesticide) (Table 2) were determined by performing toxicity bioassay (APHA 1975).

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Table 1: Lethal concentration of Aldrin for *C. batrachus* during different reproductive periods.

Reproductive periods	Sex	Lethal Concentrations		
		Sublethal LC_0	Semilethal LC_{50}	Lethal LC_{100}
Prebreeding	Male	0.002	0.0033	0.0042
Breeding	Male	0.0025	0.0035	0.0045
Postbreeding	Male	0.001	0.003	0.004

Table 2: Lethal concentration of Cythion for *C. batrachus* during different reproductive periods

Reproductive periods	Sex	Lethal Concentrations		
		Sublethal LC_0	Semilethal LC_{50}	Lethal LC_{100}
Prebreeding	Male	0.002	0.004	0.006
Breeding	Male	0.004	0.006	0.008
Postbreeding	Male	0.001	0.002	0.004

LC_0 = Concentration of pesticide that causes no death.
LC_{50} = Concentration of pesticide that kill 50% of the exposed organisms in a specific time of observation.
LC_{100} = Concentration of pesticide that kill 100% (Total) of the exposed organisms in a specific time of observation.

Experiment with both the pesticides, Aldrin and Cythion were done in all the three annual reproductive seasons- prebreeding, breeding and postbreeding. Liver glycogen content was quantitatively estimated by the method of Kernop and Andrienne(1954) as modified by Ahsan and Ahsan (1985). To collect liver tissue, fish was decapitated, liver tissue was immediately taken out, washed gently with ice cold distilled water. Known weighed liver tissue is taken in a mortar and pestle and grinded with 5 ml. of ice cold trichloroacetic acid and a pinch of pure sand. Grinded material are centrifuged at 3000 rpm in cooling centrifuge. 2 ml supernatant was taken in a hard glass test tube; to which 6 ml. conc. H_2SO_4 (Analar sp. gr. 1.84) was added.. The test tube was kept in boiling water bath for 5 minutes; allowed to cool. Optical density of the brown colour fluid so obtained is taken at 515 nm with the help of UV spectrophotometer.

RESULT AND DISCUSSION

The mean glycogen content in liver in male and female of *Clarias batrachus* in case of control, sublethal and semilethal dose of Aldrin (organochlorine pesticide) and Cythion (organophosphate pesticide) during prebreeding, breeding and postbreeding period are presented in Table 3 and 4 respectively.

Table 3 : Effects of LC_0 & LC_{50} 48-h concentration of Aldrin on Liver Glycogen in both sexes of *Clarias batrachus* during different reproductive periods .(in mg/100 ml blood)

Reproductive stages	Sex	Experimental conditions					
		Control	't'	Sublethal	't'	Semilethal	't'
Prebreeding	Male	4.46±0.17 (4.31-4.72)	0.798	2.41±0.37 (2.02-2.98)	0.468	4.36±0.45 (3.88-4.89)	0.842
	Female	4.18±0.36 (3.82-4.63)		2.32±0.24 (2.18-2.63)		3.96±0.23 (3.74-4.32)	
Breeding	Male	4.65±0.07 (4.63-4.81)	1.642	2.58±0.35 (2.13-3.09)	0.205	4.52±0.423 (4.17-5.13)	0.380
	Female	4.38±0.20 (4.12-4.73)		2.54±0.30 (2.14-2.81)		4.34±0.25 (4.53-4.64)	
Postbreeding	Male	4.24±0.27 (3.91-4.62)	2.120	2.08±0.30 (1.96-0.30)	0.328	3.33±1.344 (3.65-4.31)	0.253
	Female	3.70±0.27 (1.75-2.44)		1.96±0.30 (1.75-2.44)		3.48±0.315 (3.12-3.96)	

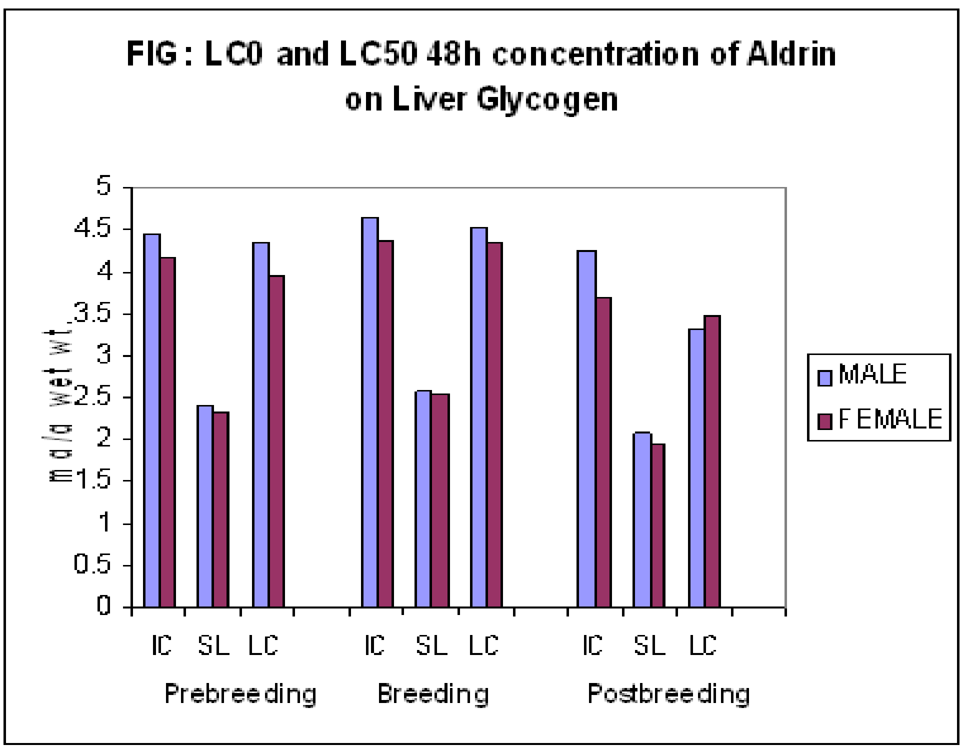
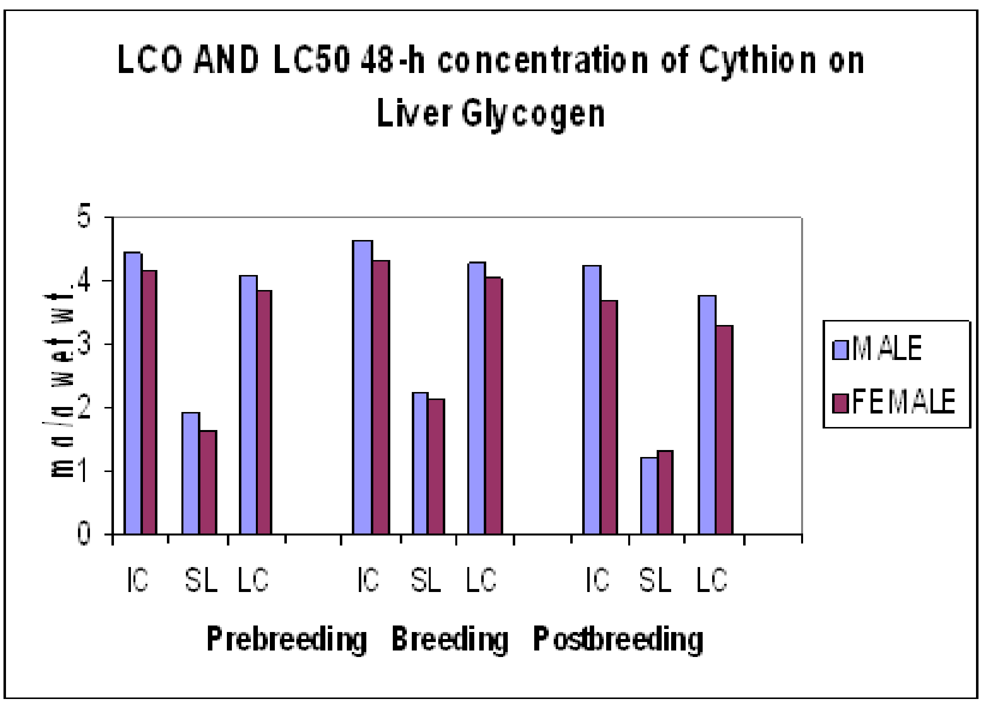


Table 4 : Effects of LC_0 & LC_{50} 48-h concentration of Cythion on Liver Glycogen in both sexes of *Clarias batrachus* during different reproductive periods .(in mg/100 ml blood)

Reproductive stages	Sex	Experimental conditions					
		Control	't'	Sublethal	't'	Semilethal	't'
Prebreeding	Male	4.46±0.17 (4.31-4.72)	0.798	1.92±0.27 (1.61-2.32)	0.188	4.10±0.39 (3.64-4.51)	0.604
	Female	4.18±0.34 (3.82-4.63)		1.66±0.32 (1.23-2.01)		3.84±0.23 (3.15-3.60)	
breeding	Male	4.65±0.07 (4.63-4.81)	1.642	2.24±0.12 (2.003-2.51)	0.372	4.28±0.23 (3.97-4.51)	0.838
	Female	4.32±0.27 (3.91-4.62)		2.14±0.22 (2.03-2.51)		4.06±0.21 (3.74-4.28)	
Postbreeding	Male	4.244±0.27 (3.42-4.62)	2.12	1.21±0.18 (1.21-0.18)	0.942	3.76±0.18 (3.51-4.02)	2.092
	Female	3.70±0.27 (3.42-4.06)		1.31±0.15 (1.12-1.56)		3.28±0.21 (3.08-3.61)	

Number of observations in each case = 10; Range in parentheses
*= P<0.05; ** = P<0.01; *** = P<0.001 (student's 't' test)



Analysis of the result of the table 3 and table 4 show that, in general liver glycogen content is higher in male than the female in all the three experimental conditions, i.e. control, sublethal and semilethal conditions in all the three annual reproductive cycle, i.e. prebreeding, breeding and postbreeding periods.. In case of both Aldrin and cythion intoxication, there is a decreasing trend in liver glycogen content in following order : control > semilethal > sublethal in all the three reproductive periods (Table 3 &4). However, in case of cythion difference in glycogen content of liver is statistically significant in control fishes ($p < 0.05$) of the postbreeding period (Table 4). When seasons are taken into account, in case of both Aldrin and Cythion intoxication, highest liver glycogen level was found during breeding period followed by prebreeding and postbreeding periods (Table 3 & 4).

In case of Aldrin intoxication, when experimental conditions are compared among themselves, in prebreeding period, difference is found significant in both the sexes ($p < 0.001$) of sublethal condition compared to control and semilethal condition (Table 5); in breeding period difference is found significant in both the sexes ($P < 0.001$) of control condition compared to sublethal condition (Table 5); when sublethal condition was compared to semilethal condition, difference is found significant in male ($p < 0.01$) and in female ($p < 0.001$) (Table 4); in postbreeding period, when control condition is compared to sublethal condition, difference is significant in both the sexes ($p < 0.001$) and when sublethal condition was compared to semilethal condition, difference is found significant only in female ($p < 0.01$) (Table 5).

Table 5: In Aldrin intoxication,variation in 't' test value of Liver glycogen in different reproductive periods when experimental conditions are compared among themselves,.

Experimental conditions	Sex	Reproductive stages		
		Prebreeding	Breeding	Postbreeding
Control vs LC_O	Male	12.59***	6.86***	9.43***
	Female	4.94***	6.38***	4.55***
LC_0 vs LC_{50}	Male	4.54***	3.90**	1.76
	Female	5.68***	5.15***	3.81**
Control vs Lc_{50}	Male	0.22	0.32	0.59
	Female	0.59	0.14	0.59

Number of observations in each case = 10; Range in parentheses
*= P<0.05; ** = P<0.01; *** = P<0.001 (student's 't' test)

Table 6: In Cythion intoxication,variation in 't' test value of Liver glycogen in different reproductive periods when experimental conditions are compared among themselves.

Experimental conditions	Sex	Reproductive stages		
		Prebreeding	Breeding	Postbreeding
Control vs LC_O	Male	8.91***	12.82***	9.71***
	Female	1.81	8.86***	8.49***
LC_0 vs LC_{50}	Male	4.88***	7.77***	14.55***
	Female	1.59	7.14***	9.55***
Control vs Lc_{50}	Male	0.87	1.81	2.96**
	Female	0.91	1.37	1.39

Number of observations in each case = 10; Range in parentheses
*= P<0.05; ** = P<0.01; *** = P<0.001 (student's 't' test)

In case of cythion intoxication, when experimental conditions were compared among themselves, during prebreeding period, difference was significant only in male ($p < 0.001$) when sublethal fishes were compared to fishes of control and semilethal conditions respectively (Table 6); during breeding priod, difference was found significant in both the sexes ($p < 0.001$) of sublethal condition fishes compared to control and semilethal conditions respectively (Table 6); during postbreeding period, difference was significant in both the sexes ($p < 0.001$) of sublethal condition fishes compared to the fishes of control and semilethal conditions respectively and only in male ($p < 0.01$) of control fishes of semilethal conditions (Table 6).

In case of Aldrin intoxication, when reproductive periods were compared among themselves, in control set fishes, glycogen content in liver was found significant in male ($p < 0.001$) and in female ($p < 0.05$) when breeding period is compared to postbreeding period (Table 7); in sublethal condition, difference was found significant in female ($p < 0.001$) of postbreeding period compared to breeding and prebreeding periods respectively (Table 7); in semilethal condition. differenc was found significant only in female ($p < 0.05$) of breeding period compared to postbreeding period (Table 7).

Table 7: In Aldrin intoxication,variation in 't' test value of Liver glycogen in different experimental conditions when reproductive seasons are compared among themselves

Reproductive stages	Sex	Experimental conditions		
		Control	Sublethal	Semilethal
Prebreeding vs Breeding	Male	1.103	o.606	0.272
	Female	0.576	0.607	1.173
Breeding vs Postbreeding	Male	6.021***	1.341	1.592
	Female	2.305*	5.145***	2.287*
Prebreeding vs Postbreeding	Male	1.357	1.390	1.355
	Female	1.195	6.197***	1.394

Number of observations in each case = 10; Range in parentheses
*= P<0.05; ** = P<0.01; *** = P<0.001 (student's 't' test)

Table 8: In Cythion intoxication,variation in 't' test value of Liver glycogen in different experimental conditions when reproductive seasons are compared among themselves

Reproductive stages	Sex	Experimental conditions		
		Control	Sublethal	Semilethal
Prebreeding vs Breeding	Male	1.103	1.084	0.422
	Female	0.576	0.649	0.818
Breeding vs Postbreeding	Male	6.021***	4.616***	2.068
	Female	2.305*	3.418**	3.228**
Prebreeding vs Postbreeding	Male	1.356	2.114*	0.825
	Female	1.195	0.264	2.148

Number of observations in each case = 10; Range in parentheses
*= P<0.05; ** = P<0.01; *** = P<0.001 (student's 't' test)

In case of Cythion intoxication, when reproductive periods were compared among themselves, in control set fishes, difference was found significant in male ($p < 0.001$) and in female ($p < 0.05$) when liver glycogen content of breeding period fishes were compared to postbreeding ones (Table 8); in sublethal condition fishes, difference was found significant in male ($p < 0.001$) of breeding period fishes compared to fishes of postbreeding period (Table 8) and in male ($p < 0.05$) of prebreeding period compared to postbreeding period fishes (Table 8); in semilethal condition, difference in glycogen content in liver was found significant in female ($p < 0.01$) of breeding period fishes compared to postbreeding fishes and in female ($p < 0.05$) of prebreeding fishes compared to the fishes of postbreeding period (Table 8).

Result show that in case of both Aldrin and Cythion intoxication, the glycogen content of liver decreases in all the three experimental conditions during all the three annual reproductive periods of the test fish. similar findings have been reported by Polat *et al.* (2002) in *Poecilia reticulate* exposed to â- cypermethrin. Bansal *et al.* (1979) in *Tilapia mossambica* exposed to chlordane; Koundinya and Rammurthi (1979) in *Tilapia mossambica* exposed to organophosphorus pesticides; Singh and Srivastava (1981) in *H. fossilis* exposed to Aldrin and Fenitrothion; Dalela *et al.* (1981) in *M. vittatus* exposed to different pesticides; Pant and Singh (1983) in *P. conchonius* exposed to carbaryl and Dimethoate. has also made similar reporting. Rojik and Boross (1983) have also reported the marked decrease in the glycogen content of liver. However, Srivastava and Singh (1981) in *H. fossilis* exposed to methyl parathion found an initial decrease in liver glycogen content after 3 and 6 hours of exposure, followed by an increase after 12 and 48 hours. Whereas Grant and Mehrle (1973) in *Salmo gairdeneri* exposed to endrin; Escoubet and Vincente(1975) in *Scorpaenia poreus* exposed to Lindane reported increase in liver glycogen content.

The breakdown of glycogen may be related to the stress caused by toxicants (Vig *et al.*, 1987); Pant and Singh, 1983; Mathur 1965, 1976). An enhanced secretion of catecholamines occur under stress condition (Mushigeri and David, 2004; Nakano and Tomlinson, 1967). The glycogenolysis may be related to an augmented supply of catecholamines as well as glucocorticoids (Khalaf *et al.* 1999). Thus the pituitary-adrenal axis is apparently stimulated in an effort to mobilize energy stores to meet the requirement of a hypermetabolic state caused by stress in the test fish.

Thus from result it seems that glycogen content has direct correlation with concentration of toxicants. Higher concentration of pesticides cause breakdown of glycogen more rapidly in the liver.

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