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Effect of varied calcium concentrations on serum calcium, sodium, potassium and protein value during the different phases of reproductive cycle in *Heteropneustes fossilis* (Bloch)

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Abstract: The serum, calcium, sodium, potassium and protein value were measured during different phases of reproductive cycle in *Heteropneustes fossilis* (Bloch) due to effect of varied calcium concentrations. The calcium value was higher then and Potassium value Protein during spawning period pointing a more demand of calcium which is necessary for the functioning of a enzyme thereby also helps in hardening of eggs while protein meets an extra energy demand during this period. An increase in serum, sodium level was also observed during spawning period than pre and post-spawning.

Keywords : *Heteropneustes*, Reproductive cycle, Serum, Calcium, Sodium, Potassium, Protein

INTRODUCTION

The present study has been planned to observe the effect of different calcium concentrations in the serum calcium, sodium, potassium and protein level in *Heteropneustes fossilis* (Bloch) during its reproductive cycle.

Bjornsson *et al.* (1989) have worked on smoltification and sea water adaptation in coho salmon *Onchorhynchus kistuch*. Plasma osmolity, gill Na^+ K^+ ATpase activity, plasma level of calcitonin and free total calcium, magnesium were also measured.

Fenwick and Brasseur (1991) have reported that effect of stanniectomy and experimental hypercalcemia on plasma calcium levels and calcium influx in American eels, *Anguilla rostrata* Intraperitoneal injection of $CaCl_2$ sub(2) in intact, shamstanni-ectomized and stanniectomized American eels, *Anguilla rostrata* resulted in a rapid elevation of plasma calcium level which was more marked and of longer duration in the stanniectomized eels.

Malhotra and Sharma (1988) have reported that the variable calcium concentration seems to be one of the factors which determines the breeding times of the fishes in the same breeding area. Tandon and Joshi (1974) have worked on seasonal variations in blood glucose and serum calcium levels of fresh water fishes. They have further reported definite seasonal variations in normal blood glucose and serum calcium level in the fish.

MATERIALS AND METHODS

Mature fishes ranging between 12 to 17 cm in length and 25-30 gm in weight were obtained from Sagar lake during the first week of every month for one complete reproductive cycle i.e. continuously for 12 months and acclimatized in tap water aquarium in laboratory conditions and treated with tetracycline to control bacteria and other outbreaks for experimental work. For experimental work eight fishes were kept in each aquarium containing 24 litre tap water i.e. 3 litre/fish. During acclimatization period fishes were fed with dried shrimps. However it was refrained throughout the experimental period. The water of each aquarium was also renewed twice a week. Three sets of experiments, containing 16 fish in each set up were arranged. For pre spawning and post spawning period.

Experiment with different calcium concentrations: It was very surprising and interesting to note the maximum calcium tolerance i.e. 65.0 m mol l^{-1} by this fish. Preliminary calcium tolerance were conducted using calcium chloride experiments with gradual fast adaptation while 62.5 m mol l^{-1} during spawning. Maximum calcium tolerance was 65 m mol l^{-1} and 62.5 m mol l^{-1} during spawning phase.

Gradually fast transfer in different calcium concentrations during post-spawning, pre-spawning and spawning period.

Sixteen fishes in total were used for this experiment i.e. eight fishes were used for experimental work and remaining for control. Each aquarium contained equal amount of water i.e., 3 litres/fish. After setting experiments the observations were taken at regular interval to note the pH, temperature and mortality of experimental and control group.

The experiments were set in the following way as per the protocol of Wendelaar Bonga *et al.* (1983):

1.Gradually fast transfer in different calcium concentrations during post-spawning period (December).The fish *Heteropneustes fossilis* belonging to experimental group were gradually adapted from 2.5 m

Table 1. Serum, calcium, sodium, potassium and protein value of *Heteropneustes fossilis* (Bloch) with exposure to calcium chloride 62.5 m mol l^{-1} or 65 m mol l^{-1} in fish.

		Post-spawning			Pre-spawning			Spawning		
		Mean	Standard Error (±)	p Value	Mean	Standard Error (±)	p Value	Mean	Standard Error (±)	p Value
Calcium (m Eq.l^{-1})	Control	5.094	0.1361	0.1558	5.9333	0.06808	0.0148	6.069	0.2179	0.7817
	Experimental	5.429	0.1557		6.262	0.1387		6.195	0.1725	
Sodium (m mol.l^{-1})	Control	130.58	7.987	0.0040	159.17	0.3757	<0.0001	143.72	13.556	0.336
	Experimental	93.705	1.652		111.06	0.6094		206.21	26.257	
Potassium (m mol l^{-1})	Control	7.82	0.254	0.1830	8.371	0.0245	<0.0001	6.505	0.131	0.3359
	Experimental	8.436	0.161		8.640	0.0137		6.667	0.165	
Protein (g l^{-1})	Control	7.792	0.861	0.1618	6.260	0.0433	0.0018	3.294	0.197	0.1867
	Experimental	5.542	1.114		4.862	0.257		2.852	0.223	

mol l^{-1}, 5.0 m mol l^{-1} upto 65 m mol l^{-1} in calcium chloride ($CaCl_2.2H_2O$, E. Merck) solution in fresh water (each step lasted for a day). In 65 m mol l^{-1} solution the animals could not survive for more than 5 to 6 hrs as the concentration was found lethal.

2.Gradually fast transfer in different calcium concentrations during pre-spawning period (April). The whole set up was described as above.

3.Gradually fast transfer in different calcium concentrations during spawning period (July). The fish *Heteropneustes fossilis* belonging to experimental group were gradually adapted from 2.5 m mol l^{-1}, 5.0 m mol l^{-1} upto 62.5 m mol l^{-1} in calcium chloride ($CaCl_2.2H_2O$, E. Merck) solution made in freshwater (each step lasted for a day). In 62.5 m mol l^{-1} solution the animals could not survive for more than 5 to 6 hrs as the concentration is found lethal.

Biochemical test: The blood was collected from caudal vein by a sterilised disposal syringe (2.0 ml Dispovan) in a small plastic tube. It was then stored in ice till actual use for calcium, sodium, potassium and protein determination. All tests were measured by a spectrophotometer (Shimadzu, Double beam, UV 190, Japan).

Calcium estimation: Span Diagnostics (Calcium Trinder's Method) kit was used for calcium estimation.

Sodium and potassium estimation: Dr. Reddy's diagnostic kit was used for Sodium and Potassium estimation.

Protein estimation: Span Diagnostics kit for Protein (Modified Biuret and Dumas Method) was used for Protein estimation in serum.

RESULTS

During the higher calcium concentration i.e., 62.5 or 65.0 m mol l^{-1} the serum calcium value of experimental group was higher in comparison to the control group. When it is compared during different phases of reproductive cycle i.e., post-spawning, pre-spawning and spawning, the experiment calcium value is significantly high during pre-spawning and spawning period in comparison to post-spawning period. (Fig.1)

At the highest tolerance limit of maximum calcium concentration i.e.,62.5 or 65.0 m mol l^{-1}, the serum sodium value of experimental group is surprisingly and interesting high and significant during spawning period in comparison to control. During pre and post-spawning, sodium value of experiment group was significantly lowered when compared to control. (Fig.2).

With the highest tolerance limit of calcium concentration i.e.,62.5 or 65.0 m mol l^{-1}, the serum potassium value of experimental group was low during pre and post-spawning than spawning phase where this value was significantly higher than control. (Fig. 3) With the highest tolerance limit of calcium concentration i.e., 62.5 or 65.0 m mol l^{-1} the serum protein value of experimental group was less in comparison to the control throughout the different phases of reproductive cycle. i.e., post-spawning, pre-spawning and spawning. Protein value is significantly high during post-spawning period in comparison to the pre-spawning and spawning. In experimental group, the protein level is high during post-spawning period and also corresponds to control value, in comparison to pre-spawning and spawning phases (Fig.4).

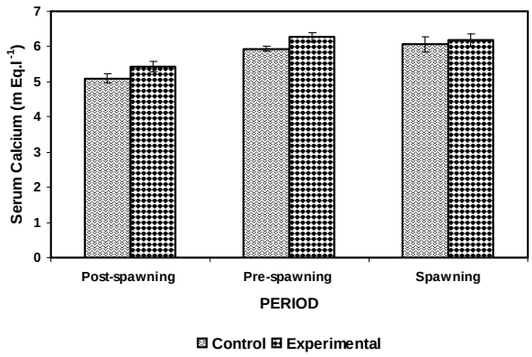

Fig. 1. *Showing the variation in calcium after exposure to calcium 62.5 m mol l^{-1} and 65 m mol l^{-1}.*

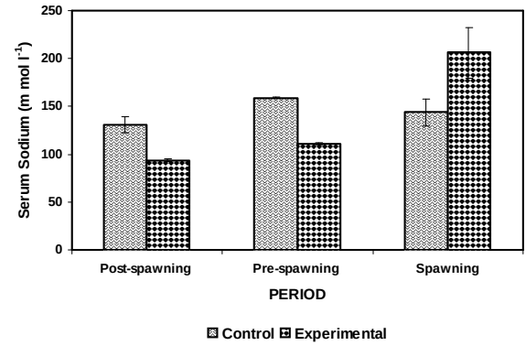

Fig. 2. *Showing the variation in sodium after exposure to calcium 62.5 m mol l^{-1} and 65 m mol l^{-1}.*

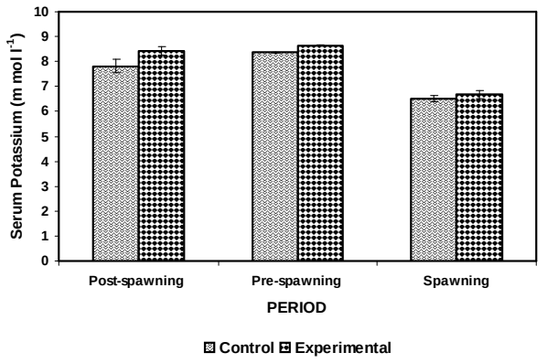

Fig. 3. *Showing the variation in potassium after exposure to calcium 62.5 m mol l^{-1} and 65 m mol l^{-1}.*

The seasonal variations of temperature and pH during experimental and control in different phases of reproductive cycle i.e., post-spawning, pre-spawning and spawning can also be well understood by the data presented in diagrams (Figs. 5 and 6).

DISCUSSION

Ahmad and Swarup (1990) have shown that the serum calcium level increased with the ovarian maturation and reaches its peak during spawning phase in *Mystus vittatus*. The maturation cycle in *Mystus vittatus* begins in March and progressively continues to May and June

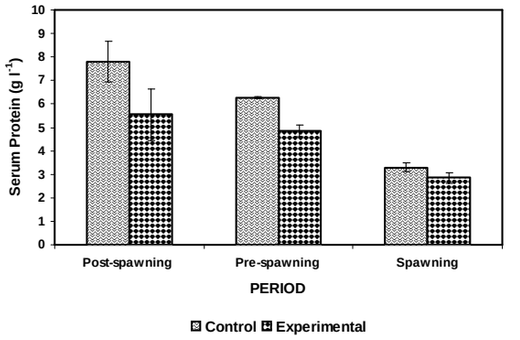

Fig. 4. *Showing the variation in potassium after exposure to calcium 62.5 m mol l^{-1} and 65 m mol l^{-1}.*

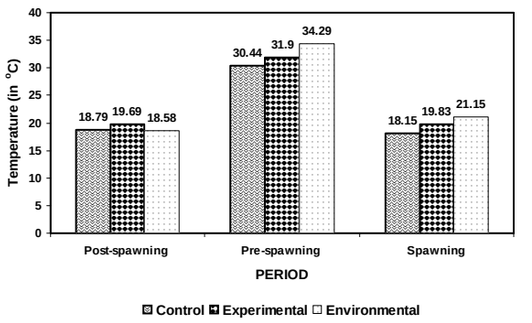

Fig. 5. *Showing the variation in temperature during different phases of reproductive cycle.*

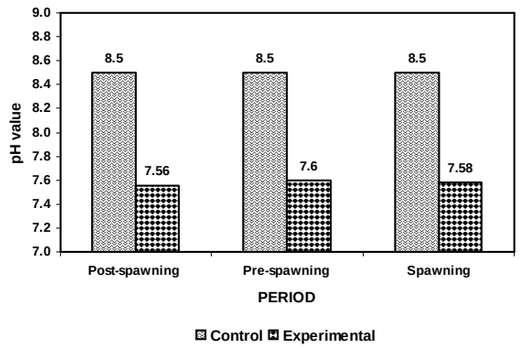

Fig. 6. *Showing the variation in pH of during different phases of reproductive cycle.*

(Pre-spawning phase) with corresponding increase in serum calcium level. The highest level of serum calcium is recorded during July and August (Spawning phase). It records a decline after spawning (September onwards) i.e. during post-spawning phase and further declines in November with significant decrease in January and February (resting phase).

Assem and Hanke (1984) have shown that injection with prolactin increase the osmolality and plasma Na^+ in fresh water and sea water Tilapia (*Sarotherodon mossambicus*). Gradual increase is observed in sodium as the calcium concentration increases during pre-

spawning, spawning and post-spawning period but in high concentration i.e.; 7.5 and 10.0 m mol l^{-1} it is significantly low in all the phase of the reproductive cycle. In different salinity concentrations there is a fall in sodium level during pre-spawning, spawning and post-spawning period.

Bjornsson *et al.* (1989) have observed that calcitonin does not participate in the regulation of physiological changes that occur following introduction of smoltified coho, salmon to sea water. During netting and confinement stress, the calcitonin is not responsible to short term increase in plasma calcium, magnesium and osmolality. They recognised that greatest hypoosmoregulatory ability occurred in April-May although sea water adapted fish had higher plasma osmolality than fresh water adapted fish at all times.

Wendelaar Bonga *et al*. (1983) have shown that adaptation to higher external calcium concentration tends to increase in plasma calcium level and decrease in mg level in *Sarotherodon mossambicus*. Similar results were also observed in our experiment with *Heteropneustus fossilis*. With different calcium concentrations during pre-spawning period of *Heteropneustes fossilis* there is rise in calcium level. The highest calcium level is recorded during pre-spawning period but gradual fall in calcium level is observed thereafter i.e., post-spawning period also suggesting the lower need of this important ion during this phase as reported above also. Calcium level was also high during spawning period in control group (Brook, 1964; Swarup *et al.*, 1986; Shrivastav and Shrivastav 1998; Pang, 1978; Tandon and Joshi, 1974 and Westin 1978 while in present study of experimental group, also the calcium level was also higher in spawning when compared to post-spawning period.

The serum sodium value of experimental group was surprisingly high during spawning when compared to the pre and post-spawning period. The serum potassium value in experimental group was higher during pre and post-spawning period than spawning period. The protein value was significantly higher during post-spawning pointing a less demand of protein during this period.

Woo (1990) have observed the changes in blood chemistry of the red sea bream after acclimation to 10, 20, 25, 30^0C for three weeks. It shows an enhancement of plasma osmolality, Na^+, K^+, Mg^+ and amino acids, protein and lipid concentrations and there is an increasing trend with rising temperature and plasma glucose concentration and the hematocrit value. However, the respective values for the 10^0C acclimated group are significantly higher than those at other temperature. At 30^0C there are signs of osmoregulatory stress as indicated by elevated plasma ion levels. Also high water temperature leads to metabolic reorganization, resulting enhanced protein and lipid utilization. At low temperatures, the fish utilizes carbohydrate as the energy source, while conserving body protein. They also suggested that there was a stepwise increase in the activity of G.6 ptase and FD ptase in the liver as the temperature increased indicating enhanced gluconeogenic activity at high temperature. Thus it seems that at high temperature, enhanced gluconeogenesis from proteins occurs at the expense of glucose utilization, resulting in overall deposition of glycogen in the liver and muscles.

It can be concluded that serum calcium in experimental group remains with in a narrow range during different phases of reproductive cycle, the fish is likely to experience.

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