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# Morphological effect of highest calcium concentrations in *Heteropneustes fossilis* (Bloch)during different periods of reproductive cycle.

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**Abstract:** The present study has been observe the morphological effect of highest calcium concentration in *Heteropneustes fossili*s. It was very surprising and interesting to note the highest calcium tolerance i.e. 65.0 m mol $l^{-1}$ ( post-spawning and pre-spawning period) by this fish. Preliminary calcium tolerance, they conducted using calcium chloride experiments with gradual fast adaptation while 62.5 m mol $l^{-1}$ during spawning period. During Exposer of maximum calcium tolerance barbels, fins are highly rupture. The mucilaginous skin are dry.

**Keywords :** *Heteropneustes*, Reproductive cycle, Calcium, Barbels, fins, Skin

## INTRODUCTION

Environment plays a very important role in the movement of different ions through various organs, responsible for the adjustment with the environment and one of the important ion which play a major role is Calcium. An important factor considering teleost Calcium regulation is the type of external environment; the hypercalcium seawater or the hypocalcemic fresh water. Superficially it would be expected that marine species would prefer “open” mode of regulation while fresh water would

conform to be ‘closed’ mode to avoid Calcium losses. One of the important fresh water teleost is *Heteropneustes fossilis* (Bloch) which forms a large proportion of fish catch and culture in many parts in our country. Interestingly and surprisingly it can tolerate a large range of Calcium variations in external milieu.

Inland saline soil generally called “users lands” are found in the semi arid areas of M.P., Chhattisgarh, Maharashtra, Haryana, Rajasthan, U.P., and Gujarat etc. is considered unsuitable for agriculture. It can be utilised culture of certain fish species like *Chanos chanos, Etroplus suratensis* (Pearl spot) clarias batrachus . This is being tried for their culture possibilities in saline water.

Seasonal diurunal variations in plasma Calcium concentration was reported by Dr. Mugiya. However, it remain unknown, how fish physiologically respond to such changes for this purpose. Moreover the differential presence of Calcium appears to be one of the factors determining the breeding time of fishes at the same breeding site (Malhora et al., 1988).

Since fish lack parathyroid hormone, several studies suggest a physological role of pituitary derived prolactin and vitamin D metabolite in fish Calcium homeostasis. Administration of mammalian prolactin as well as partially purified fish prolactin causes significant hypercalcemia in teleosts and the response is more pronounced in fishes kept in high Calcium water or normal tap water. The secretagogic effects of Calcium have been documented *in vitro* where the cation has concentration dependent effects on prolactin release from the pituitary in fishes. (Mukherjee, 2000).

Calcium is of paramount importance to a teleost fish in two different ways i.e. the solid (98-99% in endoskeleton) and dissolved (plasma). Studies indicate that resorption of scales can indeed take place during sexual maturation in female or during starvation (Ouchi and Kosaka, 1972). The endocrine regulation of Calcium balance in teleost is obviously very complex and there are numbers of endocrine glands with suspected or confirmed function in Calcium regulation. The plasma Calcium levels are under precise dual endocrine control of corpuscles of

Stannius and hypophysis while primary target organs for this endocrine control of Calcium are the gills and kidney.

Lahnsteiner.F . (2009) has reported the effect of different kinds of electrolytes and non electrolyte solution on the survival rate and morphology og Zebra Fish *Danio rerio* embryos.

## MATERIALS AND METHODS

The fish *Heteropneustes fossilis* (Bloch) were obtained from local Sagar lake, Sagar, M.P. Twenty four adult fishes were collected during the first week of every month for one complete reproductive cycle i.e.; for continuous 12 months.

During the experimental period the mature fishes ranging between 12 to 17 cm in length and 25-30 gm in weight were placed in tap water aquarium in laboratory conditions and treated with tetracycline to control bacteria and other out breaks in Post-spawning (December), Pre-spawning (April) and Spawning period (July). Healthy fishes were selected for the experimental work. Eight fishes were kept in each aquarium which contains 24 litre tap water i.e. 3 litre/fish. They were acclimatized for about a week before starting the experiment. During this period fishes were fed with dried shrimps. However, they were not fed throughout the experimental period and the water of each aquarium was renewed twice a week.

### Experiment with different Calcium concentrations

The Calcium chloride ($CaCl_2.2H_2O$, E. Merck) was used throughout the experimental period. For the preparation of Calcium chloride solutions of different concentrations the dry powder was properly weighted and dissolved in tap water.

It was very surprising and interesting to note the highest calcium tolerance i.e. 65.0 m mol $l^{-1}$ by this fish from the preliminary experiments with gradual fast adaptation during pre and post spawning and 62.5 m mol $l^{-1}$ during spawning period.

Even in the euryhaline fish, Oreochromic mossambicus (Pathak, 2002) and also in other teleosts the maximum tolerance limit do not cress beyond 10 m mol $l^{-1}$ exceeding which the animals show sign of stress thereby also start onset of mortality.

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

experimental group, temperature of control group. The environmental temperature as well as the mortality at each animal in all the aquariums were noted.

The experiments were set in following way as per the protocol of S.E. Wendelaar Bonga et al.(1983): Gradually fast transfer in different Calcium concentrations during post-spawning period (December)pre-spawing period (April) and spawing 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 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 and the concentration is found lethal.

**RESULTS AND DUSCUSSION**

During exposer of highest calcium concentration 62.5 and 65 m mol $l^{-1}$ in experimental group four pair barbels, and fins are highly rupture and skin are dry without mucilaginous comparison to control group.

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 (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 (Septemberonwards) i.e. during post-spawning phase and further declines in November with significant decrease in January and February (resting phase).

Bjornsson et al. (1989) have worked on smoltification and sea water adaptation in coho salmon *Onchorhynchus kistuch*. Plasma osmality, gill $Na^+$ $k^+$ ATpase activity, plasma level of calcitonin and free total calcium , magnesium were also measured. The study of sea water adaptability of fresh water fish by the measurement of the plasma osmolality was made after 24 hours exposure to sea water and it was observed that sea water adapted, fish show higher osmolality than fresh water adapted fish at all times. They also

recognised that plasma calcitonin level gradually increased in fresh water adapted fish during the period of desmoltification. However, no changes in plasma calcitonin levels occurred during sea water induced hypercalcemia suggesting that hormone does not play a major role in short term plasma calcium regulation in coho salmon.

Bonga, et al (1983) have studied the effect of external Mg 2+ and Ca 2+ on branchial osmotic water permeability and prolactine secretion in the teleost fish *Sarotherodan mossambicus.*

Bonga et al. (1982) have shown that adaptation to high external calcium concentration lands to increase in plasma calcium level and decrease in mg level in sarotherodon mossambicus. Similar results were observed in our experiment with Tilapia, (Oreochromis mossambicus). In different calcium concentrations during pre-spawning period with Tilapia (Oreochromis mossambicus) when there is rise in calcium level. The highest calcium level is recorded during 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. 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 aminoacids, 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 pase and FD pase 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 morphological effect of highest calcium concentration in *Heteropneustes fossili*s is very surprising and interesting to note the highest calcium tolerance i.e. 65.0 m mol $l^{-1}$ ( post-spawning and pre-spawning period) by this fish. Preliminary calcium tolerance, they conducted using calcium chloride experiments with gradual fast adaptation while 62.5 m mol $l^{-1}$ during spawning period. During Exposer of maximum calcium tolerance barbels, fins are highly rupture. The mucilaginous skin is dry. In India it is that found large quantity of sewage water this fish is suitable for culture in sewage water.

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**Figure:1**

*Heteropneustes fossilis* (Bloch) showing sexual dimorphism.


**Figure:2**

Morphological effect of 65 m mol $l^{-1}$ (post-spawning and pre-spawning period) and 62.5 m mol $l^{-1}$ (spawning period) of calcium chloride ($CaCl_2.2H_2O$) solution in experimental group in *Heteropneustes fossilis* (Bloch) showing barbells, fins are highly rupture and mucilaginous skin are dry, comparison to control group.