

**SOME BIOCHEMICAL AND PHYSIOLOGICAL ADAPTATIONS
IN CARPS (CYPRINUS CARPIO AND LABEO ROHITA) TO
HIGH ALTITUDE DURING DEVELOPMENT**

ABSTRACT

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Reproduction and development in fish are complicated processes regulated by many intrinsic and extrinsic factors. The fish embryo develops externally in water. The egg membrane is impermeable to most of the biomolecules. Therefore, maintenance and growth of the embryo proceed at the expense of nutrients, deposited in the egg during oogenesis. During the course of early development, the deposited biomolecules are degraded to supply energy for the growing embryo and also to supply precursors for synthesis of new biomolecules. Metabolism of various biomolecules varies at different stages of development, depending on the requirements of the embryo. These variations are brought about by regulation of enzyme activities which control the metabolic reactions. Differential gene expression, at different stages of development, largely controls the modulation in the levels of activities of different enzymes. However, environment also plays some important role in regulating metabolism during embryogenesis. Metabolism in fish is a labile process and shows compensatory changes with alterations in the environment. Alteration in physico-chemical conditions of water, in which the embryo develops, leads to alterations in rate of development. Beyond a certain limit of environmental fluctuations, the embryos die due to the breakdown of metabolic homeostasis. Metabolic adaptation to survive in variable conditions of the environment varies widely among different species. The developmental stages of some fishes have a wide tolerance range while for some others it is narrow.

Common carp (Cyprinus carpio) is an example of fishes having a broad range of tolerance capacity. It breeds and grows

well at low as well as at moderately high altitudes. In comparison to common carp, the Indian major carp, L. rohita (rohu), is highly sensitive to the environmental variations. It grows well in tropical warm water where it breeds well in nature. It fails to breed naturally in small confined water bodies. Adaptability to nature is different in different species and is mostly determined by genetic factors. This is observed in terms of different metabolic compensatory changes at sub-cellular level. However, the basic ontogenic patterns of metabolic and biochemical changes in fishes, in general, and in these species in particular, are limited. Therefore, the present study was undertaken to study the breeding efficiency, and rate of development and survival, alongwith certain biochemical and metabolic patterns during the early development of scale carp (a common carp, Cyprinus carpio var. communis L.) and rohu (an Indian major carp, Labeo rohita Ham.). Their response to high altitude stress, with regard to these parameters and the physico-chemical factors of the ponds were also studied.

The experiments were conducted in North-Eastern India, at Gauhati (26°11'N & 91°47'E) situated at an altitude of 100 m ASL and at Shillong (25°5'N & 91°9'E) at an altitude of approximately 1500 m ASL and Mawpun (25°40'N & 91°56'E) situated at an altitude of 1000 m ASL. The experimental ponds were prepared and managed, under identical conditions, in the Fish Farms of Governments of Assam and Meghalaya. The physico-chemical factors of these ponds at Gauhati and Shillong were recorded for 18 months during the period from January, 1979 to May, 1980 at

regular monthly intervals. It was observed that at higher altitude, temperature and pH were lower while free carbon-dioxide content was higher than those at lower altitude. The dissolved oxygen did not show any significant altitudinal difference.

Induced breeding by hypophysation and rate of spawning, development and survival of the embryos were optimum at lower altitude. Rohu showed a greater rate of development with shorter incubation period than scale carp. However, in both the species, some stage specific variations were also observed. The environment at higher altitude affected the rate of induced breeding and development of scale carp and rohu, with rohu showing more damage. At lower altitude (Gauhati), induced breeding experiments conducted during 1979-80 showed an appreciable success in both scale carp and rohu. However, at Shillong, out of the two attempts, only once (March, 1980) the induced breeding of scale carp was completely successful. Rohu breeders were found to be highly sensitive to Shillong water. In two occasions (August, 1979 and June, 1980), all breeders died at Shillong when introduced from Gauhati. In an intermediate altitude at Mawpun (1000 m ASL), once in August, 1979, all rohu breeders survived, but spawning was only partial. At the same place, in two other attempts in the next year (July & August, 1980) all female breeders died. Besides survival of breeders and spawning, the rate of fertilization, development and embryonic survival were also lower at higher altitude. During 1979 breeding, there was a high mortality of scale carp embryos due to the infection of Saprolegnia. Their mortality was considerably reduced during

1980, with appropriate measures to control the parasitic aquatic fungus. Rohu embryos were highly susceptible to higher altitude and the survival of embryos during the only successful breeding at Mawpun (1979) was negligible.

Sixteen (16) developmental stages were identified in scale carp, from the fertilized egg to the fry stages, while in rohu, three of these stages namely, 32-cells (stage-6), late gastrula (stage-10) and beginning of eye pigmentation (stage-13) could not be detected. The total incubation period was larger in both the species at higher altitude having low temperature and low pH. However, at some stages the rate of development were faster at higher altitude showing some stage specific relationship to the environment. The survival studies were conducted in nine different developmental stages. Scale carp embryos showed a higher rate of mortality, only after the 8-somite (12) stage, at higher altitude whereas all stages of rohu showed higher susceptibility to higher altitude. This showed some very close relationship with the low temperature. Mortality was higher at the neurula (11) stage when about 50% of the embryos died at 24°C, and at pre-hatching (15a) stage with a further decrease in temperature to 15°C, virtually all the embryos died indicating stage specific sensitivity to environmental stress.

The alterations in the rate of development and survival and their response to high altitude are obviously related to the biochemical and metabolic changes in the embryos. This might be reflected, grossly, in the qualitative and quantitative changes of various biomolecules which are not permeable through the

chorionic membrane. Therefore, the concentrations and compositions of amino acids, proteins and nucleic acids and the enzymes and metabolites of three metabolic pathways (ammonia metabolism, neurotransmission & tyrosine oxidation) were studied as models, from unfertilized egg till hatching (stage-15b), at both the altitude in the two species.

The ontogenic pattern of changes in various biomolecules were found to be more or less similar in the early cleavage stages in the two fishes. However, in the later stages they showed some species specific variations. The wet weight of scale carp was higher with higher water and dry matter contents. During the course of embryonic development, the wet weight and water content increased but DMC followed a reverse pattern. The reduction of DMC, which represents the nutrients in the embryo was higher in rohu than scale carp. This might be necessary for a higher energy need of the major carp embryos for their faster rate of development (12.5 hrs.) than scale carp (58 hrs.). The total-, soluble- and cytoplasmic proteins showed a common pattern of decrease, till the end of cleavage in the two fishes. In scale carp, after a temporary increase at blastulation (stage-8), they further decreased till hatching. However, in rohu, proteins again increased from blastulation. The mitochondrial protein levels increased in embryos of both the fishes, but a significant increase was observed at stage-9 (gastrulation) in rohu than stage-12 (8-somite) in scale carp. The overall increase in mitochondrial protein was higher in scale carp (385%) in comparison to rohu (293%). The changes in total free amino acids (FAA), in general, followed a reverse pattern with that of total protein in embryos of both the species. The

composition of the FAA in mature oocytes of the two fishes were different. In scale carp altogether seventeen and in rohu eighteen amino acids were identified. Cysteine and cystine in scale carp and threonine in rohu could not be detected at any stage of development. During the course of embryonic development, the amino acids changed qualitatively and also quantitatively which were also different in the two species. Estimations of DNA and RNA in the oocytes of the two species showed that the values were apparently several thousand times higher than their somatic values. This is similar to the earlier reports in other oviparous animals and some fishes. The DNA and RNA contents of embryos did not change during the early stages of development. The enhancement could be seen only from the late morula (stage-7) and late gastrula (stage-10) stages for DNA and RNA respectively in scale carp. However, in rohu the increase were earlier at 16-cell (5) stage.

The effects of higher altitude on the above parameters of carp embryos were, in general, biphasic with the cleavage stages almost showing no effect and the later stages, particularly from gastrulation till hatching, exhibiting alterations of normal pattern of changes of biomolecules. In scale carp, the rate of increase in water content and dry matter content was comparatively slow and so also the wet weight. In rohu, from neurula (11) stage the water content suddenly increased above the normal level following a simultaneous decrease in DMC. This indicated a severe depletion of the yolk substances to aid the increased rate of metabolism of the embryos under stress of higher altitude. The levels of different types of proteins significantly decreased in the post gastrulation stages of both the fishes except the mitochondrial protein in scale

carp. The decrease indicated a low rate of synthesis of protein and/or higher degradation of yolk at higher altitude. The FAA, in general, showed a higher level in embryos of both the carps at higher altitude. However, in most cases, higher altitude did not alter the relative concentrations of different amino acids except a few. The levels of total DNA and RNA were found to be lowered only towards the later phase of development before hatching, at higher altitude. This too might have been due to the low rate of their synthesis or a higher depletion of the deposited nucleic acids. The low levels of nucleic acids also indicate a low rate of protein synthesis and low growth rate of the embryos at higher altitude.

The variations observed, in the levels of different biomolecules between developmental stages, between the two species and between the two altitudes, might be due to alterations in the different metabolic pathways which, in turn, are controlled by enzymes. Ammonia metabolism, neurotransmission and tyrosine oxidation pathways are known to show developmental and adaptational changes in various other groups of animals. The mature oocyte of both the carp species contained a considerable amount of ammonia and urea which are toxic products of nitrogen catabolism. The levels of both metabolites were higher in scale carp probably due to its larger size. The concentrations did not vary when the values were expressed per gram wet weight. Both ammonia and urea levels increased in concentration with progress of development. The increase was stage specific. The rate of increase was more prominent in scale carp than rohu, showing a higher resistance capacity of scale carp embryos to ammonia or urea toxicity. The

ontogenic and species specific variations of the ammonia and urea levels might have been an independent or combined effect of differential rate of their production, elimination, or conversion to other substances.

Studies on some enzymes associated with ammonia metabolism (GDH, PDG, GS and arginase) showed different ontogenic patterns. Some of the enzymes such as arginase and GS in both the species and PDG in scale carp were present in all stage, including unfertilized egg, GDH appeared only after fertilization and PDG activity in rohu appeared only at neurulation stage. These enzyme activities, which were present in the egg or appeared after fertilization, were maintained during the cleavage stages and showed the enhancement of their activity in some stages after the morphogenetic movement started. In a few cases, the enzyme activity decreased at particular stages. The accumulation of some enzymes in active state and some in inactive state during oogenesis has been known. Some of the inactive enzyme molecules get activated at fertilization to meet the need of these enzymes at the earliest developmental stages. GDH might have activated in the same way. Besides, many masked mRNAs of the egg also start translating different proteins during the early stages of development, contributing to the appearance of some enzymes at these stages when synthesis of RNA has been suspected. Hence, due to gradual loss of these maternally deposited enzymes, the level of activity showed some decrease after some stages of development due to lack of synthesis of new molecules. However, some enzymes which were not deposited in the egg or their activity was not necessary in a particular species in the early stages of development, appeared only at a later stage, possibly

due to the expression of the embryonic gene at that particular stage of development. This might have also happened for the pre-existing enzymes to boost their activity to a higher level from a particular stage. The fluctuations in the endogenous ammonia and urea levels during the development of the scale carp and rohu and the lack of a correlation with their corresponding enzyme activities, fail to make any specific conclusion about ammonia metabolism pattern in the two species. However, species specific variations in the enzyme activities and the concentration of the metabolites have been clearly shown in the present study.

Estimations of ammonia and urea concentrations in scale carp and rohu embryos revealed that the endogenous level of ammonia at higher altitude changed at certain stages of development whereas urea showed no significant variation from their respective normal levels at lower altitude. In rohu, the 16-cell stage and all stages from neurulation till pre-hatching contained significantly higher level of ammonia. Scale carp showed better tolerance to fluctuations in the concentration of ammonia during the developmental stages and survived well, with about 3 times higher concentrations of ammonia than rohu. The alterations in ammonia level showed a close relationship with the changes in protein and FAA content of the embryos at higher altitude and to some extent with the variations in the activity of the enzymes related to ammonia metabolism.

Activity of ammonia metabolising enzymes studied (GDH, PDE, GS and arginase) showed different effects at higher altitude. The activities of some enzymes increased or decreased at some stages of development and the appearance or induction of some enzyme

activities were either postponed or preponed at higher altitude. However, the variations in general, were more pronounced during the post-gastrulation stages than the cleavage stages.

Functional differentiation during development has been coupled with differentiation of specific enzymes. AChE, is one such enzyme, associated with the functional differentiation of nerve and muscle cells. In the present study, AChE activity in embryo was detected at the onset of gastrulation in both the species. This is similar to earlier report in amphibians showing a higher induction of the enzyme activity at gastrulation. Although it is difficult to explain a definite functional significance of the appearance of AChE at gastrulation, it is true that during this stage the morphogenetic movements start determining the map of final differentiation. Estimations of ACh revealed its simultaneous rise with AChE at gastrulation. It can be suggested that perhaps at this stage, the ACh synthesis occurred at a large scale as an inducing factor for AChE gene. The substrate induction of enzyme activity has been known in enzyme regulations. In the subsequent stages, the activity of AChE increased with neural and muscular differentiation. This was almost parallel in both species indicating a species non-specific and differentiation related gene activation.

The effect of higher altitude on the ontogeny of ACh and AChE was also species specific. Scale carp did not show much variations at higher altitude whereas in rohu, the high altitude effect was prominent during the later stages of development. The appearance of AChE activity also got delayed from gastrulation to neurulation in rohu indicating the delay in functional differentiation of neural and muscular tissues.

Studies on the developmental pattern of TAT, the rate limiting enzyme of tyrosine oxidation, showed that the enzyme was present in the cytoplasmic fraction (c-TAT) in all developmental stages, including mature oocyte, in scale carp and rohu. In the mitochondrial fraction, TAT activity (m-TAT) was detected only at neurulation in scale carp and at 8-somite stage in rohu, followed by a significant increase till hatching. However, the appearance of m-TAT was correlated with the significant increase in the embryonic mitochondrial protein level indicating that this enzyme might be produced by a mitochondrial gene and was activated when new mitochondria were synthesized in the embryo. The differences in the ontogenic pattern of c-TAT and m-TAT suggested that they are two isoenzymes of TAT and have different regulatory mechanisms. The developmental pattern of c-TAT and m-TAT in scale carp and rohu were also found to be different from those of mammals, where activities of both the isoenzymes remained low and without any significant alteration till birth. The differences in the developmental pattern of c-TAT and m-TAT in scale carp and rohu, particularly the late appearance of m-TAT in the later species, suggest different regulatory mechanisms for the two isoenzymes in the two carp species.

There was no alteration at higher altitude in the appearance of c-TAT and m-TAT activities. However, during the later stages of development c-TAT activity increased in scale carp and m-TAT activities decreased in rohu at higher altitude.

The developmental pattern of all enzymes in the present study was found to show some differences with the changes in general

protein levels. The changes in general proteins and the enzyme activities were very prominent at fertilization of both the carps. The protein content was drastically reduced on fertilization whereas most of the enzyme activities were either not affected or increased. Similar contrasting changes in total protein and enzyme activities were also observed in other stages of development. These differences may be due to the independent regulation of the enzymes for specific physiological functions independent of the general protein turnover.

Therefore, the morphological and biochemical changes during the developmental stages of the two carps were mostly species specific. Besides, their adaptive capabilities were also different, possibly due to their difference in genetically controlled biochemical regulations.

One basic information which seems apparent from the present study is that, the early stages of ontogenic development of carps, dominated by fast rate of cell multiplication are less sensitive to environmental fluctuations than the later stage of development, dominated by differentiation and morphogenesis. This could be, possibly, due to the fact that besides supply of energy, the deposited yolk in the egg contains necessary precursors and enzymes for completing the cleavages and due to less number of cells and their simpler organization, the gaseous exchange and metabolic waste diffusion also take place efficiently during early stages of development. However, morphogenetic movements, started from gastrulation followed by differentiation and organogenesis, involve expression of several genes and activation of protein synthetic mechanism. These processes need a large amount of energy for which

the metabolism might have been greatly accelerated needing more gaseous exchange and disposal of metabolic wastes. The situation in vivo becomes so complicated during these stages that the interference by the alterations in environmental factors becomes easier. In these stages, the yolk perhaps plays the major role to provide energy but not the readymade precursors, the stock of which might have already depleted. However, an absolute confirmation of this proposition needs more detailed studies regarding the mode of deposition of different biomolecules in the egg and their utilization during development.

It could be seen from the studies in relation to altitude that scale carp has better adaptability for development and growth at higher altitude than rohu. It showed better spawning performance and rates of fertilization, growth and survival. These activities had significant correlations with their biochemical compositions and relative stability of metabolic pathways. The genetic variability in their capacity of adaptation might be related to the presence of excess amount of yolk substances including, perhaps, several enzymes, messenger RNA and other essential substances in the egg of scale carp, giving it more independence for its metabolism and development. Besides, biochemical regulatory mechanisms, probably, operates more efficiently in scale carp than rohu. However, studies on regulation of different key metabolic pathways with relation to different environmental factors can explain, for sure, that out of this multifactorial effect which factors are more important and whether they could be controlled by some experimental manipulation to induce adaptability in the non-adaptive rohu to higher altitude.