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

ABSTRACT

AJIT KUMAR BHAGOWATI

THESIS SUBMITTED IN FULFILMENT OF THE DEGREE OF
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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 (<u>Cyprinus</u> <u>carpio</u>) 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 Maupun (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 occassions (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 Maupun (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 metabolities 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 (98 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 those 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, PDC, 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 preponded 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 fluctustions 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 epathways 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.

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September 9, 1982

Certified that the thesis entitled "SOME BIOCHEMICAL AND PHYSIOLOGICAL ADAPTATIONS IN CARPS (CYPRINUS CARPIO AND LABEO ROHITA) TO HIGH ALTITUDE DURING DEVELOPMENT", submitted by Mr. Ajit Kumar Bhagowati for the degree of DOCTOR OF PHILOSOPHY in Zoology of the North-Eastern Hill University, Shillong embodies the record of original investigations carried out by him under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. Degree. This work has not been submitted for any degree of any other University.

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CONTENTS

Page No.

Index of fish names used in text ... a-c

GENERAL INTRODUCTION ... 1

CHAPTER-I : BREEDING AND DEVELOPMENT

INTRODUCTION ... 15
- Induced breeding ... 16
- Effect of environmental factors on breeding ... 19
- Effect of environment on fish development ... 21
- Effect of higher altitude ... 26
- Plan of work ... 28

MATERIALS AND METHODS ... 30
- Study Areas ... 30
- Ponds ... 30
- Analysis of physico-chemical factors ... 31
- Breeding ... 31
- Kinetics of development ... 34
- Survival studies ... 35

RESULTS ... 37
- Physico-chemical factors ... 37
- Induced breeding ... 38
- Rate of development ... 39
- Survival studies ... 41

DISCUSSION ... 43
- Physico-chemical factors ... 43
- Induced breeding ... 47
- Rate of development ... 50
- Rate of survival ... 52

CHAPTER-II : BIOCHEMICAL COMPOSITION

INTRODUCTION ... 57
- Intrinsic regulation ... 58
- Extrinsic regulation ... 69
- Plan of work ... 71

MATERIALS AND METHODS ... 72
  Fixation of stages ... 72
  Determinations of wet weight, dry matter content (DMC) and water content ... 72
  Extraction of biomolecules ... 73
  Estimation of biomolecules ... 75
  Chemicals and biochemicals ... 77
  Expression of data ... 77
  Statistical analysis ... 77
RESULTS ... 78
  Wet weight, dry matter content (DMC) and percent water content ... 78
  Protein content ... 80
  Free amino acids ... 81
  DNA ... 84
  RNA ... 85
DISCUSSION ... 86
  Wet weight, dry matter content (DMC) and percent water content ... 86
  Protein content ... 90
  Free amino acids (FAA) ... 96
  DNA ... 99
  RNA ... 103

CHAPTER-III : METABOLIC CHANGES

INTRODUCTION ... 106
  Changes in enzyme levels during fish development ... 109
  Effect of environmental factors ... 111
  Ammonia metabolism ... 113
    Origin of ammonia ... 114
    Detoxification of ammonia in fish tissues ... 118
    Adaptive nature of ammonia metabolism ... 121
    Developmental studies on ammonia metabolism in fish ... 123
  Neurotransmission ... 124
  Acetylcholine (ACh) and Acetylcholinesterase (AChE) ... 124

Adaptive nature of ACh and AChE ... 125

Developmental studies in ACh and AChE in fish ... 126

Tyrosine oxidation ... 126

Tyrosine aminotransferase (TAT:E.C.2.6.1.5.) ... 126

Adaptive nature of TAT ... 127

Developmental aspects of TAT ... 128

Plan of work ... 128

MATERIALS AND METHODS ... 130

Preparation of extracts ... 130

Estimations ... 131

Expression of data ... 141

RESULTS ... 142

Ammonia ... 142

Urea ... 143

Glutamate dehydrogenase (GDH) activity ... 144

Phosphate dependent glutaminase (PDG) activity ... 145

Glutamine synthetase activity ... 146

Arginase activity ... 148

Acetylcholine (ACh) concentrations ... 149

Acetylcholinesterase (AChE) activity ... 150

Cytoplasmic tyrosine amino-transferase (c-TAT) activity ... 151

Mitochondrial tyrosine amino-transferase (m-TAT) activity ... 152

DISCUSSION ... 154

Ammonia ... 154

Urea ... 157

Glutamate dehydrogenase (GDH) activity ... 158

Phosphate dependent glutaminase (PDG) activity ... 162

Glutamine synthetase (GS) activity ... 164

Arginase activity ... 165

Acetylcholine (ACh) concentration and acetylcholinesterase (AChE) activity ... 168

Tyrosine aminotransferase (c-TAT & m-TAT) activities ... 172

GENERAL DISCUSSION ... 177
TABLES ... No. 1 to 39
FIGURES ... No. 1 to 29
LITERATURE CITED ... i to xlv
APPENDIX-I : BIODATA
APPENDIX-II : LIST OF PUBLICATIONS AND REPRINTS OF PAPERS

INDEX OF FISH NAMES USED IN TEXT

A. guldenstadti	*Acipenser guldenstadti*
A. hexagonolepis	*Acrossocheilus hexagonolepis*
A. nobilis	*Aristichthys nobilis*
A. stellatus	*Acipenser stellatus*
B. bendelisis	*Barilus bendelisis*
B. boddaeri	*Boleophthalmus boddaeri*
B. icistia	*Bairdiella icistia*
B. rerio	*Brachydanio rerio*
C. auratus	*Carassius auratus*
C. batrachus	*Clarias batrachus*
C. carpio var. *communis*	*Cyprinus carpio* var. *communis*
C. carpio var. *nudus*	*Cyprinus carpio* var. *nudus*
C. carpio var. *specularis*	*Cyprinus carpio* var. *specularis*
C. catla	*Catla catla*
C. idella	*Ctenopharyngodon idella*
C. macularias	*Cyprinodon macularias*
C. moliterella	*Ctenopharyngodon moliterella*
C. mrigala	*Cirrhinus mrigala*
C. punctatus	*Channa punctatus*
C. reba	*Cirrhinus reba*

(b)

D. labrax	*Dicentrarchus labrax*
E. danricus	*Esomus denricus*
E. masquinongy	*Esox masquinongy*
G. conirostris	*Glyptothorax conirostris*
G. pectinopterus	*Glyptothorax pectinopterus*
G. reticulatum	*Glyptosternum reticulatum*
H. fossilis	*Heteropneustes fossilis*
H. ilisha	*Hilsa ilisha*
H. molitrix	*Hypophthalmichthys molitrix*
L. bata	*Labeo bata*
L. cyanellus	*Lepomis cyanellus*
L. dero	*Labeo dero*
L. dyocheilus	*Labeo dyocheilus*
L. ferruginea	*Limanda ferruginea*
L. fimbriatus	*Labeo fimbriatus*
L. gulosus	*Lepomis gulosus*
L. rohita	*Labeo rohita*
M. fossilis	*Misgurnus fossilis*
M. salmoides	*Micropterus salmoides*
M. seenghala	*Mystus seenghala*

(c)

O. gorbuscha	*Oncorhynchus gorbuscha*
O. keta	*Oncorhynchus keta*
O. latipes	*Oryzias latipes*
P. dentatus	*Paralichthys dentatus*
P. fluviatilis	*Perca fluviatilis*
P. reticulata	*Poecilia reticulata*
R. amarus	*Rhodeus amarus*
C. curvifrons	*Schizothorax curvifrons*
S. esocinus	*Schizothorax esocinus*
S. fario	*Salmo fario*
S. fontinalis	*Salvelinus fontinalis*
S. gairdneri	*Salmo gairdneri*
S. irideus	*Salmo irideus*
S. longipinnis	*Schiezothorax longipinnis*
S. plagiostomus	*Schizothorax plagiostomus*
S. salar	*Salmo salar*
S. trutta	*Salmo trutta*
T. khudree	*Tor khudree*
T. putitora	*Tor putitora*
T. tinca	*Tinca tinca*
T. tor	*Tor tor*

GENERAL INTRODUCTION

Fish, one of the largest vertebrate groups, has conquered all possible aquatic systems in the world with their diverse structural and functional adaptations. They are an important food items as a rich source of easily digestible cheap proteins, vitamins and many essential minerals for human beings. A major portion of the consumable fishes are obtained from 'capture fisheries'. However, in recent years, the world's total catch has been declining at an alarming rate mainly due to over-exploitation, pollution and decline in food for fish in nature (Bardach, 1978). A proper control over production of wild fishes is practically difficult. Therefore, currently much emphasis has been laid on the 'culture fisheries' which was once practiced by people merely as a hobby. Consequently, tremendous efforts are being made to exploit all possible water bodies for pisciculture and hence, to obtain scientific knowledge on the various aspects of fish life under controlled conditions to develop proper technology for increasing fish production. Having so much of applied importance, even basic informations about the adaptations at physiological and biochemical level during different phases of life in freshwater fishes, are very much limited.

Fish, as in any other organisms, lives in close interaction with its environment. Its morphology, anatomy or physiology is always influenced by different biotic and abiotic factors of the environment. Any deviation of the external factors evokes a series of adaptational reactions, both visible and invisible, in the organism to establish a bew rapprochement.The morphological or anatomical characters are relatively stable, whereas changes in physiological and biochemical parameters help as the immediate mediator in the rapprochement process. The changes in physiological and biochemical processes tend to take place in the favourable direction which ensures survival of the fish in an altered environmental condition. This is referred to as 'physiological' or 'biochemical adaptation' or sometimes, simply as 'adaptation' of a species (Hochachka & Somero, 1973; Prosser, 1973a; Smith, 1980). The amplitude of adaptation is a 'genetically fixed' phenomenon and it varies between species. The variation in the adaptive capacity is the reason for differential distribution of numerous fish species in different geographical regions of the world with different environmental conditions.

The efficiency for adaptation varies between the different stages of the life cycle of a fish. In salmon, the adult fish lives in sea water, but for breeding it migrates to freshwater, where eggs are laid and embryos develop. The juvenile salmon migrates back to sea water to spend its adult life. In general, reproduction and development are the two phases of fish life which are very precisely controlled by the environment (Hoar, 1969; Blaxter, 1969; Jalabert, 1976; Braum, 1978). A fish does not breed successfully or its embryos do not survive, if the external environmental conditions are not within an optimum limit. This optimum conditions are species specific. Thus, some fish breed in spring, some in autumn, while some others breed in winter. Both, biotic and abiotic factors, influence the reproduction in fish. Among the biotic factors, availability of food is considered as the prerequisites for the development of gonads (Nikolskii, 1963, 1969;

De Vlaming, 1972; De Vlaming et al, 1978). However, the abiotic factors, primarily temperature and photoperiod, are shown to be the dominant regulatory factors for the successful reproduction in many species of fish (Hoar, 1969; Sundararaj & Vasal, 1976; Billard & Breton, 1978). These two environmental factors might act independently or co-operatively. Temperature is an important factor for cyprinids whereas photoperiod is the major factor for salmonids and gasterosteids for reproduction. However, in tropical fishes, where environmental temperature is directly related to the length of the photoperiod, both the factors combinedly regulate the reproduction. Other abiotic factors have also been observed to influence successful reproduction in some species of fish (Husain, 1945; Hora, 1954; Khanna, 1958; Ruby et al, 1977, 1978). The environment influences the reproduction in fish through neuroendocrine mechanism (Hoar, 1969; Liley, 1969). The signals from the environment are received through the exteroreceptors and are conveyed to the hypothalamus which, in turn, stimulates the release of pituitary hormones. The pituitary hormones control reproduction and metabolism in fish.

All fishes freely breed in nature. Some breed in small confined water bodies in captivity under adequate environmental conditions. However, some others like the Indian major carps do not breed in captivity even all biotic and abiotic conditions being optimum. In pisciculture practices, these fishes are induced to breed by injecting an adequate dose of pituitary hormones, a method called 'hypophysation'. This method was first successfully employed by Houssay (1930) in Argentina. The method of hypophysation or induced breeding not only helps to by-pass the environmental inhibition of the breeding in captivity, but it also helps the pisciculturists to adopt suitable measures for obtaining maximum survival and growth of the fish.

The onset of sexual maturation and spawning of fish at a particular environmental condition is of great adaptive significance. It ensures the most favourable condition for development of the embryos and young fishes (Schwassmann, 1971). However, in nature, a large number of fishes die during their ontogenic development. The causes of these mortality are predation by large fishes or by other animals, disease and fluctuations in the environmental factors (Blaxter, 1969; Love, 1970; Braum, 1978). Most viable larvae are produced at an optimum condition of the environmental factors. The 'optimum condition' of a particular environmental factor, has its upper and lower 'limits of tolerance' beyond which the embryo fails to survive indefinitely. Both the 'optimum level' and 'tolerance level' are specific for embryos of a particular species. They also vary between different stages of development in a given species (Nikolskii, 1963).

Variations in environmental conditions has been shown to alter biochemical activities at cellular and subcellular level. Such alterations might have been the cause of mortality of embryos due to a shift in the normal environmental condition. The development of fish, as in any other animal, takes place through a series of biochemical changes. The different biochemical reactions which comprise the metabolism as a whole, are strictly governed by physical factors. The changes in the environmental conditions might result in complete disruption of cellular metabolism, causing death of the developing embryos in extreme cases.

The biochemical mechanism of development and differentiation is basically similar in all multicellular animals (Grant, 1978). In general, it is concerned with (a) degradation of organic molecules, like carbohydrate, lipid or protein, for supplying energy to the growing embryo and also to supply raw materials for (b) synthesis of new molecules, primarily nucleic acids and proteins- the structural and functional units of all kinds of cells, tissues and organs. These biochemical changes occur in a sequential pattern during development. The sequential events have been shown to be due to the differential activity of appropriate genes (McClintock, 1967; Gurdon, 1974; Davidson, 1976; Davidson & Britten, 1979). Informations contained in the DNA molecules are transcribed into m-RNA which translates the same into specific proteins in collaboration with r-RNA and t-RNA. These newly synthesized proteins are either structural, for building of the cell organelles or functional proteins called enzymes, which catalyze different biochemical reactions. The presence of a specific set of proteins or enzymes in a particular organ of an eukaryote animal enables the organ to perform a specific function. During embryonic development, the differentiation of a particular group of cells, along a specific line, is synchronized by incorporation of a specific spectrum of enzymes (Whitt, 1981a). In fact, the relationship between certain enzymes and morphological differentiation is so good that, often the appearance of a specific enzyme is considered as 'biochemical marker' of differentiation of a particular kind of morphological structure (Monroy, 1973; Brachet, 1974). Enzymes, besides giving the functional entity of diverse kinds of cells, also play an important role in regulation of metabolism of different biomolecules during differentiation. The rate of metabolism varies during different periods of development (Love, 1970; Moog, 1971; Balinsky, 1976) in accordance with the changes in demand for energy by the embryo and the precursors necessary for the synthesis of new molecules. This control is achieved through the pre-existing enzyme molecules and also through the synthesis of varieties of new enzymes.

Although the biochemical process of differentiation is basically similar in all animals, it is more complicated in the oviparous ones including fish. In the absence of any direct supply of nourishment or precursors for synthesis of new molecules, as in viviparous animals, the embryo of oviparous animals depends primarily on the amount of yolk materials deposited during vitellogenesis for its successful development. Needham (1931) calls the externally developed eggs as a 'closed box' because it is sealed from any external supply of nourishment and many other materials necessary for development. Studies on the biochemistry of ontogenesis in oviparous animals have been exclusively conducted in echinoderms and amphibians with the sea urchin and Xenopus, respectively, as typical examples (Brachet, 1974; Denis, 1974; Gurdon, 1974; Paul, 1974b). In fish, these studies have been restricted to only a few species such as salmonids, medaka (O. latipes) and loach (M. fossilis). On the other hand, fishes are known for their diverse type of adaptations to different aquatic habitats. Therefore, more informations are required from more representative groups of fish from different habitats.

The development of oviparous animals proceeds at the expense of yolk substances deposited in the egg during oogenesis. In fish, yolk materials consist mainly of lipoproteins and phosphoproteins (De Vlaming et al, 1980) and some amount of free amino acids, fat and glycogen (Balinsky, 1976). Interestingly, the eggs of oviparous vertebrates also accumulate a large amount of DNA and RNA during oogenesis (Tyler, 1967; Denis, 1974; Paul, 1974b). In fish, the amount of DNA in eggs far exceeds their somatic values by 10^3 to $3X10^4$ times or more, and RNA values are several times more than DNA (Neyfakh & Abramova, 1974). The increase in DNA is partly due to the amplification of chromosomes in the nucleus. However, the major deposition of DNA occurs in the cytoplasm. The high DNA values has been shown to be due to amplification of certain genes such as r-RNA gene and due to the increase in mitochondrial number in echinoderms and amphibians. RNA increase is mainly due to the accumulation of different types of RNA (Denis, 1974). Protein synthesis in sea urchin (Brandhorst, 1976), Xenopus (Ballantine et al, 1979) and also in some species of fish (Neyfakh & Abramova, 1974) is shown to be highly activated only at or around the gastrulation in the embryo. However, in salmon (S. gairdneri), Zeitoun et al (1977) have shown an immediate intensive rise of protein content following fertilization. This might perhaps indicate a high activation of the protein synthesis at fertilization in salmon eggs. The yolk protein is gradually degraded with the progress of development and new proteins are synthesized. Depending upon the balance of degradation and synthesis, the protein content decreases or increases with development. The free amino acids also change quantitatively and qualitatively depending upon their production from yolk breakdown, new synthesis and rate of their incorporation into the newly synthesized proteins (Zeitoun et al, 1977). Nuclear DNA synthesis begins at the early cleavage stages (Denis, 1974; Sarkar et al, 1979), but quantitatively DNA content of the egg does not show any increase till the number of cells or in other words the nuclear DNA becomes more than the deposited DNA (Neyfakh & Abramova, 1974). The beginning of the synthesis of RNA in the early development varies from the cleavage stages to gastrulation in different species of fish (Neyfakh & Abramova, 1974; Zeitoun et al, 1977; Chaudhury et al, 1979; Sarkar et al, 1979).

The unfertilized egg of oviparous vertebrates contains a large store of enzymes of various metabolic pathways (Brachet, 1974). Besides, many new enzymes are also synthesized during development. The activity of some enzymes increase, some decrease, some show fluctuations, while some remain constant. The bulk of such studies during fish development deals mainly with the enzymes of glycolysis, TCA cycle or respiratory chain (Hishida & Nakano, 1954; Terner, 1968; Neyfakh & Abramova, 1974; Shaklee & Whitt, 1977; Whitt, 1981a). Informations regarding the enzymes of other metabolic pathways during the development of fish are very little.

Besides carbohydrate and lipid, protein is an important source of energy for developing fish embryo (Hayes, 1949; Smith, 1958; Harmor & Garside, 1977). Thus, yolk protein is catabolized by proteolysis, amino acids so liberated are transdeaminated or directly deaminated liberating α-ketoglutarate and ammonia. α-ketoglutarate, so formed, enters the TCA cycle to liberate energy. The ammonia content in the eggs may also be supplemented by deamination of glutamine (with the help of glutaminase) or by deamination of nucleotides (with the help of nucleodeaminases), which are known to be ammonia producing reactions in adult fish tissues (Forstnr & Goldstein, 1969; Watts & Watts, 1974). Ammonia is a very toxic product and cannot be retained in the cell for a long period. It has been reported in salmon that ammonia could be liberated through the chorionic membrane (Rice & Stokes, 1973; Fedorov & Smirnova, 1978). However, Smith (1947, 1957) observed that ammonia failed to come out of salmon eggs. In the event of ammonia diffusion rate is nil or less in the embryo, ammonia is expected to be converted either to a less toxic form such as urea (through ornithine-urea cycle) or to a non-toxic form such as glutamine (with the help of glutamine synthetase). The conversion of ammonia to urea via the ornithine-urea cycle in teleost fishes is still an enigma. In developing embryos of salmon, Rice and Stokes (1973) reported the absence of a functional O-U cycle. However, recently, Dépeche et al (1979) have shown the presence of the pathway during some stages of development of salmon. Literature on this pathway in the developmental phases of other teleost fishes, and the participation of glutamine synthetic pathways in ammonia detoxification during the development of any fish, are extremely limited.

Neurotransmission is another important physiological process in an animal. Acetylcholine is one of the important neurotransmitters. It is synthesized with the help of the enzyme choline acetyltransferase and degraded by the enzyme acetylcholinesterase. Acetylcholinesterase is known as a marker enzyme for neural and muscle tissue differentiation (Brachet, 1974). Not many reports are available on the developmental pattern of acetylcholinesterase in fish (Uesugi & Yamazoe, 1964; Whitt, 1981a).

The induction or inhibition of the activity of enzymes during development is due to the activation or repression of respective genes (Markert, 1965, 1973; Scandalios, 1979; Whitt, 1981a,b). The functions of genes, in turn, are dependent on the physical and chemical stimuli (Moscona, 1972). In eukaryotic animals, the mechanism of gene regulation is quite complicated. Some model enzymes (Markert, 1970, 1973; Davidson, 1976; Davidson & Britten, 1979) having adaptive characters have been used to study regulation of specific genes. Tyrosine aminotransferase (TAT) is one such enzyme, which in higher vertebrates is quickly induced by physical and chemical stresses (Gelehrter, 1971; Thompson, 1979). The induction of TAT during the stress takes place through the release of corticosteroids which cause transcription of TAT gene (Gopalakrishnan & Thompson, 1977). Therefore, TAT has been exhaustively used as a model enzyme to understand the mechanism of gene regulation in eukaryotes. It has been reported that the regulation of TAT in adult fish and other lower vertebrates is different from the mammals. Glucocorticoids failed to induce TAT in these animals (Chan & Cohen, 1964; Fellman *et al*, 1971; Ohisalo & Pispa, 1975). In a report from this laboratory, it has been shown that the enzyme was inhibited during hypoxic stress in adult *C. carpio*, a phenomenon opposite to that observed in the stressed mammals (Ratha & Bhagowati, 1981). It is known that the gene activity changes during the life time of an animal. Therefore, it might be interesting to see the ontogenic changes in TAT activity in fish to get some idea about its regulation in lower vertebrates.

The role of extrinsic factors on the rate of development and survival of embryo has been studied in a large number of fish species in both freshwater and marine environments (Alderdice *et al*, 1958; Forrester & Alderdice, 1966; Alderdice & Forrester, 1968, 1971a,b; Blaxter, 1969; Love, 1970; Braum, 1978). The embryos have been shown to be very sensitive to the fluctuations in environmental factors. As mentioned earlier, the sensitivity of fish embryos to the external factors might be a reflection of the lability of metabolism, taking place at the cellular and subcellular levels. There are some sporadic reports on the gross changes in metabolism of fish embryos as measured by indirect methods such as estimating the rate of oxygen consumption under different environmental conditions. It has been shown that the rate of oxygen consumption varies inversely with temperature (Lindroth, 1942; Holliday _et al_, 1964). The maximum efficiency of yolk utilization has been shown at an optimum temperature range (Hayes & Pelluet, 1945; Blaxter & Hempel, 1966; Marr, 1966; Ryland & Nichols, 1976). However, there are some reports on yolk utilization independent of temperature, by embryos of some fishes (May, 1975b; Howell, 1980; Johns & Howell, 1980; Johns _et al_, 1981). Low salinity induces an efficient utilization of yolk in _B. icistia_ (May, 1975b). In Atlantic salmon, _S. salar_, reduced oxygen supply caused a low rate of yolk translocation (Harmor & Garside, 1977).

Studies on adult fishes have shown that metabolic adaptation to the variations in environmental condition is a complex process (Hochachka & Somero, 1971, 1973; Vernberg & Vernberg, 1972; Prosser, 1973a). During this, some metabolic processes are activated, some are reduced, while some others do not show any compensatory change. This is reflected by different pattern of changes of the metabolites and the activity of the enzymes involved in different metabolic pathways. To speak in general, studies on the biochemical adaptations during the developmental stages of freshwater fishes are extremely limited and are very much wanting.

Plan of work :

Cyprinus carpio var. communis L. (common carp or scale carp) and Labeo rohita Ham. (rohu, an Indian major carp) are two important species of culturable freshwater fishes in India. The former is an 'eurythermal' species which has the capacity to withstand a wide range of environmental temperature. It breeds and grows well at warmer climate. However, it is also found to adapt well in upland waters at moderately high altitude where the environmental temperature is comparatively low. Therefore, often it is termed as a 'cold-water fish'. On the other hand, the Indian major carp, rohu, has so far been cultured only at lower altitudes in tropical climate. It does not naturally breed under captivity. But, it can be induced to breed successfully only at warm temperature. In India, many upland water bodies are situated at a medium altitude zone. It is felt that such water bodies could also be exploited for culture of the major carps including rohu (Jhingran & Dehadrai, 1974). A fruitful way to make such an attempt is however dependent on the breeding performance, survival of embryos and growth of the fishes in the prevailing physico-chemical conditions of those water bodies at higher altitude. It may be presumed that 'physiological adaptations' would play a great role in determining the acclimatization capacity of the adult fishes or of their embryos to survive and grow under the high altitudinal conditions. This capacity is obviously possessed by the common carps but no attempt has yet been made to find out their molecular mechanism of this adaptation. There is practically very little information available on the developmental physiology and biochemistry in these two (scale carp and rohu) freshwater species.

Keeping these in view, the present work has been planned to study the breeding efficiency, survival and rate of development of the embryos of scale carp (*Cyprinus carpio* var. *communis* L.) and rohu (*Labeo rohita* Ham.) at lower and higher altitude and to correlate these events, if possible, with some molecular and biochemicals changes in them. The higher altitude where the present experiments were conducted were of 1500 metres ASL (at Shillong, 25°5'N & 91°9'E) and at 1000 metres ASL (at Mawpun, 25°40'N & 91°56'E). The lower altitude was almost at sea level at Gauhati (26°11'N & 91°47'E).

The findings have been presented in the three chapters of this thesis as follows :-

CHAPTER-I (Breeding & Development) presents (a) a comparative account of the physico-chemical conditions of managed fish ponds at low and higher altitude (1500 m) and (b) observations on breeding performance and rate of fertilization, development and survival of embryos of scale carp and rohu at different altitudes.

CHAPTER-II (Biochemical Composition) deals with the pattern of changes in wet weight and quantitative changes in dry matter, water, total-, soluble-, cytoplasmic- and mitochondrial- proteins, free amino acids, DNA and RNA, and qualitative changes of free amino acids during early development (from unfertilized egg till hatching) of the two fishes at lower and higher altitude.

CHAPTER-III (Metabolic Changes) deals with the ontogenic changes in the activity of some enzymes and concentration of metabolites related to ammonia metabolism (ammonia, urea, glutamate dehydrogenase, phosphate dependent glutaminase, glutamine synthetase and arginase), neurotransmission (acetylcholine and acetylcholinesterase) and tyrosine oxidation (cytoplasmic and mitochondrial tyrosine aminotransferase), during the early development of the two fishes at lower and higher altitude.

CHAPTER I

BREEDING & DEVELOPMENT

INTRODUCTION

The fishes, like any other vertebrates, reproduce sexually. They are, mostly dioecious. The ova and spermatozoa are produced in separate individuals, and the fertilization and development of the zygote are external. The mature gametes, thus released to the surrounding water, are subjected to the fluctuations in different environmental factors, including predation by other animals. If these conditions are not optimum, the successful development of the next generation gets severely affected (Blaxter, 1969; Nikolskii, 1969). The oviparous fishes, therefore, produce a large number of eggs to balance the mortality during development (Hoar, 1969). However, in the process of evolution, fishes have been successful in restricting their breeding to a particular period of the year when the conditions are most favourable for the development of their embryos (Schwassmann, 1971; De Vlaming, 1972; Crim, 1982). The optimal conditions required for their reproduction and breeding varies considerably in different species (Billard & Breton, 1978). The fish fails to breed in its normal breeding period of the year if the environmental conditions are not optimum (Jalabert, 1976). The effect of different environmental factors both biotic and abiotic, on the reproductive events have been studied in different fishes.

Sufficient experimental evidences are now available to show that the environmental factors influence the reproduction in fish through the neuro-endocrine mechanisms acting primarily along hypothalamic-hypophysial-gonadal axis (Hoar, 1965, 1969; Liley, 1969; Dodd, 1972; De Vlaming, 1972; Donaldson, 1973; Peter & Crim, 1979; Peter, 1982). The environmental factors stimulate the hypothalamus through the exteroceptors which in turn influence the pituitary gland to release the pituitary hormones. Colombo and Belvedere (1976) have reported that the pituitary of teleosts secretes a complement of hormones showing similar functions as seen in other vertebrates. Gonadotropin functions like FSH and LH have been clearly shown in the hormones released from the fish pituitary. However, it is still not clear whether these two functions are shown by two different gonadotropins or only by one species of hormone. The pituitary hormones, through the gonadal steroids and thyroid hormones, control the gametogenesis, metabolism and the reproductive behaviour including spawning in fish. Therefore, in recent years pituitary extract has been widely used to induce successful breeding even in difficult to spawn species of fish in captivity.

Induced breeding :

The fishes breed successfully under optimal natural conditions and produce a large number of eggs. However, variations in the environmental factors drastically affect the rate of spawning, and changes in hydrobiological conditions and predation pressure result in the loss of a large number of eggs or young ones produced in the nature (Wagler, 1933; Elster, 1944; Nümann, 1961; Braum, 1978). It is indeed, difficult to determine the actual cause of such happenings, the extent of loss incurred and to adopt suitable control measures in the large aquatic systems. However, the demand for fish as a major source of protein nutrition for the growing human population has been increasing enormously. This has led to culture fish under controlled conditions in confined water bodies like ponds and reservoirs.

The success of fish culture depends, to a large extent, on the availability of enough good quality fish seeds which are usually obtained from two sources (Das & Khan, 1962). (1) In the earlier days and even now, for many species which fail to breed in captivity, the seeds are collected from nature during the breeding season. This process is not very efficient because the seeds collected are often mixed with many unwanted species, and a large scale mortality takes place during the transport and domestication of the seeds during their early development. (2) The second source which has been developed using scientific knowledge on fish reproduction is by induced breeding of fish in captivity with the injections of pituitary extract (hypophysation) or different hormones.

The use of pituitary injection for induced breeding of fish as reported by Das and Khan (1962) dates back to 1930 when Houssay in Argentina successfully ovulated Cnesterodon decemmaculatus by intra-peritoneal injection of pituitary to gravid females. Following Houssay's experiments, Brazilians in 1934 induced ovulation in Prochilodus using fish pituitary extracts and since then hypophysation has become a regular practice amongst fish culturists of Brazil (Jhingran, 1982). The Russians were next to introduce induced breeding in fish culture with successful hypophysation of sturgeon (Gerbilskii, 1938). This technique started in U.S.A. when Hasler et al (1940) successfully induced spawning in Esox masquinongy by intra-peritoneal injection of pituitary. The hypophysation technique was first introduced in Japan by Kawajisi et al (1948a,b). In India, Khan (1938) was the first to obtain successful breeding by hypophysation in mrigal (C. mrigala) in which mammalian pituitary was used. Chaudhuri (1955) introduced in India the use of fish pituitary (*C. catla*) in induced breeding of *Esomus danricus*. Now, the use of hypophysation technique in fish breeding has been widely used.

The Indian major carps and some exotic chinese carps (*C. catla*, *L. rohita*, *C. mrigala*, *H. molitrix*, *A. nobilis*, *C. moliterella* and *C. idella*) do not breed naturally in ponds. Cytological studies on developing ovary have shown that the final stage of maturation is never attained in ponds resulting in the breakdown and resorption of the eggs internally (Chaudhuri, 1976). The induced breeding of some Indian carps such as *L. rohita*, *C. mrigala*, *C. reba* and *L. bata* was initiated by Chaudhuri and Alikunhi (1957) using fish pituitary hormones. The exotic chinese carps were successfully bred in India by hypophysation in 1962 (Alikunhi *et al*, 1963a,b). At present, the induced breeding of carps are routinely done by hypophysation all over this country (Jhingran, 1982).

Besides carps, many other commercially important fishes in this country have been induced to breed. Some of them are *H. fossilis* (Ramaswami & Sundararaj, 1956; Sundararaj & Goswami, 1969; Thakur *et al*, 1977). *C. batrachus* (Ramaswami & Sundararaj, 1956, 1957), *H. ilisha* (Malhotra *et al*, 1969) and *T. putitora* (Pathani & Das, 1979).

With the rising demand for fish pituitary gland and due to the difficulties in their mass procurement in recent years, a need for substituting pituitary was increasingly felt by the fish culturists. Various mammalian and synthetic hormones have been tried to induce breeding in fish. Human chorionic gonadotropin (HCG or CG), prolan and antuitrin-S have brought about ovulation and spawning in several species (Pickford & Atz, 1957). Khoo (1974) observed that treatment of gravid goldfish (C. auratus) with progesterone and corticosteroids induced ovulation. The mammalian hormone flavolutan (for females) and perandren (for males) were found to induce spawning in the mahseer, T. putitora (Pathani & Das, 1979). Liu (1963) successfully induced spawning of some fishes using choriogonin (a trade name for CG) and SZhk (prepared from pregnant mare serum). HCG alone could not induce successfull spawning in the Indian major carps but when a low dose of carp pituitary extract (3-6 mg/kg + 1 mg HCG) was used, it effected spawning. Synahorin (a mixture of CG and mammalian pituitary extract) were equally ineffective but in combination with fish pituitary extract it induced spawning in L. rohita (Anonymous, 1968). Lutocycline or ovocycline alone did not have any effect (Anonymous, 1967). The use of synthetic and mammalian hormones do not always yield successful breeding. Due to the uncertain results with pure hormones, crude pituitary extract is still more popular and widely used by fish culturists all over the world in induced breeding operations in fishes to produce quality seeds in captivity (Chaudhuri, 1976; Hao-Ren, 1982; Lam, 1982; Larkin, 1982).

Effect of external factors on breeding :

In the nature : Successful breeding of fishes can be obtained from gonadially mature and ripe fishes. Growth and maturation of gonads and the ovulation are brought about by various biotic and abiotic factors of the environment. Nutrition has been considered as the most important among biotic factors (Nikolskii, 1963). Decrease in food availability has resulted in the regression of gonads (Scott, 1962; Clemens & Reed, 1967; De Vlaming, 1971;

De Vlaming et al, 1978) and delayed the onset of sexual maturation in different species of fish (Alm, 1954; Wilkins, 1967). The abiotic environmental factors such as temperature, photoperiod, pH, salinity etc. have shown regulatory effects on the breeding cycles in fish. They have been reviewed from time to time by different workers (Aronson, 1965; Schwassmann, 1971; De Vlaming, 1972, 1974; Jalabert, 1976; Billard & Breton, 1978; Scott, 1979). Photoperiod and temperature, among other factors have been considered to be most important in the reproduction of teleost fishes. In salmonids and gasterosteids, photoperiod exercise a dominant role (Hoover, 1937; Alison, 1951; Carlson & Hale, 1973; Whitehead et al, 1978; Erikson & Lundquist, 1980; Whitehead & Bromage, 1980), whereas in cyprinids temperature seems to be more important (Bullough, 1939; Medlen, 1951; Shrode & Gerking, 1977). Both temperature and photoperiod play important roles in gonadal development and spawning in many fishes in the tropical regions where warm temperature is the consequence of long photoperiod (Yoshioka, 1962, 1963; Baggerman, 1969; Sundararaj & Sehgal, 1970; Kaya & Hasler, 1972; Sundararaj & Vasal, 1973, 1976; De Vlaming & Paquette, 1977). Besides photoperiod and temperature, water pH has also been observed to influence the reproduction in some fishes (Ruby et al, 1977, 1978; Craig & Baksi, 1977). In the Indian major carps, water pH or dissolved oxygen have shown to have no regulatory role in gonadal maturation and spawning (Jhingran, 1982). However, their sexual maturation and spawning are shown to be evoked by monsoon flood (Husain, 1945; Hora, 1954; Khanna, 1958).

Induced breeding : The effects of different environmental factors on the induced breeding of fishes have not been clearly understood. Alikunhi et al (1964) observed successful induced spawning of

L. rohita at 28°C and the success was inhibited when the temperature increased by 2°C. In experiments conducted by Central Inland Fisheries Research Institute, Barrackpore, India, 6 sets of rohu induced at a temperature of 28–31°C spawned successfully, whereas out of another 6 sets induced at 30–34°C, only 4 sets spawned (Anonymous, 1967). Ibrahim *et al* (1968) reported the lack of correlation between induced spawning and water temperature in *L. rohita*, *C. catla* and *C. mrigala*. Their observations were in the temperature range of 26.5 to 35°C. Recently, the ovulatory response of *C. auratus* was reported to be enhanced after pituitary treatment at low temperature (Stacey *et al*, 1979). Clemens (1967) suggested that an adequate dose of pituitary can overcome the impact of any adverse conditions of environment. However, sufficient experimental evidences are lacking to substantiate this hypothesis. Chaudhuri (1976) suggested that temperature is an important factor for the success of induced breeding in fish. Photoperiod may also play an important role in the success of induced spawning in fish. In Indian major carps, hypophysation gave better results when done in cloudy days and evening time (Chondor, 1970).

Effect of environment on fish development :

The use of induced breeding techniques have helped to a great extent in controlling the 'environmentally regulated reproduction' in fish. However, the survival of the embryos and the proper development of the young ones depend largely on the environmental conditions. Reports on the population dynamics of fish development indicate that sometimes heavy mortality, as high as 100%, occurs between egg to adult stages in some species of freshwater fishes (Wagler, 1933; Elster, 1944; Alikunhi *et al*, 1952;

Nümann, 1961). The early stages of development are highly sensitive to the changes in environmental factors. Hence, mortality during these stages increases when the conditions deviate from optimum. It is difficult to assign any specific condition as optimum condition for survival of the ontogenic stages because, neither the developing stages nor the external factors are static in nature (Braum, 1978). Laboratory experiments in recent years, are trying to identify the unfavourable conditions of the various environmental factors which cause abnormalities and death of the developing embryos. This has been reviewed from time to time by Alikunhi et al (1955), Nikolskii (1963, 1969), Blaxter (1969) and Braum (1978). The different environmental factors studied can be broadly grouped into two categories as biotic and abiotic factors.

Biotic factors : Predation and food deficiency, according to Nikolskii (1969) are the two most important biotic factors influencing survival and growth during early development. The major predators of the developmental stages are the larger fish of the same or different species (Alikunhi et al, 1955), frogs (Huet, 1970; Waynarovich, 1975) and aquatic insects (Khan & Hussain, 1947; Julka, 1965, 1969). The damage due to predation becomes more during the pre-hatching stages when the embryos are immobile than the free swimming post-hatching stages (Nikolskii, 1969).

Lack of proper nutrition influences the survival of both pre-hatching and post-hatching stages (Braum, 1978). According to Nikolskii (1963) sufficient yolk reserve in the egg improve the embryonic survival. Fabre-Domergue and Biétrix (1897) formulated the term 'critical period' for marine fishes when the fish larvae shifts from yolk to exogenous food. The absence of suitable external nourishment at the end of yolk nutrition causes catastrophic mortality of these post-hatching stages (Hjort, 1914; Marr, 1956; May, 1974a).

Besides these two biotic factors, the occurrence of disease (Bauer et al, 1973) or parasitism of the embryos by aquatic fungi (Hoffman, 1969; Willoughby, 1969) also reduce the survival and growth of embryonic stages.

Abiotic factors : The ambient temperature is the most important among the various abiotic factors, affecting the developmental process (Krough, 1914; Blaxter, 1969). The incubation time, in general, is prolonged at low temperature and accelerated at higher temperature (Embody, 1934; Kinne & Kinne, 1962; Volodin, 1980). Moderate increase in temperature enhances the metabolic rate of the embryos. However, efficient yolk conversion occurs at an optimum temperature range within the zone of tolerance of the species (Johns et al, 1981). Each species has a definite tolerable temperature range beyond which the embryos fail to survive (Combs, 1965). The lethal temperature varies with the level of their adaptation (Blaxter, 1969).

Dissolved oxygen content plays a critical role in the general metabolism of fishes and particularly so for the embryos in which the rate of metabolism is high. A richly oxygenated environment increases the rate of metabolism and the rate of yolk conversion into body tissues resulting in the increase in the rate of development (Garside, 1959; Braum, 1978). At low dissolved oxygen level, the respiration rate is gradually lowered leading to the death of the embryos (Wickett, 1954; Hamdorf, 1961; Shumway et al, 1964). The low level of dissolved oxygen at 2-3 mg/1 can produce normal larvae particularly in salmonid species (Anonymous, 1973). Some non-salmonid species can survive at dissolved oxygen content below 2 mg/l. However, in most of the non-salmonid species normal development requires a minimum oxygen level of 4-5 mg/l (Adelman & Smith, 1970). It has also been observed that oxygen supersaturation is also not favourable and can be lethal for developing embryos (Bishai, 1960a).

In addition to temperature and oxygen content of water, pH also affects the embryonic development in fish. Dahl (1927) observed that acid water was primarily responsible for heavy mortality of eggs and hatchlings of salmon and trout in Southern Norway. Many reports have appeared on the effect of pH on the embryonic development which have been reviewed by Bishai (1960b) and Jacobsen (1978). It has been shown that embryonic development takes place at a definite range of pH, specific to a species. Particularly at low pH, the acid-base balance of the embryo gets disturbed (Bua & Snekvik, 1972) and the hatching enzyme chorionase is inhibited resulting in delayed hatching (Peterson et al, 1980).

Salinity is an important factor for the development of fishes and its effect is associated with osmoregulation in fish embryo (Holliday & Blaxter, 1960; Kinne, 1960). The salinity tolerance of the ontogenic stages are extremely high for some species being 45-50% or even a higher range for a short period of exposure (Holliday, 1969). However, the change of survival is maximum near the optimal salinity which is species specific (Holliday & Blaxter, 1960).

Developmental abnormalities and death may also occur due to the adverse effect of light and photoperiod. Strong visible light cause early hatching and poor growth (Hamdorf, 1960). Ultraviolet light causes abnormalities of embryos, premature hatching and high mortality (Bell & Hoar, 1950; Marinaro & Bernard, 1966). Darkness or underlighted conditions have shown to result in poor survival rate in grunian (McHugh, 1954) and herring (Blaxter, 1956). Longer photoperiod has shown to induce the rate of growth but poor survival of sea bass embryos (Borahona-Fernandes, 1979).

Different environmental factors do not work independently in the nature and interact with each other to produce the combined effect on the organism (Kinne & Kinne, 1962). Therefore, attempts are being made to study the effect of combination of different factors on the developmental processes (Blaxter, 1969). Kinne and Kinne (1962) used combination of temperature, salinity and saturation of air during the development of the desert minnow C. macularius. Mortality was least at near lethal temperature, when salinity was held at 50% and 35% of sea water. The lethal temperature was lowered at low oxygen concentration. Brooke and Colby (1980) used various combinations of oxygen concentration and temperature during the development of lake herring. The hatching percentage was higher at 6°C and 8°C, than at 2°C and 4°C when DO was 4 mg/l or higher. Conversely, at DO concentrations of 1 mg/l, survival was nil at 6°C and 8°C and was 18.7% at 4°C and 32.4% at 2°C. Similarly, optimal condition of various combinations of environmental factors have been shown for a variety of species (Alderdice & Forrester, 1968, 1971a,b; Alderdice & Velsen, 1971; Alderdice, 1972; May, 1974, 1975; Kwain, 1975; Tay & Garside, 1975; Harmor & Garside, 1976; Clarke et al, 1981; Morgan & Rasin, 1981). It becomes evident from these studies that the effects of one factor may be modified considerably due to the interactions of other factors in the natural environment.

Effect of higher altitude :

The environmental condition at higher altitude is different from that at sea level in having reduced atmospheric pressure, low temperature, strong wind and higher intensity of light (Mani, 1974). The conditions encountered at higher altitude have adverse effects on normal functioning of those species which are adapted to live at lower altitudes (Nelson et al, 1975). Yet, many species of plants and animals successfully live at high altitude. The composition of the flora and fauna at different altitudes are different depending on their adaptive characteristics (Mani, 1974). The aquatic environment at higher altitude is comprised of mostly streams, lakes and man-made reservoirs. The physico-chemical conditions of these water bodies differ from each other in their dissolved oxygen concentration, nutrients and velocity of water current (Edmonds & Hutchinson, 1934; Pennak, 1941). They have in common a low temperature profile in comparison to that at low altitude. At 3,000 metres or above, the water bodies may remain frozen or covered with ice for most part of the year. Besides low temperature, the pH of water is generally low at high altitude where the trees are dominated by pine and the soil is acidic. Due to low temperature and low pH, the biotic composition in aquatic bodies are rather sparse (Jhingran & Sehgal, 1978). Fish constitutes a major part of the upland aquatic fauna. In comparison to that of low altitude the high altitudinal fish community presents only a limited number of species. The most common and relatively abundant upland fishes, as listed by Jhingran & Sehgal (1978) are brown trout (*S. trutta*), rainbow trout (*S. gairdneri*), snow trouts (*S. esocinus*, *S. plagiostomus*, *S. curvifrons* and *S. longipinnis*), mahseers (*T. putitora*, *T. tor*, *T. khudree* and *A. hexagonolepis*), minor carps (*C. carpio*, *L. dero*, *L. dyocheilus*), lesser barils (*B. bendelisis*), sucker-heads (*Garra* sps.), loaches (*Nemacheilus* sps.) and the glyptosternoid fishes (*G. reticulatum*, *G. conirostris* and *G. pectinopterus*). The adaptive significance of these fishes to high altitude is mainly their lower temperature tolerance for which sometimes they are called as cold water fishes. The cold water fishes may be eurythermal - having a broad temperature tolerance range such as common carp or stenothermal - having a narrow temperature (cold) tolerance range as in trout. The growth rate and the natural yields of cold water fishes at higher altitude is lower than their counterparts at lower altitude. However, efforts are being made to use proper technology for successful commercial exploitation of some cold water fishes. Trout is one of such species which is widely dispersed in cold waters throughout the world. There was little interest in culture of trout for food purposes till the early fifties. The idea of commercial culture of trouts got a boost with the formulation and development of an 'industrial trout food' which was found to enhance considerably the growth rate of trouts. In the recent years, intensive trout culture has revolutionised the fish culture in Europe, North-Asia and North-America. Common carp is also another commercially important cold water fish species. This species includes three phenotypes viz. scale carp (*C. carpio* var. *communis*), mirror carp (*C. carpio* var. *specularis*) and leather carp (*C. carpio* var. *nudus*). All the three varieties can be easily bred and grown well under controlled conditions.

Both trout and common carp are exotic in India. Trout was first introduced as early as in 1899 from England and common carp was brought later from Sri Lanka in the year 1939. Most of the high altitude fisheries comprise of these two species in India. However, it has been suggested that the belt of medium altitude zone in India may be tried for culturing the other commercially important fishes such as Indian major carps (Jhingran & Dehadrai, 1974). Sincere efforts to introduce such fishes in these water bodies have not yet been done and a few attempts made have proved unsuccessful. At Shillong, which is situated at an altitude of about 1,500 metres ASL in the North-Eastern part of India, grass carp (*C. idella*) was introduced in 1973. Most of the introduced fishes died and a few which survived did not grow properly. Hence, there is a need to understand the problems of such fishes which failed to adapt to this moderately high altitude conditions. Once the problems at cellular or sub-cellular level are understood, attempts could be made to induce adaptability into these fishes. Besides, such studies will also fill the gap in the present day knowledge on the effect of higher altitude on warm water fishes.

Plan of work :

Cyprinus carpio var. *communis* (a common carp) and *Labeo rohita* (rohu) an Indian major carp have been used for the experiments. Both the species are known to breed and grow well at low altitude in tropical climates. *C. carpio* is well adapted to moderately high altitude but *L. rohita* has not so far been tried in such conditions. The different aspects of the work embodied in this chapter are as follows :-

(1) Study of the physico-chemical factors of the managed ponds, at a low (100m ASL) and moderately high (1500m ASL) altitude in order to obtain a picture of hydrological conditions at the two altitudes.

(2) Observations on the response of breeders to hypophysation, rate of fertilization, development and survival of the embryos till hatching of C. carpio and L. rohita at lower and higher altitudes.

(3) The water temperature and duration of different developmental stages from fertilization till hatching were recorded at lower and higher altitudes.

MATERIALS AND METHODS

Study areas :

The experiments were conducted in the Assam Government Fish Farm at Gauhati and Meghalaya Government Fish Farms at Shillong and Mawpun in the North-Eastern part of India. Gauhati is situated at 26°11'N and 91°47'E and at an altitude of approximately 100m ASL in the State of Assam. Shillong is situated at 25°5'N and 91°9'E and at an altitude of approximately 1,500m ASL and Mawpun at 25°40'N and 91°56'E and at an altitude of 1,000m ASL. Shillong and Mawpun are in the State of Meghalaya.

Ponds :

The ponds used in the three altitudes were kaccha nurseries. They were maintaining the required level of water throughout the year at Gauhati. However, at Shillong and Mawpun, due to heavy seepage, the ponds were connected by a stream to maintain the required water level. All the ponds were prepared and managed under almost identical conditions following conventional methods (Jhingran, 1982). They were first dewatered and left to dry. After about two weeks, quick-lime was applied on the pond bed. The rate of liming was 200 kg/ha. At Shillong and Mawpun a high dose of liming (700 kg/ha) was applied because of the acidic conditions of water. The ponds were then allowed to fill-in with water. Organic and inorganic fertilizers were applied as cowdung at the rate of 5,000 kg/ha/year and super phosphate at the rate of 250 kg/ha/year in equal monthly instalments. At Shillong and Mawpun, a second dose of lime (250 kg/ha) was applied to the pond water in November, 1979 (after 10 months of the 1st liming).

Analysis of physico-chemical factors :

The physico-chemical factors of the pond water were studied at Gauhati and Shillong during the period from January, 1979 to July, 1980 at an interval of 30±2 days. Air and water (surface) temperatures were recorded between 9-10 a.m. in the field by an ordinary mercury bulb thermometer graduated from 0-100°C. Water samples were collected in polythene bottles, immediately transported to the farm laboratory and pH was measured with a pH Meter. For estimations of dissolved oxygen (DO) and free carbon-dioxide (CO_2) surface water samples were collected carefully and fixed separately in 125 ml glass bottles. The concentrations of DO and free CO_2 were estimated following the methods given in APHA standard method (Anonymous, 1955).

Breeding :

(i) Fish : The induced breeding experiments were conducted on the scale carp (Cyprinus carpio var. communis L.) and on an Indian major carp, rohu (Labeo rohita Ham.). Fully ripe brood fishes were obtained from the Assam Government Fish Farm at Gauhati. Breeders were kept in a stocking pond at Gauhati and fed everyday with rice bran and mustard oil cake (1:1) at 1% of their body weight for 1-2 months prior to breeding. A ripe female was characterized by soft, bulging and round abdomen, reddish vent and smooth dorsal surface of the pectoral fins. The ripe males were distinguished by freely oozing milt with rough dorsal surface of the pectoral fins.

For transportation of the breeders to higher altitudes, they were caught with utmost care to avoid any injury and were conditioned by keeping them in a large cloth hapa for 5-6 hours without any food in the stocking pond at Gauhati. The conditioned breeders were put singly in polythene bags containing artificially oxygenated water. The bags were put in fish carrying tins and transported immediately with care in a jeep. Transportation usually took about 3 hours to Shillong and about 2½ hours to Mawpun. The polythene bags were first put over the surface of the introducing water so as to bring a temperature equilibrium between the water inside the polythene bags and in the pond. The fishes were released into the respective ponds within one hour of their arrival.

(ii) Induced breeding : Induced breeding was done at Gauhati in January, 1979 and March, 1980 for scale carp, and July, 1979 and June, 1980 for rohu. At Shillong, scale carp breeding was done in the month of May both in 1979 and 1980. Induced breeding of rohu was done at Shillong during August, 1979 and June, 1980 and at Mawpun during August, 1979 and twice in 1980 during July and August. In all cases, breeding was induced by hypophysation following Chaudhuri, 1963. Pituitary glands were collected from mature and freshly killed rohu at Gauhati fish market. They were kept in absolute alcohol for dehydration immediately after collection and the alcohol was changed for further dehydration after 24 hours. The glands were then preserved in a refrigerator at 0-4°C in dark phials containing fresh absolute alcohol. The required quantity of glands were taken out at the time of injection to the breeders and the alcohol was allowed to evaporate. The glands were weighed and homogenized in an all glass homogenizer in double distilled water. The homogenate was centrifuged and the clear supernatant was used as pituitary extract for injection.

Females were given a primary dose of 2-3 mg/pituitary/kg body weight and after an interval of 5-6 hours, a final dose of 5-8 mg/pituitary/kg body weight. Two males per female were also given, each, a single dose of 2-3 mg/pituitary/kg body weight at the time of second injection to the females. The first injection was given given between 2-3 p.m. and the second injection between 8-9 p.m. The injected males and females were kept for spawning in one set (1 female : 2 males) in a breeding hapa. For the attachment of eggs in scale carp, water hyacinth at Gauhati and rotala weed at Shillong were used inside the hapa.

A close watch was kept for the time of spawning which was marked by vigorous splashing of water due to their mating behaviour. The mouth of the hapa was gently opened without disturbing the breeders to collect samples of hapa water for observation. As soon as ovulation occurred, some eggs were taken for various developmental studies. The brood fishes were gently removed from the hapa after spawning and the eggs were transferred to a hatching hapa in the morning.

(iii) Number of eggs produced : The female breeders of scale carp were weighed before and after spawning, and the loss in their weight were used to calculate the total number of eggs released. The calculation was made at the rate of 700 eggs per gram decrease in weight of female breeder (Alikunhi, 1966) and the number of eggs released was presented as the total of all the sets bred at a time.

In case of rohu, the eggs were collected in buckets with a mug before transferring them from breeding to hatching hapa. The number of eggs per mug was counted and the total number of eggs released was calculated as follows :-

Total number of eggs = Number of eggs per mug X Number of mugs filled with eggs

Kinetics of development :

The following studies were conducted in the scale carp embryos obtained during 1980 breeding both at Gauhati and Shillong and rohu embryos obtained during June, 1980 at Gauhati and August, 1979 at Maupun.

(i) **Percentage of fertilization** : 3-4 samples of about 100 eggs were examined under microscope and the number of unfertilized eggs counted. Fertilized eggs were identified by their swollen nature due to the formation of perivitelline space. Percentage of fertilization was calculated as follows :-

$$\text{Percentage of fertilization} = \frac{(\text{Total number of eggs studied} - \text{Number of unfertilized eggs}) \times 100}{\text{Total numbers of eggs studied}}$$

(ii) **Percentage of hatching and fry obtained** : After hatching, the hatchlings escaped to the outer hatching hapa through the holes in the inner hapa. The inner hapa, containing the eggs cases and spoilt eggs, was removed after the hatching was complete. This time taken for completion of hatching was different under different conditions. The spawns were collected over a piece of wet cotton cloth and scooped into a spawn measuring cup of known capacity. The total number of spawns were calculated as follows :-

$$\text{Total number of spawns} = \text{Number of spawns per cup} \times \text{Number of cups filled with spawns}$$

The spawns were released back into the outer hapa for 7-15 days and then released into the nursery ponds at the rate of 1,000/ha. Supplementary feeding was done using a mixture of rice bran and oil cake (1:1) at the rate of 1% body weight. The fry stage was characterized with attainment of full characteristics of an adult fish. They were then caught with a fine sieved net and the number was counted.

Percentage of hatching and percentage of fry obtained were calculated taking the total number of eggs released as 100 percent.

(iii) Rate (time course) of development : Fertilized eggs were collected immediately after fertilization in an enamel tray containing respective pond water and the rate of development was studied continuously till the completion of hatching. The tray water was changed with fresh pond water every hour. The developmental stages were designated in scale carp as per Verma (1970) and in rohu according to the most easily visible morphological characters as no standard table was available. The rate of development of individual stages were recorded in terms of post-fertilization time taken for the commencement and duration of each developmental stage with corresponding temperature in the medium till hatching. The time of beginning and completion of hatching was also recorded.

Survival studies :

(i) Survival of embryos till hatching : To study the rate of survival of a particular stage, samples containing about 100 embryos of that stage were taken in a big petridish containing respective pond water and examined under a microscope. The embryos which failed to progress to the next stage were separated into another petridish. The death of such embryos were confirmed when they became completely opaque in case of scale carp and slowly started rotting with breaking of the egg case in rohu. The survival percentage was calculated as follows :-

$$\text{Percentage survival} = \frac{(\text{Number of embryos studied} - \text{Number of dead embryos}) \times 100}{\text{Number of embryos studied}}$$

The average survival percentage for a particular stage was calculated from 3-5 sets of observations.

(ii) Survival of hatchlings and fry : Survival percentage of hatchlings and fry were calculated as follows :-

$$\text{Survival percentage of hatchlings} = \frac{\text{Total number of fry} \times 100}{\text{Total number of hatchlings}}$$

$$\text{Survival percentage of fry} = \frac{\text{Total number of 2-month old fish} \times 100}{\text{Total number of fry}}$$

(iii) Survival of young rohu at Shillong when introduced from Gauhati: The survival rates of 1-, 2- and 3- month old rohu brought from Gauhati and introduced at Shillong were studied. Fishes of the 1979 brood at Gauhati were caught and conditioned by putting them in a hapa without food. The fishes were then put in polythene bags containing artificially oxygenated water. Each bag contained not more than 50 fishes. They were then transported to Shillong and were conditioned to pond water as done for breeders. The fishes were then released in a big hapa. The number of dead fishes were counted till 2 days and the surviving fishes were released in the managed pond. Supplementary feeding was done with rice bran and oil cake (1:1) at 1% body weight. Further, mortality was observed by searching out every morning the dead fishes floating at the sides of the pond. The survival rate was finally calculated by finding out the actual numbers of fishes living in the pond by catch method every month as follows :-

$$\text{Percentage survival} = \frac{(\text{Number of fish introduced} - \text{Number of fish survived}) \times 100}{\text{Number of fish introduced}}$$

RESULTS

Physico-chemical factors :

The variations in the physico-chemical factors such as air and water temperature, dissolved oxygen, free carbon-dioxide and pH of water were recorded at monthly intervals in the nursery ponds at Gauhati and Shillong from January, 1979 to July, 1980 (Table-1; Fig. 1).

Temperature : Air and water temperature, in general, were 5-15°C higher at Gauhati than Shillong. At both the places the temperature, as usual, were lower during the winter months (January & February) and higher in summer (June & July). The air and water temperature varied between 15-34°C and 15-35°C respectively at Gauhati and between 12-24°C and 12.5-24°C respectively at Shillong. At Shillong, the circannual temperature changes were at two levels, the lower level (12-16°C) between November to February and higher level (19-24°C) between March to October. However, at Gauhati the temperature range showed a gradual increase from January to February till June to July when they reached the peak level and then gradually decreased to its lower level in January to February in the annual cycle.

Dissolved oxygen : The concentrations of dissolved oxygen (DO) in water in different months were relatively higher at Gauhati in comparison to Shillong. The annual variation was between 7.2-11.6 mg/l at Gauhati and 5.2-12.0 mg/l at Shillong. The oxygen level at Gauhati was more stable than Shillong where the variation range was comparatively more wider and a few sharp fluctuations observed. In July, 1979 there was a sudden increase in DO at Shillong reaching 12.8 mg/l and in February, 1980 it decreased to the lowest level of 5.2 mg/l.

Free Carbon-dioxide : The level of free carbon-dioxide (CO_2) was much higher in Shillong pond water than Gauhati throughout the study period. The range at Gauhati was within 1 mg/l for 6 months from February to July which was then increased suddenly to its peak of 3.2 mg/l in August. Later it decreased but maintained at a fairly higher level around 2 mg/l. In Shillong pond, the carbon-dioxide concentration was much higher between 3.0-7.0 mg/l without any definite annual pattern.

pH : The pattern of pH change followed almost a reverse order to that of free carbon-dioxide concentration. The pH of water at Gauhati pond was higher and more stable between 7.1-8.2 whereas at Shillong the pH remained in the acidic side for most part of the year. The range of pH at Shillong pond was between 5.8-7.1.

Induced breeding :

Scale carp (*Cyprinus carpio*) : The results of induced breeding experiments in *Cyprinus carpio* conducted at Gauhati and Shillong are shown in Table-2. The temperature varied between 17-23.5, 19-28, 16-20 and 17-22°C during the breeding in January, 1979 and March, 1980 at Gauhati and May, 1979 and 1980 at Shillong. Each time 3 sets of breeders were used and complete breeding success was achieved by hypophysation. However, the total number of eggs produced were only 50% in the breeding of May, 1979 at Shillong. The rate of fertilization and later developments were very much successful at Gauhati than at Shillong. In Shillong, during May, 1980 the results were better than May, 1979, and in both cases the results were far below the Gauhati level. The percentage of fertilization, percentage of hatching and percentage of fry obtained were 90, 60 and 52.5 respectively in 1979 and 94.28, 93.68 and 61.03 respectively in 1980 at Gauhati. However, the respective values at Shillong were 55, 20 and 6 in 1979, and 74, 34.5 and 20 in 1980. In May, 1979 the eggs and embryos at Shillong were infected by a fungi of Saprolegnia species, probably resulting in the large scale mortality.

Rohu (Labeo rohita) : Table-3 presents the results of induced breeding of rohu at three different altitudes (Gauhati, Mawpun and Shillong). The water temperature were different at the different breeding time and place. In each case, two sets of breeders were used except in Gauhati during June, 1980 when three sets were used. The success was complete only at Gauhati. All breeders died at Shillong. At Mawpun the breeding was successful only in August, 1979 and during the two other trials the female breeders died. The number of eggs produced were 10,000 per set at Gauhati and much less (7,500 per set) in the only successful breeding at Mawpun. The rate of fertilization were 70 and 55 percent at Gauhati and 50 percent at Mawpun. Further developments were normal at Gauhati showing about 50% hatching and about 40% frys obtained. However, at Mawpun there was a very high rate of mortality between the developmental stages after fertilization (Table-8; Fig. 5) and the percentage of hatching and fry obtained were 0.67 and 0.24 percent respectively.

Rate of development :

(i) Developmental stages : The rate of development was studied along with the alterations in the water temperature and different developmental stages were identified with their significant morphological characters (Table-5; Figs. 2a-c & 3a-b). In scale carp, 15 pre-hatching and two post-hatching stages were identified till the fry stage. However, in rohu, three of these pre-hatching stages such as stage-6 (32-cells stage), stage-10 (late gastrula) and stage-13 (beginning of eye pigmentation) could not be identified in the present study.

(ii) Hatching time : The time required for hatching was different during different breeding and showed some correlation with the temperature of the medium (Table-4). The hatching was quicker at higher temperature. In scale carp, at Gauhati in 1979 and at Shillong in 1980, the hatching time were similar (about 80-85 hrs.) with the temperature in both the places ranging between 17-23.5°C. However, at Gauhati in 1980, the hatching time for scale carp embryos was reduced to 58-60 hrs. with a higher temperature range between 18.5-26°C and at Shillong the time was extended to 126-131 hrs. with the temperature range falling to 16-20°C. Similar temperature related variations in hatching time was observed in rohu. But in general, the hatching time for rohu embryos were much lesser than the scale carp. At Gauhati, rohu embryos hatched comparatively early (in 12-14 hrs.) both in 1979 and in 1980 than at Mawpun in 1979 where they took 18-21 hrs. The temperature range were 24-31°C at Gauhati and 15-25°C at Mawpun.

(iii) Rate of development of different stages : The rate of development of individual stages with corresponding water temperature has been presented in Tables-6 & 7 and Fig. 4. In scale carp commencement of most of the stages were earlier at Gauhati than Shillong showing a positive correlation with higher temperature. However, the deviation was seen in stages-11 and 12 which appeared early at Shillong though the corresponding temperature was higher at Gauhati. The incubation periods were also lower at Gauhati with comparatively higher temperature than Shillong. However, at stages-9, 10 and 11 the rate of development became slower with larger incubation time at Gauhati even with higher temperature.

In rohu, the rate of development was similar in both the places and was indicative of its temperature dependence. During hatching when the temperature suddenly decreased to 15°C at Mawpun, the incubation time almost became twice that at Gauhati.

Survival studies :

(i) Developmental stages : Survival studies were carried out for 9 selected developmental stages (stages 1,4,7,9,11,14,15a,15b & 16). The survival pattern of the selected developmental stages studied in scale carp and rohu at the two different altitudes are presented in Table-8 and Fig. 5.

In scale carp, the survival percentage was about 99% till the stage-11 in both the altitudes though there were considerable variations in the water temperature between 17-27°C. In Shillong the water temperature was 2-5°C lower than Gauhati. The survival percentage gradually decreased after stage-11 at Gauhati upto 82% at stage-16 with the temperature between 26-28°C. However, at Shillong the survival percentage decreased significantly and irregularly upto 52% at stage-16 with water temperature going up but remaining between 18-21°C.

In rohu, the survival percentage was between 98-100% upto the stage-15b with water temperature ranging between 24-31°C at Gauhati. At stage-16 the water temperature suddenly fell from 31°C to 28°C resulting in the decline of survival percentage to 74%. Survival of rohu embryos at Mawpun showed a clear susceptibility to low temperature and the mortality was much higher than at Gauhati at different stages of development. At neurulation stage, though there was not much differences in the water temperature, the mortality was observed to be of 49%. A catastrophe took place at stage-15a (hatching) when the temperature decreased suddenly to as low as 15°C resulting in a mortality of 93% of the embryos. The survival rate of the post-hatching stages studied gradually improved with the gradual improvement in water temperature.

(ii) *Young rohu introduced in Shillong* : Five hundred (500) hatchlings of rohu from 1980 breeding were brought to Shillong at the age of one month and the same number of frys brought at the age of two months died within 1-2 days of their introduction in Shillong pond. However, out of 500 frys brought at the age of three months only about 200 died and the rest (60%) was found to survive well in the Shillong pond and were used for growth studies.

DISCUSSION

The successful existence and development of an organism largely depends on its interaction with the environment. Adaptation ensures the survival of a species under the conditions to which it is exposed. All organisms live in a definite zone of tolerance to the environmental variables, bounded by limits, beyond which the organism may not survive indefinitely. There may be more restricted zones within the zone of tolerance in which, different biological processes function efficiently. The magnitude of response to the changing environmental factors varies between different species of fish and also between various stages of their life cycles (Nikolskii, 1969). As discussed in the introduction, reproduction and development are more precisely controlled by the environment (Blaxter, 1969; Schwassmann, 1971). Any alteration of the physico-chemical factors of water can alter the reproduction and developmental process in fish.

It was seen from the present study that the hydrological conditions in the low and moderately high altitude ponds exhibited considerable differences, particularly as regards to temperature, free carbon-dioxide and pH of water. Such differences might have resulted in variations in the rate of breeding, development and survival of the studied fishes, scale carp and rohu, at the lower and higher altitude.

Physico-chemical factors :

Analysis of the physico-chemical factors in the two ponds at Gauhati and Shillong for a period of 18 months have revealed that temperature and pH remained at a higher level at Gauhati while free carbon-dioxide was higher at Shillong. Dissolved oxygen, in general, did not show much variation between the two altitudes (Table-1; Fig. 1).

Temperature : The environmental temperature gradually decreases with increasing altitude (Mani, 1974). A similar result was obtained in the present study with atmospheric temperature being in a higher level ranging from 15-34°C at low altitude (100 m) at Gauhati in comparison to a lower level ranging between 12-24°C at higher altitude (1,500 m) at Shillong. The water temperature followed similar pattern like air-temperature in the fish ponds studied in the two altitudes. These ponds were shallow water bodies and according to Welch (1952), the water temperature tends to follow the atmospheric temperature in shallow water. A close relationship between air and water temperature has been reported by several workers at different altitudes (Zafar, 1955; Ganapati, 1960; Munawar, 1970). At lower altitude, during most part of the year, the air and water temperature remained near the higher range whereas at higher altitude, the same remained in the lower range. This study was necessary to select near optimum time of the year to do induced breeding experiments at both the altitudes for scale carp and rohu. Scale carp is capable of breeding at as low as 17-18°C, whereas rohu needs temperature about 24-25°C.

Dissolved oxygen (DO) and free carbon-dioxide (CO_2) : In general, the water temperature and concentrations of DO and free CO_2 have an inverse relationship, with decreasing temperature water should dissolve more gases (Welch, 1952). This has been found very correct for free CO_2 with a very high concentration observed throughout the year at higher altitude (1,500 m), in comparison to lower altitude. However, DO did not follow this physical law and the DO concentration was comparatively higher at lower altitude (100 m) in spite of higher temperature throughout the year. The biological activities like growth of algae or decomposition of organic matters have been shown to regulate the DO and CO_2 concentrations in water bodies (Rawson, 1939; Michael, 1969; Prabhavathy & Sreenivasan, 1977). With higher algal population, the CO_2 is utilized and oxygen is produced significantly in water due to photosynthetic activities. On the reverse, during organic decomposition oxygen is utilized and CO_2 is produced into the aquatic system.

Algal growth has been reported to be inhibited at lower temperature (Fritsch, 1907; Pearshall, 1932; Ganapati, 1960; Verma, 1964). Verma (1964) also suggested the most favourable temperature for algal growth was between 24-31°C. At the higher altitude pond at Shillong, the water temperature was always below 24°C and at lower altitude at Gauhati it was above 24°C ranging upto 35°C during most part of the year. Hence, the low production of phytoplankton at higher altitude could be one of the factors for the observed CO_2 and DO situation. Kaur (1981) and Thapa (1981) have also reported lesser amount of phytoplankton production in some other water bodies at Shillong. This might be also due to low pH and acidic water. Hannon *et al.* (1979) have reported that the decomposition of bottom deposits and suspended materials increases at lower temperature. This also could be an additional factor for lower DO and higher CO_2 at higher altitude ponds. CO_2 might also be contributed from other sources at Shillong. Rain as it precipitates, absorbs some amount of gas from the atmosphere and delivers this to the water when it falls (Jhingran, 1982). Shillong being situated near the world's highest rainfall area, Cherrapunji, also experiences very high rainfall which might contribute to the concentration of free CO_2 in the high altitude pond at Shillong. As the pH was mostly in the acidic range in the high altitude pond, the bicarbonate might dissociate to release free CO_2. Such phenomenon has been suggested by Hutchinson (1967).

Therefore, the lowest recorded concentration of free CO_2 at higher altitude (1,500 m) was 3.0 mg/l which was very close to highest level (3.2 mg/l) observed at lower altitude (100 m).

<u>pH</u> : The pH range at higher altitude was much lower than that at lower altitude. A large part of this is due to the higher CO_2 concentration which makes the water acidic. The concentration of free CO_2 can also indicate the concentration of dissolved CO_2. At higher altitude, the pH was so strongly acidic that heavy liming and repeated liming could not make the water always alkaline. Bhattacharya (1980) and Thangkhiew (1981) have reported acidic pH in other fish ponds in the same fish farm at higher altitude. Thangkhiew (1981) also reported that even after using heavy dose of lime (1,000 kg/ha), the water pH remained in the range of 6.3-6.9. In the present study with a heavy dose of lime (700 mg/ha) in December, 1978 followed by another dose of liming at the rate of 250 kg/ha in mid-November, 1979 could only make the water pH neutral or slightly alkaline at higher altitude. However, these shifts were short lived, mainly due to the constant flow of acidic stream waters to the ponds to maintain the level due to seepage. The pH of the stream water has been recorded during 1977-78 to be as low as 5.8 (Thangkhiew, 1981). This low pH of natural streams at Shillong has been attributed to the fact that the soil of this place is acidic and the pines which are the endemic plants, produce

the needles which are highly acidic. Hence, with repeated liming, pH of high altitude pond could not be made alkaline which is suitable for fish production and growth.

Induced breeding :

Successful breeding of fish occurs only under appropriate environmental conditions in the nature. Induced breeding by hypophysation has been suggested to by-pass the adverse effects of some of the sub-optimal environmental conditions (Clemens, 1967). However, the environmental factors have to be maintained within a particular minimum and maximum limit to get the fish spawned. Scale carp and rohu have been reported to breed under a fairly wide range of pH and DO (Khan & Jhingran, 1975; Jhingran & Sehgal, 1978). However, their spawning is highly influenced by environmental temperature. Between these two species, scale carp which is considered to be eurythermal, has a wider optimum temperature range than rohu which spawns within a narrow range of temperature. Alikunhi (1966) has reported that the favourable temperature range for spawning in scale carp varies in different parts of the world such as, 15-18°C in Europe, 17-19°C in U.S.S.R., 12-30°C in Japan, 19-30°C in Indonesia, 26-29°C in Thailand, 23-35°C in Israel, 20-25°C in South America and 18-35°C in India. The temperature range favourable for the breeding of Indian major carp, rohu, has been shown to be between 24-31°C (Khan, 1945; Chaudhuri, 1960). Several workers have tried induced breeding under different temperatures and considering these results, Chaudhuri (1976) suggested that temperature is an important factor for the success of induced breeding. Therefore, induced breeding by hypophysation, which is now commonly practised in India in fisheries, are done in appropriate time of the year with favourable temperature.

Out of the four attempts made to spawn scale carp by hypophysation (Table-2), the two at lower altitude (Gauhati) in January, 1979 and March, 1980 and one of the two attempts at higher altitude (Shillong) in May, 1980 were completely successful. The temperature ranged between 17-28°C. However, in May, 1979 the number of eggs produced at Shillong in scale carp was only 50% with the temperature ranging between 16-20°C. It may be that the temperature during this period was even lower than the normal lower limit for Indian scale carp (18°C).

In case of rohu, the induced breeding (Table-3) performed twice at Gauhati during July, 1979 and June, 1980 were completely successful within the temperature range of 24-31.5°C. However, in two occasions at 1,500 m altitude (Shillong) in August, 1979 and June, 1980, all the breeders died after their introduction from Gauhati when the temperature at the time of introduction was 23 and 21°C respectively. Out of the three attempts taken at 1,000 m altitude (Mawpun) to spawn rohu by hypophysation onle once, in August, 1979, the spawning was partially successful with the temperature around 25°C at the time of introduction and spawning. In the other two attempts, the female breeders died though the temperature was at 25°C and 27°C at the time of their introduction. This indicates that rohu is extremely sensitive to fluctuations in temperature and particularly the breeders which not only failed to breed successfully but also failed to survive under slight temperature stress. Though the effects of transport stress cannot be ruled out for the death of breeders but that cannot be the only cause as in one occasion they survived and responded to hypophysation. The complete death of all breeders at 1,500 m altitude at Shillong must be primarily due to the lower temperature to which the breeders failed to adapt.

Besides spawning, the rate of fertilization, hatching and fry obtained were also recorded in different altitudes in scale carp and rohu (Tables-2 & 3). From all these results, a positive correlation with temperature could be clearly seen in both the species studied in different altitudes. Both higher and lower temperature from the optimum range are known to affect the fertilization and development of different organisms. It could be seen in rohu that, during the only successful partial spawning at higher altitude (1000m) at Maupun during August, 1979, the rate of spawning was not much different than that at lower altitude, the temperature being around 24°C. However, the hatching rate reduced to a significantly low level due to drastic mortality of the embryos with a sudden decrease in temperature to a low level at 15°C. Apart from temperature, pH variations in water also has shown to affect fertilization in many fishes and neutral or slightly alkaline pH has been recommended for successful fertilization (Saha et. al., 1957). Thus acidic pH might have caused a synergistic effect to reduce fertilization alongwith low temperature at higher altitude. During May, 1979, the embryos of scale carp also got infected by a fungi of Saprolegnia species resulting in the catastrophic decrease in the larvae production. Parasitic infection has been reported earlier (Hoffman, 1969 ; Willoughby, 1969) and they have also been seen to increase at low temperature.

The hatching time from fertilization required for the scale carp and rohu embryos (Table-4) varied in different altitudes which was possibly influenced by the variation in temperature. The prolongation in incubation time with low temperature have been recorded for many marine and freshwater fishes (Blaxter, 1969; Braum, 1978). Several workers have reported the relationship between the temperature and incubation time in scale carp and rohu. They are 180 hrs. at 12°C and 84 hrs. at 20°C (Jhingran, 1982), 96-104 hrs. at 19.7-22.6°C and 48-96 hrs. at 23-33°C (Jhingran & Sehgal, 1978), 78 hrs. at 19-23°C (Verma, 1970) and 62 hrs. 30 min. at 22.1°C (Neudecker, 1976) for scale carp, and 15-20 hrs. at 31°C (Chaudhuri, 1960), 14-17 hrs. at 23-28°C (Chakraborty & Murty, 1972) and 17-18 hrs. at 28-31°C (Kaur, 1978). However, our results seems to be little different from these with 58-60 hrs. at 18.5-26°C, 80-83 hrs. at 17-23.5°C, 82-85 hrs. at 17-21°C and 126-131 hrs. at 16-20°C for scale carp, and 12-14 hrs. at 25-31°C, 12.5-14 hrs. at 24-31°C and 18-21 hrs. at 15-25°C for rohu.

Rate of development :

The unfertilized egg gets fertilized to form a zygote which divides and differentiates to form a small organism. These different stages of development are marked by specific conspicuous morphological characters and represented by numbers. In the present study, to compare the data the embryos could be divided into 16 developmental stages in scale carp with specific identification marks of each stage (Table-5; Fig. 2a-c) with unfertilized egg as stage-0 and fertilized egg as stage-1. In rohu, (Fig. 3a-b), three of these stages (stages-6, 10 & 13) could not be detected either due to lack of clear identification mark or due to quick developmental process during these stages.

The time requirement for the attainment of most of the developmental stages in the present study was, in general, more at higher altitude in both the species (Table-6-7; Fig.4). The progression of one stage to another was also rapid and the incubation time was shorter at Gauhati with higher temperature in comparison to those at higher altitude. However, the neurula (11) stage and the 8-somites (12) stage of scale carp were observed to appear earlier and the incubation time of stages-10, 11 and 12 were shorter at the higher altitude, inspite of the fact that water temperature during the development of the corresponding stages were higher at lower altitude (Gauhati). This indicates stage specific relationship of the developmental stages to environmental factors. According to Nikolskii (1969) each stage of development possesses its own relationship with the environment. It may be possible that higher temperature increases the rate of developmental process in general, but there may be particular stages with broader or reverse temperature relationship. However, it is not possible at this stage to explain satisfactorily the deviations observed in the general pattern of altitude or temperature effect. In following chapters, some attempts have been made to correlate them with changes in their biochemical constituents and metabolism.

Besides low temperature, low pH has also been reported to cause slow rate of development in fish. Runn *et al* (1977) have shown delay in hatching in perch, *P. fluviatilis*, at low pH. Peterson *et al* (1980) also observed that hatching of Atlantic salmon (*S. salar*) embryos was delayed or prevented when exposed to low pH water. They suggested that the activity of hatching enzyme chorionase which digest the chorionic envelope was inhibited at low pH causing delay in hatching. It was observed during the present study that the hatching was severely affected in the rohu embryos at higher altitude during May, 1980 when the temperature reduced to 15°C during hatching. This might be due to the inhibition of the activity of the enzyme chorionase. From these gross observations it may be assumed that the lower temperature and lower pH might cause the cumulative effect on the developing embryos to result in slower rate of development at higher altitude.

Rate of survival :

The rate of survival of nine selected embryonic stages was observed during the development of scale carp and rohu at both lower and higher altitude (Tables-2, 3 & 8; Fig. 5). The results indicate that the embryos of both the species survived better at lower altitude. Between the two species, rohu showed greater susceptibility to higher altitude conditions in terms of survival of breeders, embryos and juveniles in comparison to scale carp. The survival rate not only varied between the two species, but also varied between different stages of development in each species.

The differential tolerance of the ontogenic stages might be due to their individual and independent relationship with the environment, as suggested by Nikolskii (1969). Variability in the degree of susceptibility of the developmental stages to environmental fluctuations has been known in many fishes. In rainbow trout (Gottwald, 1965) and lake herring (Brooke & Colby, 1980), the early developmental stages are highly susceptible to reduced dissolved oxygen level. However, in the present study due to repeated changing of incubating water, the DO level was not that low to be considered as a serious limiting factor for the embryonic survival. The early cleavage stages of atlantic salmon were highly susceptible to pH stress than the later stages of development (Daye & Garside, 1977). The detrimental effect of low pH on embryonic survival has been reported in several fishes (Krishna, 1953; Bua & Snekvik, 1972; Johansson et al, 1973). Peterson et al (1980) reported that at acidic pH the hatching enzyme chorionase was inhibited resulting in prevention of hatching of the embryos. The failure of regulation of their internal ion concentration due to external low pH has been reported to be lethal for the developing embryos (Brown, 1981). The low pH observed in the present study at the higher altitude might have contributed either directly or indirectly to the reduced survival of the developing embryos at different stages of development.

The high mortality of the embryos at higher altitude in the present study has shown some close correlation with temperature fluctuations. The critical role of water temperature on the embryonic development has been documented in many fishes (Blaxter, 1969; Braum, 1978) which have clearly shown that a precise control of temperature within a certain limit, specific for a species, is most important for obtaining maximum survival and production. Combs (1965) found pacific salmon eggs were more tolerant to low temperature beyond the 128-cell stage. High mortality prior to eye pigmentation, and development of resistance capacity after this stage has been reported in the atlantic salmon (Peterson et al, 1977). The relationship of water temperature to survival was most evident in rohu embryos in the present study. Its different stages showed appreciable survival at 24°C. At the neurula stage, which is a stage hypersensitive to environmental stress (Patten & Carlson, 1958), a reduction of temperature to 23.5°C coincided with 49% mortality of the embryos at higher altitude. It was observed that prior to hatching the water temperature suddenly dropped from 25°C to 15°C resulting in catastrophic death of rohu embryos. Such short fluctuations of temperature is not uncommon at Shillong (Kaur, 1981) and also at

Mawpun (Thangkhiew, 1981). According to Braum (1978), the sudden change of temperature is most lethal for the developing embryos. This was true in the present study as the sudden temperature reduction had killed virtually all the embryos of rohu at higher altitude.

There was a large scale mortality of scale carp embryos at higher altitude (Shillong) during the breeding of May, 1979. This coincided with the time of an external appearance of *Saprolegnia* on the egg surface. *Saprolegnia* causes the Saprolegniasis disease resulting in heavy mortality of fish and their eggs (Hoffman, 1969; Bauer *et al*, 1973; Waynarovich, 1975). The growth of *Saprolegnia* is favoured at low temperature and acidic pH of water (Bauer *et al*, 1973). Suzuki (1976) found that the production of *Saprolegnia* in a Japanese lake was at peak when the water temperature was 16-20°C and pH was 4.0 to 6.0. Moreover, resistance of the host to Saprolegniasis may decrease at low temperature. Observations on large mouth bass (*Micropterus salmoides*) have shown that *Saprolegnia* invasion causes a high mortality only at lower water temperature, due to lower resistance of the host (Inslee, 1974). Therefore, it may be assumed that the low temperature and low pH might have made the conditions favourable for the infection of *Saprolegnia* to cause mortality of developing embryos along with the unfavourable physico-chemical factors at higher altitude.

Survival of young rohu introduced in Shillong :

In the present study, none of the 1- and 2-month old rohu survived on introduction in Shillong water from Gauhati, whereas 60% of the 3-month old fishes survived under similar conditions. Although, transportation acts as a stress to the fish seeds

(Jhingran, 1982), in our experiments maximum care was taken to minimise the stress during carriage. Only the healthy and conditioned fishes were transported in artificially oxygenated water and overstocking was avoided by putting only limited number of fishes per carrying container. There was no mortality or sign of exhaustion during transportation or immediately after the introduction to water. This may indicate a healthy condition of the fishes during transport. The physico-chemical conditions of the introducing water which was different from the conditions at low altitude at which they were adapted, might have acted as the major stress. The response of the fishes to adjust under such stress might be dependent on age. It is generally known that young fishes are more vulnerable to any environmental stress than the older fishes (Cairns & Scheir, 1958; Smith & Oseid, 1974). Therefore, the mortality of 1- and 2-month old rohu at higher altitude might be due to the lack of an adjustment capacity to the high altitudinal conditions and this capacity might have appeared in the fish as it grew older.

Survival of breeders :

Not only the survival of embryos but also the survival of the breeders at the two altitudes varied between the species (Table-2 & 3). The scale carp breeders were found to be more resistant to the high altitude as evident by their absolute survival at Shillong. However, rohu breeders showed greater susceptibility which was more for females than males to high altitude. It has been suggested that during the breeding time fishes become physically weak due to the large depletion of the chemical substances for the preparation of the gonads (Love, 1970). At this stage, fishes become more vulnerable to any types of physical or environmental stress. In the present study, it was not clear whether transportation had any role in mortality of breeders. Maximum care was taken to minimise the transportation stress. Perhaps the environmental conditions at higher altitude was the major determining factor for the survival of the breeders. Further, transported under similar conditions all rohu breeders survived in 1979 at Maupun, which is situated at about 1,000 m ASL. This indicated that the survival of the rohu breeders decreased with the increase in altitude. The tolerance capacity in them was found to vary between the two sexes. At Maupun, the females were more vulnerable than males. This might be related to a comparatively higher depletion of reserved energy and a physically weak state of the female breeders. Higher depletion of chemical substances and a physically weak stage of females over the males, have been shown in many other fishes during the breeding period (Love, 1970).

The present study on the breeding performance, developmental rate and survival of scale carp and rohu has clearly shown the adverse effect of higher altitude on these fishes. However, assessment of the biochemical and physiological parameters, which modulate the adaptive capacity of an organism, may be more sensitive indicators to explain such effects of higher altitude. Therefore, studies on the alterations of some biomolecules and metabolic pathways were carried out during the embryonic developmental stages of scale carp and rohu at higher and lower altitude. These studies are discussed in the following two chapters to find out the possible biochemical or physiological effects of the higher altitude on the embryonic development of *Cyprinus carpio* and *Labeo rohita*.

CHAPTER II

BIOCHEMICAL COMPOSITION

INTRODUCTION

The development of a multicellular animal takes place from a fertilized egg through a series of complex changes. The zygote undergoes regular and extensive cell division accompanied by structural and functional differentiation involving histo- and organogenesis (Balinsky, 1976). The mechanism by which such drastic and orderly changes occur during the development is more or less similar in all eukaryotes, and is known to be biochemical in nature (Grant, 1978). However, the mechanism is more complicated in the oviparous animals in comparison to that in the viviparous ones. The embryo in the viviparous animals develops internally under the complete protection of the mother. However, the development of the oviparous animals is external and, therefore, remains under the direct influence of the variable conditions of the environmental factors. Some oviparous species have developed parental care to protect their developing embryos whereas in most others, the embryonic development takes place at the mercy of the nature. In terrestrial oviparous animals, the eggs in general, are with hard outer covering called egg shell and the eggs in some are incubated by the parents to give optimum conditions for hatching. In aquatic oviparous animals, particularly in fishes, where both fertilization and development are external, the environmental factors play a major role in regulating the embryonic development. The regulation of the embryonic development in the oviparous animals can be divided into two broad categories such as, intrinsic regulation and extrinsic regulation.

'Intrinsic regulation' denotes the control of development at the molecular level inside the egg or embryo in accordance with the inherited pattern. This includes the regulation of the gene activity, and synthesis and degradation of various biomolecules. On the other hand, the 'extrinsic regulation' refers to the modulation of the expression of the inherited pattern by different external environmental factors (Frisancho, 1977).

Intrinsic regulation :

The intrinsic regulation of the developmental process is basically similar in both oviparous and viviparous animals (Grant, 1978). The developing embryo is a living organism and requires nutrition for its vital functions. The necessary food materials in the egg are deposited in the form of yolk during oogenesis. The developing embryos of the oviparous animals get very little exogenous supply of the necessary substances. Their development depends, to a large extent, on the amount of yolk deposited during oogenesis. The process of oogenesis has been divided into two major phases such as, pre-vitellogenic phase and vitellogenic phase on the basis of histological and electron microscopic studies (Raven, 1961). During the first phase, the diameter of the oocyte increases, the nucleus enlarges and the cytoplasm becomes almost entirely free from endoplasmic reticulum, golgi bodies and ribosomes in amphibians (Balinsky & Devis, 1963). Mitochondria is the only type of organelles which is abundant in small oocytes (Balinsky & Devis, 1963). During the second phase (vitellogenesis), the oocyte becomes progressively loaded with ribosomes and various kinds of inclusions, yolk platelets, pigment grains, oil droplets and glycogen granules (Denis, 1974). A female specific serum protein, vitellogenin, which contains phosphorus, lipid, carbohydrates, calcium and iron has been identified as the egg yolk precursor in amphibians and some other vertebrates (Wallace, 1978). Vitellogenin, synthesized in the liver in response to estrogen stimulation, is released into the circulation and comes into the oocyte cytoplasm through pinocytosis (Gapp et al, 1979; Hori et al, 1979). Vitellogenin accumulates in the crystalline form as yolk platelets in amphibian oocytes (Brachet, 1974). In a mature amphibian egg, yolk constitutes 45% of the dry weight and only 20% of the dry weight in active cytoplasm (Barth & Barth, 1954). In echinoderms and lower chordates (amphioxus, tunicates), the yolk is relatively small (Kerr, 1919). In the sea urchin, Arbacia, the yolk granules take up about 27% of the total volume of the egg (Harvey, 1956). In addition to the yolk platelets, the amphibian egg contains lipid, in the form of lipochondria (Holtfreter, 1946) and glycogen (Barth & Barth, 1954), which primarily serve as the energy source for developing embryos. Besides these macromolecules, a large amount of amino acids are also deposited during oogenesis.

One of the most interesting events during the oogenesis of the oviparous animals is the accumulation of a large amount of nucleic acids, DNA and RNA, along with other biochemical substances in the oocyte. The matured oocyte of many oviparous animals have shown far more DNA content than that of a somatic cell of the same species (Tyler, 1967). The DNA content in a matured echinoderm oocyte has been reported to be 8 to 37 times of the haploid amount by different workers (Hoff-Jorgensen, 1954; Sugino et al, 1960; Baltus et al, 1965; Piko & Tyler, 1965). In amphibians, the DNA amount has been shown to be 500 times as much as in a diploid cell (Dawid, 1970). The nuclear DNA in an amphibian oocyte is only 5-10% higher than that of DNA of a somatic cell and the rest of the oocyte DNA is located in the cytoplasm (Dawid, 1966; Denis, 1974). During vitellogenesis, the oocyte chromosomes expand considerably and take a peculiar lampbrush shape (Brachet, 1974). These lampbrush chromosomes are made of two chromatids, each made up of a single giant fibre of DNA; the DNA fibre is repeatedly coiled at the chromomere regions and condensed into granular forms (Gall & Callan, 1962). They contain 4 times the DNA content of a haploid cell in amphibians (Brachet, 1974). Further, during the pachytene stage of meiosis, a conspicuous mass of extrachromosomal DNA appears in the nucleus which condenses in the form of crescent-shaped 'cap' (Gall, 1968). In the late pachytene and early diplotene stage, a few nucleoli begins to appear inside the DNA cap. Soon after, the DNA cap disperses and the nucleoli become free in the nuclear sap (Brachet, 1974). A matured amphibian oocyte contains more than 1,000 nucleoli and each nucleolus is shown to contain RNA and some amount of DNA originally present in the cap (Gall, 1968). The extra chromosomal DNA, which is often known as the 'amplified DNA', has been shown to be a product of amplification of r-RNA genes. Therefore, they are also called as r-DNA. In a matured oocyte of Xenopus, these genes may be $2X10^6$ in number in comparison to just $1X10^3$ number in its somatic cell (Brown & Weber, 1968).

The substantial portion of extra DNA in the matured oocyte is contributed by the mitochondria which accumulate during oogenesis (Tyler, 1967; Denis, 1974). Matured egg of Xenopus contains about $1X10^4$ times as much mitochondria as that of a liver cell (Chase & Dawid, 1972). The mitochondrial DNA in this amphibian is 65% of the DNA present in the egg (Dawid, 1966). The rest of the cytoplasmic DNA is located in the yolk platelets (Tyler, 1967). In the sea urchin, Lytchinnus, about $8X10^4$ yolk granules are present per egg, each containing $2.3X10^{-17}$ grams of DNA. The origin of the yolk DNA is still not clear. Brachet (1974) suggests that the yolk DNA probably originates from the liver of the female. When the liver is most active in the synthesis of vitellogenin, many nucleated red blood cells and liver cells along with the blood stream come to the oocyte and there DNA is probably taken up by pinocytosis into the growing oocyte and incorporated into the yolk platelets.

In comparison to DNA, the RNA accumulation in the oocyte of oviparous animals is exceedingly high. In a single egg of *X. laevis*, the RNA content is about $3X10^4$ times higher than the amount in its somatic cell (Brown & Littna, 1964). This large amount of egg RNA is mainly r-RNA which is about 95% in the amphibian oocytes. During vitellogenesis, a heavy synthesis of the 28S and 18S RNA takes place in the nuclear sap which move into the nucleolus. They finally become segregated into the larger ribosome sub-unit at the end of the assembly process (Denis, 1974). The accumulation of ribosomes in this way is as high as $3X10^6$ times as in a liver cell in *X. laevis* (Denis, 1974). t-RNA and DNA like RNA (d-RNA) also accumulate during vitellogenesis. The t-RNA amount in amphibian oocyte corresponds to a ratio of about 10 molecules per ribosome and accounts for about 2% of the total RNA present in the mature oocyte (Mairy & Denis, 1971). d-RNA is reported to account for 2-5% of the total RNA in an amphibian egg (Davidson & Hough, 1971). The true nature of this DNA like RNA is yet to be properly understood (Denis, 1974). Some evidences indicate that these are nothing but messenger RNA which are present in a masked form (mm RNA) (Tyler, 1967). mm RNA in the sea urchin oocyte is shown to contain the informations required for the synthesis of several proteins and enzymes involved in the process of DNA replication, cell division and embryonic metabolism at a very early stage of development. In the snail, *Ilyanasa obsoleta*, cleavage and gastrulation occur in presence of actinomycin-D which is a protein synthetic inhibitor at transcriptional stage (Feigenbaum & Goldberg, 1965). In insect eggs too, development proceeds upto gastrulation after injection of Actinomycin-D into the egg (Lockshin, 1966). Similar results have also been reported to nematodes (Kaulenas & Fairbairn, 1966) and in amphibians (Denis, 1974). However, there is no direct evidence convincing the true m-RNA nature of the d-RNA in these animals (Denis, 1974).

The mature oocyte with its reserve yolk materials remains in a dormant state till fertilization. Fertilization induces many physical and physiological manifestations making the zygote highly active (Balinsky, 1976). An immediate visible morphological change which occurs following fertilization is the swelling of the egg due to water imbibition which might be needed to accelerate simultaneously the embryonic metabolism. Rapid cell division and drastic biochemical changes have been reported by several workers in different oviparous animals. The wet weight and dry matter content (DMC) during the development indicate the gross constitutional changes in the developing embryo due to metabolism.

The metabolism of deposited food materials takes place partly by its degradation to release energy and partly utilized in the synthesis of different biomolecules and cell organelles. The total protein concentration temporarily decreases after fertilization due to proteolysis of the yolk in the sea urchin (Metz & Monroy, 1967). Protein is the building block of all kinds of biological structures and also acts as biological catalysts in forms of enzymes to accelerate biochemical reactions. Hence, among the various substances, synthesized during development, protein occupies a central place (Paul, 1974a). The level of protein synthesis increases significantly after fertilization in echinoderm egg (Epel, 1966). However, in amphibians, protein synthesis begins at the onset of cleavage

(Neyfakh & Abramova, 1974). Synthesis of new proteins begins only at gastrulation in sea urchin (Brandhorst, 1976) and Xenopus (Ballantine et al, 1979). In the early stages of development, protein synthesis involves only translation and not transcription. This has been evident from the fact that use of specific transcriptional inhibitor could not stop the development at the early stages (Paul, 1974b). The mm RNA accumulated during oogenesis perhaps gets activated and translates the proteins in the early stages of development (Brown & Gurdon, 1964).

Synthesis of chromosomal DNA begins soon after fertilization and continues in the further stages of development (Brachet, 1974). DNA polymerase remains present in the cytoplasm and moves to the nucleus at each cell division to bring about the replication of DNA (Brachet, 1974). Mitochondrial DNA (m-DNA) is also reported to be synthesized during the early cell divisions in the eggs of Xenopus (Chase & Dawid, 1972) and sea urchin (Bresch, 1973). An extremely low level of RNA synthesis has been detected in unfertilized echinoderm egg (Levner, 1974) and the synthesis of this becomes measurable only after fertilization (Mizuno et al, 1974). The three types of RNA (r-RNA, t-RNA and m-RNA) synthesis commences at different post-fertilized stages. Activation of r-RNA synthesis does not occur during cleavage in sea urchin and amphibians (Brown & Littna, 1964). The ribosomal genes become active at the beginning of gastrulation (Abe & Yamana, 1970) but the total r-RNA concentration increases only after the end of neurulation in echinoderms (Brown & Littna, 1964; Denis, 1974). In Xenopus, the r-RNA content doubles only by the time of commencement of feeding of tadpoles (Brown & Littna, 1964). Synthesis of t-RNA begins a few hours after the synthesis of r-RNA (Brachet, 1974). New m-RNA synthesis in amphibians starts during early cleavage. Howeve in sea urchin alongwith the synthesis of new m-RNA after 4-cell stage, the existing species of m-RNAs are also synthesized in the developing embryo (Tyler, 1967). The gradual synthesis of different types of RNAs are necessary to synthesize the spectrum of different proteins needed for differentiation after gastrulation. Cells which differentiate along a specific line are, apparently, characterized by the spectrum of specific proteins they synthesize (Paul, 1974a). Predominant synthesis of hemoglobin takes place during erythrocyte differentiation and muscle proteins such as actim and myosin are selectively synthesized during myogenesis. Therefore, differentiation is viewed at the molecular level, as an orderly appearance of different species of proteins. The sequential appearance of different proteins has been known to be due to sequential activation of specific sets of genes during different stages of embryonic development (Gurdon, 1974). Besides the synthesis of proteins, the synthesis of carbohydrates and lipids are also important for the embryonic development (Love, 1970). However, Salinsky (1976) considers the synthesis of carbohydrates and lipids less important than the synthesis of protein and its machinery, because synthesis of carbohydrates and lipids occur under the control of enzymes which are protein in nature.

The patterns of intrinsic regulation during ontogenic development of the oviparous animals have been elaborately discussed in the echinoderm, sea-urchin and the amphibian, *Xenopus laevis* (Tyler, 1967; Brachet, 1974; Denis, 1974; and Paul, 1974b). The informations available on the intrinsic regulation of the developmental process in fish are comparatively much lesser than echinoderms and amphibians. The bulk of the study so far done, has been confined to the salmonid species, medaka (*Oryzias latipes*) and the loach (*Misgurnus fossilis*) (Neyfakh & Abramova, 1974).

The fish egg is a closed system (Needham, 1931) and its development is dependent on the yolk and other materials deposited during oogenesis. The deposition of materials in the developing oocyte takes place at the expense of the materials from other tissues, such as liver and muscle. The depletion of specific components like glycogen, protein, lipid, nucleic acids and amino acids in liver and muscle tissues of various fishes have been established during the period of sexual maturation (Love, 1970). The major portion of the depleted substances are mobilized into the serum and subsequently deposited in the developing oocytes. A part of the depleted substances are also utilized for energy production during maturation. Yolk comprises the major bulk of the mature oocyte in fish and consists of mainly phosphoprotein and lipoproteins (Campbell & Idler, 1976; Hara & Hirai, 1978; Hori *et al*, 1979). The mechanism and site of synthesis of yolk in fish have been shown to be similar to amphibians. Vitellogenin is the yolk precursor in the goldfish, *C. auratus* (De Vlaming *et al*, 1980) and in the catfish *H. fossilis* (Nath & Sundararaj, 1981; Sundararaj & Nath, 1981) and its synthesis occurs in liver in response to estrogen stimulation. It is transported by blood to ovary where it gets deposited in the oocytes in form of yolk platelets. The yolk platelets are formed inside the modified mitochondria (Yamamoto & Oota, 1967). The deposited yolk gets segregated from the cytoplasm and concentrates in the inferior of the egg in teleosts, while most of the cytoplasm forms a thin surface layer covering the yolk (Yamamoto & Oota, 1967). Fat also gets deposited in the form of large droplets inside the yolk mass (Balinsky, 1967).

Accumulation of a large amount of DNA and RNA has also been reported during oogenesis in fish like other oviparous vertebrates

(Neyfakh & Abramova, 1974). The amount of DNA deposited varies from 10^3 to $3X10^4$ times higher than the DNA content of a diploid cell in loach and sturgeon. Still higher level of DNA content has been reported in oocyte of trout, *S. gairdneri* and *S. trutta* (Hagenmaier, 1969; Zeitoun *et al*, 1977) and in the Indian catfish, *H. fossilis* (Sarkar *et al*, 1979). The total amount of RNA in mature oocyte has been reported to be 2.6 - 2.8 μg in loach (Neyfakh & Abramova, 1974) and 77.2 μg in rainbow trout, *S. gairdneri* (Zeitoun *et al*, 1977). The bulk of RNA in loach belongs to r-RNA whereas t-RNA makes upto about 1.5% of the total RNA. The rest of the RNA is DNA like RNA (Neyfakh & Abramova, 1974).

The permeability of the chorionic membrane changes on fertilization allowing only water, urea, glucose, salts and certain dyes to enter the egg (Blaxter, 1969). Fertilization also initiates several metabolic activities resulting in changes in the water content and DMC in the developing embryo of all animals (Balinsky, 1976). Observations on various species of fish, such as *C. carpio* (Peňáz *et al*, 1976; Moroz & Luzhin, 1976), *S. salar* (Hayes, 1930), *S. gairdneri* (Suyama & Ogino, 1958) and *O. keta* (Yoneda, 1977) showed that the wet weight of the embryos increases significantly at fertilization and at hatching it decreases. This increase and decrease are, to a large extent, due to the formation of the perivitelline membrane at fertilization and its elimination at hatching, respectively. The water content is generally increased with development except for the loss at hatching (Love, 1970; Peňáz *et al*, 1976). Such increase has metabolic implications. Water is an essential constituent of a living cell providing the aquatic media for metabolic reactions to occur (Harper *et al*, 1979). The DMC, on the other hand, follows a reverse pattern than the water content, decreasing with development (Faustov & Zotin, 1965; Peňáz *et al*, 1976; Moroz & Luzhin, 1976; Johns & Howell, 1980). The DMC in the embryo mainly represents the vast yolk reserve of the egg (Johns & Howell, 1980). The alterations of DMC during development indicate the rate of yolk utilization and synthetic activities in the embryos.

The available informations on the changes in total protein content of the developing fish embryos do not follow any definite pattern. In one species of salmon (*S. gairdneri*) the protein content increased at fertilization and then remained constant till the pre-hatching stages. At hatching it declined significantly (Zeitoun *et al*, 1977). In another species of salmon (*S. trutta*), the protein content gradually declined with development till hatching to half the concentration present in the egg (Phillips *et al*, 1958; Suyama & Ogino, 1958). The protein content in the common carp (*C. carpio*) drastically decreased at fertilization and was maintained at an unstable level till hatching when it increased significantly (Moroz & Luzhin, 1976). Sarkar *et al* (1979) reported in Indian catfish, *H. fossilis*, a continuous increase in the protein content during development. Protein synthesis and utilization of the yolk also shows wide variations in the developing stages of different species. Protein synthesis in loach eggs is not activated at fertilization or during early cleavage. It starts at blastulation and becomes intense during late gastrula after which it falls. It increases again during pre-hatching stages (Neyfakh & Abramova, 1974). In salmon (*S. gairdneri*), the pattern of protein synthesis was different but similar to its pattern of protein content during development (Zeitoun *et al*, 1977). The synthesis of protein is mostly accomplished *in blastoderm cells and can also be detected in yolk mitochondria*

(Neyfakh & Abramova, 1974). In the early stages of development, till the late blastula (Neyfakh & Abramova, 1974) or till the beginning of blastulation (Sarkar et al, 1979), the synthesis of protein is suggested to be independent of nuclear control (transcription). It proceeds at the expense of templates (DNA like RNA or mm RNA) stored during oogenesis.

The ontogenetic pattern of the individual amino acids also does not show uniformity in different fish species (Love, 1974). The biggest all round change of the free amino acids takes place from the egg to the larva. The increase or decrease of the free amino acids may be the net result of the yolk degradation and protein synthesis (Zeitoun et al, 1977).

Total DNA content per embryo does not increase linearly with cell multiplication. This happens mainly due to the higher accumulation of DNA in the cytoplasm of egg during oogenesis. The role of this DNA has not been clearly understood (Dontsova et al, 1970; Zeitoun et al, 1979; Sarkar et al, 1979). However, the nuclear DNA steadily increases with development. Synthesis of new DNA has been shown to begin at the earliest cleavage stage (Neyfakh & Abramova, 1974). Pheleomycin, a potent inhibitor of DNA synthesis, blocks the development of H. fossilis at early cleavage stage indicating that new DNA synthesis is essential for cleavage during early development. Different species of fish, however, show different patterns of alterations in total DNA content in the developing embryos.

Accumulation of a large amount of RNA in the mature egg has been known in fishes. This RNA population consists mainly of r-RNA and mm RNA or DNA like RNA. Synthesis of new RNA starts from the cleavage to blastula stage in different species of fish (Timofeva & Kafiani, 1964; Neyfakh & Abramova, 1974; Choudhury *et al.* 1979). The sequence of synthesis of different types of RNA also varies in different fishes. In loach, t-RNA synthesis begins first, followed by m-RNA synthesis at the early and mid-blastula stages, whereas in trout, r-RNA synthesis starts first (Neyfakh & Abramova, 1974). The changes in total RNA content per embryo also varies in different species of fish.

Extrinsic regulation :

In externally developed animals, the 'intrinsic control' is further regulated by the external factors of the environment. The genomic expression in fish is known to be very plastic in nature and it alters in response to changes in the environment (Hochachka & Somero, 1973; Whitt, 1981a). The various metabolic processes which comprise the 'intrinsic control' are basically chemical in nature and are governed by the physical laws. Alterations in different environmental factors influence the rate of development and survival of the fish embryo (Blaxter, 1969; Love, 1970; Braum, 1978). The metabolic activity is also shown to alter with the change in environmental conditions, thereby influencing the chemical constituents of the developing embryo. The rate of oxygen consumption has been used by many workers to show the metabolic variations under different environmental conditions (Blaxter, 1969). Direct studies on metabolism of different chemical substances during fish development under variable conditions of environment are limited. Studies on the adult fishes, however, show that metabolic changes with variations in environmental conditions are more complex (Vernberg & Vernberg, 1972; Prosser, 1973a). During the biochemical adaptation to environmental changes, some metabolic processes are activated to a large extent, some remain unchanged and some others are reduced in activity (Hochachka & Somero, 1971). There are a few informations available on the yolk utilization of the fish embryos under different environmental conditions (Kinne, 1970, 1977). Yolk is most efficiently utilized at certain optimum temperature which falls somewhere in the middle of the thermal tolerance range. Above and below the optimum temperature, metabolic utilization of yolk is proportionately greater than its utilization for tissue formation, resulting in smaller larvae. However, there are reports of temperature independent yolk utilization during development of some fishes. Johns and Howell (1980) noticed that in winter flounder, *P. dentatus*, degradation of yolk and yolk utilization did not vary at two experimental temperatures of 16° and 21°C. Wood (1932) reported temperature independent yolk utilization efficiency for trout (*S. fario*) embryos when incubated at 3°, 7°, 10° and 12°C. Similarly, the embryos of *B. icistia* (May, 1975b) and yellowtail flounder, *L. ferruginea* (Howell, 1980) do not show any temperature dependence on yolk utilization within the zone of tolerance. The possible mechanism for such temperature independent yolk utilization has been related to some physiological adjustments, resulting in an equilibrium between the rate at which yolk is degraded for energy requirements and the rate at which it is converted for synthesis of other substances (Blaxter & Hempel, 1966; Johns & Howell, 1980; Johns *et al*, 1981). The efficiency of yolk utilization has been seen to be different under different salinities (May, 1975b) and variable oxygen supply (Harmor & Garside, 1977) in different fishes. Therefore, the water content, DMC and concentrations of different chemical constituents in the egg not only changes with development but also varies with environmental changes and also in different species.

Plan of work :

In the earlier chapter it has been reported that the rate of development and survival of the two carp species (*C. carpio* & *L. rohita*) were lower at the higher altitude. The intensity of the impact of high altitude was more in rohu than in scale carp. Besides, the informations on the biochemical alterations during ontogenesis and the effect of environment have been very less in freshwater fishes. Therefore, the present study has been planned to find out the pattern of changes in some important chemical constituents during the early development of the two carps species at a favourable low altitudinal (100 m ASL) environment at Gauhati, and at higher altitudinal conditions at Shillong (1500 m ASL) or Mawpun (1000 m ASL).

The following studies have been conducted.

1) The wet weight, water content and dry matter content of the developing embryos have been estimated.

2) The concentrations of total-, total soluble-, cytoplasmic- and mitochondrial- proteins have been measured during the process of development.

3) Quantitative and qualitative alterations of free amino acids have been followed in the developing stages.

4) The concentrations of total DNA and RNA per embryo have been estimated in different developmental stages from egg to the freshly hatched larva of the two species at low and high altitude.

MATERIALS AND METHODS

The eggs and embryos obtained from the induced breeding of scale carp (*Cyprinus carpio*) during March, 1980 at Gauhati and May, 1980 at Shillong and of rohu (*Labeo rohita*) during June, 1980 at Gauhati and August, 1979 at Mawpun (Chapter-1) were used in the following studies.

Fixation of stages :

Unfertilized eggs were obtained by stripping the females immediately after spawning. Fertilized eggs were collected within 10-15 minutes after fertilization. The subsequent developmental stages of both the species were followed as described in Chapter-1. About 500 embryos of each stage were rapidly removed from water, put separately in chilled bottles and placed inside ice in a thermos flask. The collected samples were brought to the laboratory and kept at -15°C till the analysis was made. The newly hatched embryos (stage-15b) were collected after about 2 hours of the first occurrence of hatching. In rohu, embryos of this stage (stage-15b) could not be collected from high altitude because only a few numbers of the post-hatched larvae were obtained in the only successful breeding at Mawpun as described in Chapter-1.

Determination of wet weight, dry matter content (DMC) and water content :

Samples of 10-15 deep frozen eggs or embryos of each developmental stage were thawed at room temperature. The water sticking to the outer membrane was removed by rapidly drying on a filter paper. The wet weight of the whole sample was determined by using a single pan electrical microbalance (K. Roy Model-K.16).

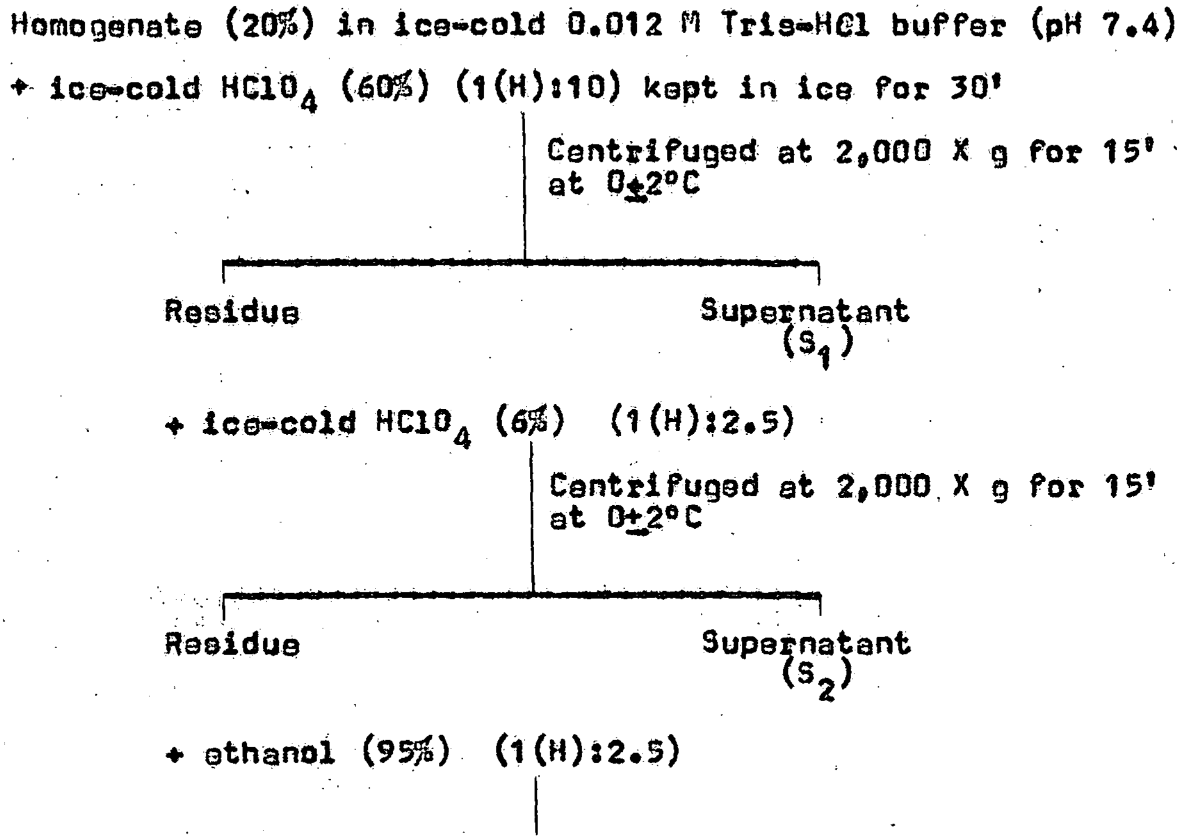
The dry matter content (DMC) was then determined by drying it at 60°C, till a constant weight was reached. DMC was substracted from the wet weight to obtain the water content.

Extraction of biomolecules :

A. Extraction of total protein, DNA and RNA :

50–60 deep frozen eggs or embryos of each developmental stage were thawed on ice and 20% homogenates of each sample were prepared in ice-cold 0.012 M Tris-HCl buffer (pH 7.4) using a pre-cooled all glass hand operated Potter Elvehjem type homogeniser. Total protein, DNA and RNA were extracted following Schneider (1957).

The schematic representation of the extraction procedure is given below.



Homogenate (20%) in ice-cold 0.012 M Tris-HCl buffer (pH 7.4)

+ ice-cold $HClO_4$ (60%) (1(H):10) kept in ice for 30'

Centrifuged at 2,000 X g for 15' at 0±2°C

Residue

Supernatant (S_1)

+ ice-cold $HClO_4$ (6%) (1(H):2.5)

Centrifuged at 2,000 X g for 15' at 0±2°C

Residue

Supernatant (S_2)

+ ethanol (95%) (1(H):2.5)

Centrifuged at 2,000 X g for 15' at 0±2°C

Residue

Supernatant (S_3)

+ ethanol-ether (1:1) (1(H):1)
warmed at 60°C for 15'

Centrifuged at 2,000 X g for 15' at 0±2°C

Residue

Supernatant (S_4)

+ $HClO_4$ (10%) (1(H):2.5)
Heated with occasional stirring with a glass rod at 90°C for 15'

Centrifuged at 2,000 X g for 15' at 0±2°C

Residue

Supernatant (S_5)

+ $HClO_4$ (5%) (1(H):1)

Centrifuged at 2,000 X g for 15' at 0±2°C

Residue

Supernatant (S_6)

Pooled for DNA and RNA estimations.

dissolved in KOH (1N) (1(H):1)

used for estimation of total protein.

8. Extraction of total soluble protein :

The soluble protein was estimated in a 20% homogenate of about 100 eggs or embryos of each developmental stage made in ice-cold 0.25 M Sucrose solution.

C. Extraction of Cytoplasmic (c-) and Mitochondrial (m-) protein:

A 20% homogenate of about 100 eggs or embryos of each stage was prepared in ice-cold 0.25 M Sucrose. The 20% homogenate of each stage was centrifuged at 600 X g at 0±2°C for 15 minutes and the residue was discarded. The supernatant was again centrifuged at 14,000 X g for 30 minutes to sediment mitochondria. The supernatant thus obtained was used for the estimation of cytoplasmic protein. The mitochondrial sediment was suspended in 0.25 M Sucrose solution to make 20% suspension with the help of a glass homogeniser and mitochondrial protein was estimated.

D. Extraction of free amino acids (FAA) :

Free amino-acids were extracted following the method of Buslow (1967). 40-50 eggs or embryos of each developmental stage were homogenised in 95% ethanol and 0.1N HCl (1:1). After the sample was cooled in ice for 30 minutes, it was centrifuged at 2,000 X g for 20 minutes. The clear supernatant was used for qualitative and quantitative estimations of free amino-acids.

Estimation of biomolecules :

A. Estimation of protein :

Total, soluble (s-), cytoplasmic (c-) and mitochondrial (m-) proteins were estimated following the method of Lowry et al (1951) using Bovine Serum Albumin (BSA) as the standard. A linear standard graph was obtained using 10 to 70 µg of BSA.

B. Estimation of free amino acids :

(a) Total free amino-acids : Total free amino acid was estimated following the method described by Spies (1957). Glycine in 5 µg to 30 µg concentrations was used to prepare a linear standard graph.

(b) (i) Individual FAA (Qualitative) : Ascending two-dimentional paper chromatography was employed for the qualitative detection of amino-acids. Extracted samples containing about 100 µg of free amino-acids were applied as a compact spot on Whatman Paper No. 1 using a micro-pipette. n-butanol-glacial acetic acid-water (4:1:5) was run as the first solvent for 10 hours and water saturated phenol was run for 8 hours as the second solvent at room temperature (23-25°C). Ninhydrin solution (0.25%) in 95% ethanol was used as the colour developing reagent. Different amino-acids were identified by comparing the Rf values of the individual amino-acids with the standard chromatogram. The standard chromatogram was prepared in the same way as described above using standard amino-acids (BDH, England).

(ii) Individual FAA (Quantitative) : Quantitative estimation of the different amino acids identified by paper chromatography were quantified individually following the method as described by Habibulla (1970). Each amino-acid spot, after identification, was cut and eluted in 2.0 ml of 95% ethanol containing 0.1% $CuSO_4$ (5:1). The elution was done by centrifuging at 1,000 X g for 3 minutes. The intensity of the colour was measured at 610 nm in a double beam Spectrophotometer (Beckman Model-26). The quantity of the obtained individual amino acid was calculated from a standard graph by spotting 10, 20 and 30 µg of each standard amino-acid (BDH, England) and employing similar treatments as described for the unknown sample.

C. Estimation of DNA and RNA :

DNA and RNA were estimated by diphenylamine and orcinol method respectively, following Schneider (1957). Calf thymus DNA in concentrations ranging from 50-200 µg and yeast RNA in concentrations ranging from 10-50 µg were used to prepare the standard graphs which were linear.

Chemical and biochemicals :

All chemicals and biochemicals used were of analytical grade, purchased from either Glaxo Laboratories, India; BDH, England; Sigma Chemical Company, U.S.A.; E. Merck, Germany; Centron Laboratories, India; Loba-Chemie, India or C.S.I.R. Centre for Biochemicals, India. Double glass-distilled water was used for all estimations.

Expression of data :

Wet weight, dry matter content, protein and total free amino acid of each developmental stages were presented as mg per embryo. Water content was expressed as percentage of the wet weight of the embryo. The individual amino acid was presented as percentage of total free amino acids. Nucleic acids were shown as µg DNA or RNA per embryo.

Statistical analysis :

The data obtained from 4-5 sets of observations were statistically analysed according to Bailey (1965). The mean and standard deviation (S.D.) of the mean for each set of data, and the level of significance between 2 sets of data were calculated. p values at 5 percent or lower for 2 sets of data were taken as significant.

RESULTS

Wet weight, dry matter and percent water content :

The results on the alterations in the wet weight, dry matter and percent water content of the embryos of the two species of carps at different altitudes are presented in Tables-9-11 and Figs. 6-8.

It could be seen that the wet weight (Table-9; Fig. 6) of the unfertilized egg of C. carpio and L. rohita were between 1.6-1.7 mg and about 0.87 mg, respectively. There was no significant variation in their weights at the two altitudes studied. In scale carp, the wet weight of the egg suddenly increased after fertilization (25%) and this continued significantly at Gauhati till the stage-3 after which the level reached a plateau till the pre-hatching stage (15a). At Shillong, the increase in the wet weight of the scale carp embryos was biphasic in nature. Once it increased (18%) at fertilization and again (about 10%) after stage-11 till the pre-hatching stage (15a). Between the stage-2 and 13, the wet weight of the scale carp embryos were significantly higher at Gauhati in comparison to the high altitude, and after that the differences were insignificant till hatching. In rohu, there were significant increase in the wet weight of the embryos after fertilization till stage-3 in both the altitudes. It remained almost constant till the pre-hatching stage (15a) at Gauhati. However, at Mawpun, the wet weight of the embryos started decreasing significantly from stage-11 till hatching.

In all cases, the wet weight suddenly decreased on hatching to a level between the weight of unfertilized and fertilized eggs.

The dry matter content (DMC) (Table-10; Fig. 7) was 31.6% and 33% of the wet weight of the scale carp at Gauhati and Shillong respectively. In rohu, the DMC was comparatively higher than the scale carp eggs and was about 43% at both the altitudes. The DMC gradually decreased after fertilization in all cases showing generally a biphasic pattern. One significant decrease was observed after fertilization and another generally took place after neurulation (stage-11). There was not much variation between the rohu embryos at two altitudes whereas in scale carp the DMC of the embryos at Gauhati were generally higher than those at Shillong.

The overall decrease in the DMC from the unfertilized egg till hatching was 30-32% in scale carp and 40-54% in rohu at both the altitudes.

The percentage water content (Table-11; Fig. 8) in the unfertilized egg of scale carp produced at Gauhati and Shillong were 68.39% and 66.98% respectively. In case of rohu, the values were about 56.9% at both Gauhati and Mawpun. There was a sudden increase in the percent water content in all cases after fertilization and then a very slow increase continued till the pre-hatching stage (stage-15a). At hatching, the water content significantly decreased. However, in rohu embryos, there was a sudden increase (10.24%) in percent water content at stage-10 at Mawpun which remained significantly higher than the corresponding values at Gauhati. The increase at fertilization was in the order of 10-12% in scale carp and about 9% in rohu at both the altitudes. Except between the stage-11 to 15a in rohu, there was no significant altitude related difference in the percent water content in the embryos of the two species studied.

Protein content :

The levels of total, soluble, cytoplasmic and mitochondrial protein were estimated at different stages of development in scale carp and rohu at different altitudes (Tables-12-15; Figs. 9-10). In all parameters and in all cases, the protein content in scale carp embryos were higher than the rohu.

The pattern of change in total, soluble and cytoplasmic protein levels, in general, followed similar pattern of change in scale carp. There were significant sharp decrease in protein levels in all cases of fertilization which were followed by a slow decreasing pattern till stage-7. At stage-8 (Blastual) there was an enhancement in the concentrations of the proteins which started decreasing again till the newly hatched larval stage. There was not much difference in these protein levels in the earlier developmental stages of scale carp at the two altitudes studied. However, the total protein level became significantly lower at pre-hatching (15a) and early post-hatching stage (15b) at the higher altitude. The levels of soluble and cytoplasmic protein were significantly lower from stage-12 till the early post-hatching stage (15b) of the scale carp embryo at higher altitude. The decrease in cytoplasmic protein was more prominent in comparison to the decrease in total and soluble protein levels in the later developmental stages at higher altitude.

In rohu, the three protein levels also declined very significantly immediately after fertilization and continued to decrease slowly till stage-7 at both the altitudes. The protein levels started increasing in all cases between stage-8 and 9. This increase continued significantly for total protein at Gauhati till the pre-hatching stage (15a). However, for soluble and cytoplasmic proteins the concentration showed an increasing trend only till the stage-14 after which they remained almost unaltered. At Mawpun, the concentrations of the three groups of proteins again declined at neurulation (stage-11) which were restored in case of total and soluble proteins. However, the cytoplasmic protein level declined continuously till the stage-15b. Thus, the concentrations of the three groups of proteins were significantly lower from stage-11 onwards in rohu embryos at higher altitude (Mawpun) than those at Gauhati.

The mitochondrial protein level, on the other hand, went on increasing from the zygote stage till the completion of hatching in the embryos of both the species at different altitudes studied. The level significantly increased at stages-12, 15a and 15b at Gauhati and stage-13 at Shillong in scale carp. However, the mitochondrial protein levels in rohu embryos significantly increased from stage-9 onwards. There were not much difference in the mitochondrial protein concentrations of the embryos at the two altitudes in scale carp. However, the same became significantly lesser at higher altitude in rohu after neurulation (stage-11) till hatching.

Free amino acids :

Total free amino acids : The concentration of total free amino acids was found to be higher in the different developmental stages of scale carp than rohu (Table-16; Fig. 11). On fertilization, the total free amino acid level increased at both the altitudes in the two species. This enhancement was more prominent in rohu eggs than scale carp. In rohu, the increase was about 67% at Gauhati and 31% at Mawpun. However, in scale carp the increase was much lesser as about 6% at Gauhati and 9% at Shillong.

Till stage-8, in both the species, the total free amino acid levels in the embryos were either similar or more at Gauhati than at the higher altitude. The pattern got reversed from stage-9 onwards when the levels were higher at higher altitude than that at Gauhati. In both the species at Gauhati, there was a significant decrease in free amino acid level between stage-8 which continued to decrease slowly in rohu. In scale carp, the level went up again at Gauhati from stage-12 upto stage-15a (pre-hatching stage). At Shillong, the amino acid level of scale carp embryos went up very slowly upto stage-10 after which it showed a very quick increase upto the pre-hatching stage (15a). In rohu, the level was higher at Shillong than Gauhati with too much fluctuations from stage-7 onwards.

At hatching, the total free amino acid level decreased in all cases. This decrease was significant (24-28%) in scale carp both at low and high altitudes.

Individual free amino acids : The free amino acids were qualitatively separated by two dimensional paper chromatography and quantified in different developmental stages of *C. carpio* and *L. rohita* at both low and high altitudes (Tables-17-20).

During different developmental stages altogether seventeen amino acids could be detected in scale carp and eighteen amino acids in rohu. Cys. and Cys.-cys. could not be detected in scale carp and thr. could not be detected in rohu embryos. In unfertilized egg of scale carp, his. and trp. could not be detected and only appeared after fertilization. In rohu, his., pro., ala., and trp. could not be detected in the unfertilized egg. His. and pro. appeared after fertilization and ala. and trp. appeared at stage-2 (2-cell stage) only. This pattern was same in both the altitudes.

Glutamic acid was one of the predominant amino acids in both embryos with asp. in scale carp and arg. and ileu. in rohu. Lys., leu., ala. and pro. were present in a moderately high concentration in scale carp embryos only. The relative concentrations of the free amino acids varied considerably between the two species but not much between the two altitudes. Different amino acids changed differently during the process of development in the two fishes at the two altitudes. Some amino acids continued to increase, some gradually decreased, some did not change and some other fluctuated with the progress of development. The changes were apparent at three phases of development - at fertilization (stages-1-2), during blastulation and gastrulation (stages-7-10) and at hatching.

At fertilization, his. and trp. appeared in scale carp at both the altitudes whereas val. and asp. decreased only at Gauhati. In rohu, his., pro., ala. and trp. appeared after fertilization and level of arg. went up both at Gauhati and Mawpun. Ileu. increased at Gauhati but decreased at Mawpun. At Gauhati, there were decrease in the proportion of asp., ser., gly., lys. and cys. whereas at higher altitude none of these except cys. decreased.

At the middle stages of development (stages-7-10), most of the amino acids showed some variations in their proportions which could be marked from the tables. Again at hatching (stages-15a-15b), several amino acids showed definite increases or decreases in their proportions. Pro. showed significant increase in both scale carp and rohu embryos at both altitudes. However, at Gauhati the increase was more significant than at higher altitudes. Besides pro., leu. also showed significant increase during hatching in rohu at Gauhati.

In scale carp besides pro., ileu., met., lys., phe. and gly. increased and arg., ser., thr., val., leu., tyr., his. and trp. decreased at Gauhati. At Shillong, the proportion of ileu., gly., phe., his. increased besides pro. in scale carp whereas ser., arg., thr., ala., val., leu., tyr. and trp. decreased.

In rohu, the changes involved less number of amino acids during hatching. Besides pro. and leu., the amino acids whose proportions increased at Gauhati were glu., arg., tyr. and cys., whereas at higher altitude the increase involved ileu., lys., tyr., trp., cys. and cys.-cys. besides pro. The proportion of ileu., gly., val. and phe. decreased at Gauhati and glu., ser., met., leu. and phe. decreased at high altitude. It is significant to see that at hatching glu. and leu. increased at low altitude and decreased at higher altitude. On the other hand, ileu. which decreased at low altitude increased at higher altitude.

<u>DNA</u> :

The DNA content per embryo of different developmental stages of scale carp and rohu at both lower and higher altitude have been presented in Table-21 and Fig. 12. The pattern of change of the DNA content per embryo was species specific and similar at both altitudes. Altitude showed no effect except in the last three stages studied (stages-14, 15a & 15b) where in both the species, the DNA content was significantly lower at higher altitude. The DNA concentration in the unfertilized egg was more with 0.593 µg and 0.613 µg in rohu than scale carp with 0.462 µg and 0.477 µg at corresponding lower and higher altitudes.

The DNA content remained stable upto stage-6 of development after which it started increasing. In scale carp the increase was continuous with the rate becoming faster at later stages of development. However, in rohu, the increase was stepwise, once from stage-5 to 9, next from stage-11 to 12 and finally from stage-14 to 15b. In this case also, the rate of increase was faster in later phases. The overall increase in the DNA content per embryo increased from unfertilized egg to pre-hatching stage (15a) by 28 and 21 times in scale carp and 24 and 18 times in rohu at lower and higher altitudes respectively.

RNA :

The total RNA content per embryo during developmental stages of scale carp and rohu at lower and higher altitudes has been shown in Table-22 and Fig. 13. The changes in the RNA content were species specific and the altitude had no effect on this pattern of change.

The RNA content in the unfertilized egg was about 3.8 to 3.9 µg in C. carpio and 2.5 to 2.6 µg in rohu. There was no significant change in this concentration till stage-10 (completion of gastrula) in scale carp and stage-5 (16-cells stage) in rohu. The RNA concentration was higher in the embryos at lower altitude than higher altitude in both fishes and these differences were significant generally in the last four developmental stages studied. In scale carp, the RNA content increased from stage-11 and in rohu from stage-7. The overall increase in RNA content from the unfertilized egg to the pre-hatched embryo (stage-15a) was about 5 and 3.5 times in scale carp and 17 and 13 times in rohu at low and high altitude respectively. The increase in the RNA content of the embryos during their development was more significant in rohu than scale carp at both the altitudes.

DISCUSSION

The development of teleost fishes, as in other animals, takes place from the fertilized egg through a network of sequential biochemical and physiological changes. The yolk material in the egg is degraded to provide energy to the growing embryo and also to supply raw materials for the synthesis of new molecules during development and differentiation. Degradation or synthesis of different biomolecules during the development is genetically programmed. In a given species, a specific biomolecule is degraded or synthesized only at a specific stage of development. Therefore, the alterations in the different biochemical constituents during the early development of scale carp and rohu can give a gross idea about the changes in metabolic activities and molecular mechanism of differentiation in these two fishes. The two species of fish, *C. carpio* and *L. rohita*, are well adapted to the altitude nearer to sea level where they show better survival, growth and ontogenic development. At higher altitude, both the fishes have shown either increased mortality or inhibition in their rate of development at different embryonic stages (Chapter-1). The studies on the variations of different biochemical constituents in the developing embryos of the two species at lower and higher altitude are expected to throw some light on the interruptions in the normal physiological and biochemical processes at the higher altitude which might have resulted into the adverse effects on the embryonic development and survival, as reported in the previous chapter.

Wet weight, dry matter and percent water content : (Tables-9-11; Figs. 6-8)

The wet weight of any living organism represents the total of its water and dry matter content. The alteration in the proportions of these three parameters generally indicate the gross changes in synthesis or degradation of substances in the organism. Particularly in developmental stages of aquatic oviparous animals, measurements of these components could help to find out the rate of utilization of the yolk for energy production or its conversion to other biomolecules.

The eggs of scale carp and rohu contained about 32% and 43% of dry matter, and the water content was higher with about 68% and 57% respectively. The dry matter represents mainly the large amount of yolk present in the mature oocyte. The wet weight and total DMC of scale carp egg were much higher than rohu egg indicating the large amount of yolk present in scale carp than rohu. The period of ontogenic development is also larger in scale carp than rohu with total hatching time as about 58 hrs. and 12.5 hrs. respectively (Gauhati, 1980). Higher yolk content could be the reason for better adaptability of scale carp embryos to stressful environmental factors than rohu. The significant increase in wet weight and water content after fertilization and decrease at hatching has been reported in several fish embryos (Hayes, 1930; Suyama & Ogino, 1958; Peňáz *et al.* 1976; Moroz & Luzhin, 1976; Yoneda, 1977). The formation of the fertilization membrane and imbibition of water immediately after fertilization are the main causes of increase in wet weight, and the elimination of the chorionic membrane alongwith the perivitelline fluid during hatching is the main cause of decrease in wet weight. Fertilization is known to induce an array of molecular events in the egg for mobilising the yolk materials to meet the heavy demand of metabolic energy and also to start several biosynthetic processes.

This has resulted in a sudden and significant loss of yolk resulting in the decrease in DMC after fertilization, though the wet weight of the embryo increased due to the large scale imbibition of water. Some amount of this water might have been utilized in helping the high rate of metabolism in the post-fertilization embryo. Though, there were variations in the wet weight of the post-fertilization stages in the two fishes, the percent water content remained fairly stable with altitudinal variation. This indicates that the embryonic development needs a particular proportion of water to be maintained for normal metabolism. This, more or less constant level of water, might have been maintained by the regulation of the permeability of water through membrane. The fish egg membrane is permeable to water (Blaxter, 1969). The DMC decreased slowly during the cleavage stages and significantly after gastrulation (stages-9-10) and further stages of development which involve actively in differentiation and organogenesis. In the earlier phases, the yolk materials might have got converted mainly to new substances and during later stages, the catabolic processes to release energy for differentiation and complex developmental process might have dominated over the synthetic processes. The wet weight of the embryo did not show much variation in rohu and in scale carp at lower altitude where the development was normal. Hence, to compensate the decreased DMC, total water content might have increased to maintain the wet weight of the embryo. Calorimetric measurements carried out by Faustov and Zotin (1965) on the eggs of *M. fossilis*, *A. stellatus* and *A. guldenstadti* showed that the calorific value of the egg declines steadily from the time of fertilization to the point of hatching. The DMC of the eggs also slowly decreased in concentration. Similarly, in the summer flounder

(*P. dentatus*), Johns and Howell (1980) showed a significant decrease in the yolk reserve of the egg from fertilization till the late larval development. That they attributed to its utilization as an energy source. Studies on the common carp, *C. carpio* have shown that the newly hatched embryos lose as much as 23.12% (Peňáz *et al.* 1976) or 28.47% (Moroz & Luzhin, 1976) of DMC from the level in its unfertilized oocyte. In the present study, the decreasing level of DMC from unfertilized egg till hatching in scale carp (*C. carpio communis*) was found to be 31.45%, and in rohu it was 40.32%. The comparatively higher reduction of the DMC in rohu is indicative of the high energy expenditure of the embryo which developed at a faster rate in 12.5 hrs. than that of the scale carp (58 hrs.) and also, contained less amount of yolk. At hatching, scale carp lost more wet weight than rohu but the DMC and percent water content did not alter much. The scale carp might have a thicker chorionic membrane and more perivitelline fluid than rohu which are lost during hatching.

At higher altitude, the embryos did not show much variations in wet weight, DMC and percent water content till the neurulation (11) stage except in the total wet weight of scale carp. Some significant variations were seen after the stage-11 in the two altitudes. The wet weight of scale carp at lower altitude remained almost constant from stage-3 till the pre-hatching stage. However, at higher altitude, it showed a very slow increase from stage-1 till stage-10. A significant increase started from neurulation (stage-11) till the pre-hatching (15a) stage when the altitudinal difference became insignificant. Thus, there was a biphasic water imbibition in scale carp at higher altitude where temperature was comparatively low. The DMC and percent water content were not significantly different at the two altitudes though the levels, in general, were lower at higher altitude during the cleavage stages. This might have resulted due to the higher rate of energy releasing metabolic degradations of the yolk at higher altitude as an adaptation to the low temperature and other metabolic activities. Increase in energy production has been reported with variations in environmental factors by measuring the oxygen consumption in animals (Love, 1970).

Rohu showed almost no effect of altitude till the gastrulation (10) stage. From neurulation (stage-11), the effect of altitude became significant. The water content increased at higher altitude though the DMC decreased significantly in all stages after neurulation (stage-11), resulting in a decrease in the wet weight of the embryos. The increased mortality of the rohu embryos were also very conspicuous from the neurula (11) stage at higher altitude (Fig. 5). There might be a severe depletion of the yolk to enhance the metabolism in these stages under the influence of the adverse environmental conditions at higher altitude.

Protein content :

Among different macromolecules in the cell, protein is most predominant having both structural and functional roles to play. The chorionic membrane being impermeable to nutrients (Neyfakh & Abramova, 1974), the complete development of fish embryo till hatching depends on the amount of yolk deposited in the egg. The major constituent of the yolk is protein, besides some amount of lipids, carbohydrates and nucleic acids. The protein content in the mature oocyte was about 58% and 36% of the dry weight in scale carp and rohu respectively. Scale carp egg with higher concentration of protein, showed longer incubation period and better tolerance to environmental fluctuations than rohu. The total amount of protein per rohu egg was about 50% of scale carp values. In the egg and during the early stages of development, the total protein consisted of mostly the cytoplasmic proteins. A small part was contributed by mitochondrial proteins and insoluble proteins. Unlike amphibians, there might not have been excess of mitochondrial accumulation during vitellogenesis. However, it is difficult to make a clear statement on this subject with the available informations.

At fertilization, a significant decrease in total, soluble and cytoplasmic protein fractions occurred in both scale carp and rohu to the extent between 30-40%. However, mitochondrial protein did not change significantly. This significantly decreasing protein level might be due to sudden utilization of the proteins after fertilization. The alterations in protein level during fertilization and during the early development have been reported to be variable in different species. In *S. trutta*, a continuous degradation of the protein level has been reported by Phillips *et al* (1958) and Suyama & Ogino (1958). Moroz and Luzhin (1976) showed a significant decrease (69%) of the protein content of *C. carpio* egg on fertilization which increased again in the pre-hatching stage after an intermediate unstable period. In *H. fossilis* (Sarkar *et al*, 1979) and in *S. gairdneri* (Zeitoun *et al*, 1977), the protein level increased after fertilization which continued in *H. fossilis* throughout the development till hatching.

In the present study, *C. carpio* and *L. rohita* showed almost a common pattern till the end of cleavage and then the patterns of protein content were different. The protein concentrations were maintained at a low level or decreased slowly after fertilization to reach a low level at morula or blastula (stage-7-8) stage. The mitochondrial protein level remained fairly unchanged in both the species during this period.

There was a significant increase in the level of total, soluble and cytoplasmic proteins in C. carpio at stage-8 which gradually decreased to a lower level in the freshly hatched larva (15b). This rate of decrease was more conspicuous in cytoplasmic protein followed by soluble and total proteins. The trend in rohu was very much different with the protein concentrations gradually increasing from stage-8 to the peak at pre-hatching (15a) stage. However, the cytoplasmic protein did not increase that significantly as total protein. The increase in the total protein in rohu from the level at fertilization till hatching was about 45%, whereas the increase in soluble protein was about 10%. This might be due to the increase in the insoluble proteins which mainly belongs to the group of structural proteins (Zaitsev et al, 1969). When the size and number of the cells in the embryo increases, more structural proteins might have been synthesized, resulting in an increase in the insoluble fraction of the total protein.

The mitochondrial protein in both the species showed a gradual enhancement from gastrulation (stage-9) onwards till the freshly hatched embryo (stage-15b). The overall increase in mitochondrial proteins was above 385% in scale carp and 293% in rohu.

These alterations indicate that the proteins are utilized during the early cleavage period to supply the necessary energy for the active mitosis. Protein has been considered as the vital source of energy for development in fish (Blaxter, 1969; Love, 1970). The presence of protease activity in the egg has been correlated with the degradation of the yolk protein (Neyfakh & Abramova, 1974). The protein content change indicates the predominance of protein synthesis or degradation at different stages of development. Except for blastula (8) stage, there is predominance of protein degradation than synthesis in *C. carpio*. However, in *L. rohita*, the protein synthesis increasingly dominates over the protein degradation after the blastulation (stage-8). The synthesis of new proteins in different fish has been reported to start sometime during late cleavage phase and intensified at gastrulation during development (Neyfakh & Abramova, 1974; Zeitoun *et al.* 1977; Sarkar *et al.* 1979). The amount of total protein in *C. carpio* and *L. rohita* embryos at hatching are similar though the egg of *C. carpio* contained twice the amount of protein present in *L. rohita*. This high amount of protein present in scale carp might have served as the energy source throughout the development and in rohu, the energy source might have changed from protein after the blastula (8) stage.

The increase in mitochondrial protein from gastrulation (stage-8) indicates the synthesis of fresh mitochondria to help the high energy need for the differentiation and organogenesis during later stages of development. The rate of respiration during fish development has been shown to increase by many folds (Neyfakh & Abramova, 1974) and this might have some correlation with the increase in mitochondria. At hatching, (stage-15b), there was a significant decrease in protein content except mitochondrial protein, which might have been due to the loss of the proteins present in the chorionic membrane which is thrown off during hatching.

The variations in the alteration pattern of protein content of the developing embryos of the fishes are species specific and might have some correlation with the protein concentration in the egg.

The effect of higher altitude was different in the two species and was also dependent on the stages of development. It is quite interesting to note that the earlier stages of development till gastrulation (stage-10) was not affected by the higher altitude. The rate of development and survival also were, in general, not affected by the high altitude during these stages. The effect was significantly clear during the later stages of development after neurulation (stage-11). The concentrations of different types of proteins studied in the two species significantly decreased during these stages of development except the mitochondrial protein in scale carp. The yolk utilization which has been predominant during the early cleavage stages in some species of fish has been shown to be independent of environmental temperature within the tolerance zone (May, 1975b; Howell, 1980; Johns & Howell, 1980; Johns et al, 1981). In the present study, the development was carried out within the optimal temperature zone and that might have helped in the proper utilization of the yolk resulting in parallel alterations in protein concentrations at both the altitudes at the early stages of development.

Gastrulation and later stages of development, involving differentiation and organogenesis, need a large number of new proteins to be synthesized and also a large amount of energy to be produced. Thus, these stages involve the activities of several enzymes which are known to alter with alterations in the environmental conditions (Hochachka & Somero, 1971, 1973). Therefore, the soluble cytoplasmic protein levels which showed greater inhibition at higher altitude might be due to the lesser amount of metabolic proteins. The structural protein part which is comparatively more stable with environmental fluctuations, might have maintained its level, thereby lowering the intensity of decrease in the total protein concentration. The synthesis of new proteins needs a large number of enzymes and a large amount of energy. Therefore, it could be possible that due to lower temperature at higher altitude, the protein synthesis might have been inhibited and yolk utilization activated for metabolic adaptations resulting in lower protein concentration. The decrease in the mitochondrial protein level in rohu embryos at higher altitude indicates that synthesis of fresh mitochondrial might have been inhibited at higher altitude. Alterations in protein synthesis rate has been studied in adult fishes with relation to temperature (Das & Prosser, 1967; Haschemeyer, 1968, 1969a,b; Hilmy et. al. 1978) which have shown enhancement of protein synthesis with decreasing temperature. However, the mechanism in adult animals might not be similar in embryos.

The decreasing levels of protein at different stages of development in both scale carp and rohu coincided with those stages where the mortality was seen to be greater (Chapter-I). Therefore, it makes one to believe that the alterations in the protein levels have important role in the metabolic process of the developing embryo. Detailed studies on protein synthesis and degradation pattern could explain this clearly.

Among the two species, rohu was found to be more susceptible to higher altitude with greater mortality rate of developing embryos than scale carp. This was parallel with the greater inhibition of protein level of the later developmental stages in rohu. Particularly, the mitochondrial protein level which did not change in scale carp, decreased significantly in rohu. This might have resulted into the failure of the rohu embryos to meet the metabolic energy need in higher altitude embryos, resulting into their death.

Free amino acids :

Total free amino acids (FAA) :

Free amino acids serve mainly as the precursors for protein synthesis. Besides, they also serve as energy source under specific conditions and as precursors for the synthesis of several metabolic or physiological regulators. The level of total free amino acids vary greatly under different environmental conditions depending on the relative abundance of protein synthesis, amino acid synthesis, degradation of amino acids for energy release and their conversion to other metabolites or regulator molecules. During vitellogenesis, FAA accumulate in the egg of fish (Maslennikova, 1978) and its pool size varies in different species. The FAA concentration in the egg of scale carp was found to be significantly higher than that of rohu. The level increased significantly on fertilization. However, the increase was about 67% in rohu and only 6% in scale carp at lower altitude resulting in a near equal level of FAA in the fertilized egg of the two species. Örström (1941) reported an increase in the FAA level within 10 minutes of fertilization due to the splitting of yolk proteins. The FAA pool in the developing embryo was in the reverse order of the total protein alteration pattern. The decreased protein level coincided with increased FAA pool. This might be due to the fact that amino acids are mainly contributed in the developing embryo of these fishes from the degradation of proteins. The depletion of FAA level at specific stages of development might be due to the utilization of the amino acids for energy production. Such mobilization has been reported in fish (Atherton & Aitken, 1970; Knapp & Wieser, 1980).

At hatching, scale carp showed a significant decrease (about 25%) in the FAA concentration whereas no significant change could be observed in rohu. This shows that a substantial amount of amino acids might be associated with the chorionic membrane and perivitelline fluid of scale carp embryo which is lost at hatching.

The FAA level has been known to be an important indicator of physiological stress in fishes. Alterations in FAA level under different environmental conditions have been reported in fish tissues (Atherton & Aitken, 1970; Love, 1970; Forster & Goldstein, 1976; Squires et al, 1979; Knapp & Weiser, 1980). In the present study, the higher altitude did not alter significantly the changing pattern of FAA concentration during development. However, the level was comparatively lower during cleavage stages and significantly higher during later stages of development at higher altitude in both the species. These changes were inversely related to the variations in protein level.

Individual free amino acids :

Proportions of various amino acids vary in different fishes and at different stages of development. The amino acids such as arg., his., leu., ileu., lys., met., phe., thr., trp. and val. are not synthesized in fish, and therefore, have been considered as essential amino acids (Watts & Watts, 1974; Ketola, 1980). Reports on the amino acid composition in different fishes showed that the non-essential amino acids are usually more than the essential amino acids. However, their individual proportions varied between species. In the rainbow trout (S. gairdneri), Zeitoun et al. (1977) reported nine essential and eight non-essential amino acids. Cys.-cys. was not identified at any stage of development. The relative concentration of the non-essential amino acids was higher (72.26%) than the essential amino acids (27.74%). In chum salmon, O. nerka, the free amino acids extracted with TCA showed the presence of only seven essential and eight non-essential amino acids (Yoneda, 1977). His., cys., and cys.-cys. were not identified till the 50 days of development.

In the present study, there were significant differences between the proportions and compositions of essential and non-essential amino acids in the two fishes. In scale carp, except his. all other essential amino acids were identified in the unfertilized egg. Among the non-essential amino acids, cys. and cys.-cys. could not be identified throughout the development. Quantitatively, the relative contribution of the essential and non-essential amino acids in the scale carp egg was found to be 47.62% and 52.38% respectively. The acidic amino acids (glu. & asp.) were most abundant. Among the neutral amino acids ala., leu., pro. and the basic amino acid lys. were present in moderate concentrations. In rohu, three essential amino acids (thr., his. & trp.) and two non-essential amino acids (ala. & pro.) could not be detected in the egg. The concentrations of the essential amino acids was higher (59.4%) than the non-essential amino acids (40.6%) in rohu egg. On the other hand, the neutral amino acid ileu. and the basic amino acid arg. occurred in much higher proportions in the FAA pool of mature rohu egg. The concentration of sulphur containing amino acids (met., cys. & cys.-cys.) were higher in rohu than in scale carp egg. Such species specific and stage specific variations in the proportions of different amino acids have been reported in many other fishes (Love, 1970). The number of amino acids in mature egg was seventeen in *B. boddaerti*, twelve in *L. fimbriatus* and eleven in *M. seenghala* (Krishnamoorthi, 1958). The constituents of the FAA pool were different in different species and also at different stages of development. The variations among the amino acids in the two species studied were quite significant during fertilization which initiates a high level of metabolic activities, at gastrulation when morphogenetic movements of the cells leading to differentiation starts requiring varieties of new proteins to be synthesized and higher amount of energy to be released, and during hatching when the embryo comes out of the chorionic membrane for an independent life.

In general, altitude did not alter the relative concentrations of different amino acids, except in a few cases. However, it is difficult to precisely explain the phenomenon with the existing informations.

DNA :

The presence of large amount of DNA, many times higher than that of a diploid somatic cell, has been reported in the mature egg of several oviparous animals including fishes (Neyfakh & Abramova, 1974; Denis, 1974). The DNA content of a diploid somatic nucleus has been reported in large number of fishes to be between 0.8 to 8.8 pg and in carp, the value ranged between 3.04 to 3.83 pg (White, 1973). The total DNA content per egg of scale carp and rohu observed in the present study are several thousand times higher than the values for a diploid somatic nucleus. Hence, there must be a large amount of DNA accumulated in the yolk and in the cytoplasm of the mature egg. The information about the cytoplasmic DNA in fish is very much limited (Sarkar et al, 1979). However, it has been established in other aquatic animals that a large amount of DNA accumulation takes place in cytoplasm in form of amplified genes, increased number of mitochondria or/and as some granules in the yolk (Dawid, 1966; Brachet, 1974; Denis, 1974). Indirect evidences have been presented to show a large amount of DNA associated with yolk of some fish egg by estimating the DNA content in normal and yolk free embryos (Shmerling, 1965; Hagenmair, 1969; Dontsova et al, 1970). However, the excess amount of cytoplasmic DNA in scale carp and rohu egg can neither be assigned any specific site in the egg cytoplasm nor any function could be definitely attached to it. The origin, location and function of this excess DNA is yet to be understood in fishes.

Between the two species, rohu egg contained much more DNA than the scale carp, even though the wet weight of the rohu egg was much lesser than the scale carp. Therefore, not only the nuclear DNA level is different in different species but also the accumulation of cytoplasmic DNA during oogenesis varies in different species. Timofeeva and Kafiani (1964) suggested that probably the reserve DNA in the egg was to construct the genetic material of the newly formed nuclei during early cleavages.

The total DNA content did not change till some late cleavage stages in both the species, though the number of cells and the nuclei increased at each cleavage stage. Such non-linear increase in DNA level has been reported in several fishes (Dontsova *et al*, 1970; Zeitoun *et al*, 1977; Sarkar *et al*, 1979). However, in *S. gairdneri*, a sharp decline in DNA level was reported during the early cleavage stages of development due to fast degradation of the DNA (Zeitoun *et al*, 1977). The synthesis of DNA has been shown from the earlier cleavage stage by using radioactive DNA precursors in loach egg (Aitkhozhin *et al*, 1964). It has also been confirmed that this DNA synthesis is essential for cleavage to continue. Specific DNA synthesis inhibitor stopped the development in the early cleavage stages *H. fossilis* (Sarkar *et al*, 1979). This increase of nuclear DNA was so negligible in relation to the heavy load of cytoplasmic DNA that the method used for estimation was not sensitive enough to detect them in the present study during the early developmental stages. In the later stages when the number of cells increased greatly and the cytoplasmic DNA might have been degraded, the increase in the DNA content of the embryo could be observed with progress of development.

Higher altitude had no effect on the total DNA content per embryo of both the species for most part of the developmental stages studied. However, during the later developmental stages around hatching (stages-14, 15a & 15b), the DNA content per embryo was significantly lower at higher altitude in both the species. The difference was more prominent in scale carp than rohu. The DNA content normally does not change in a somatic cell under environmental stress (Hotchkiss, 1955). However, the situation in the embryo, is different, having a large number of cytoplasmic DNA which might breakdown to meet some metabolic need of the embryo. Besides, different tissues and at different stages of growth, the DNA contents have been shown to vary significantly (Bulow, 1970; Love, 1970; Jafri & Mustafa, 1976; Satomi & Ishida, 1976; Mustafa, 1977; Bhagowati & Ratha, 1982). Therefore, the variation in the DNA content at higher altitude might be the result of decreased DNA synthesis due to reduced cell growth or increased metabolism of stored DNA.

RNA :

The concentration of RNA in a particular cell or tissue, generally, indicates its protein synthetic potential (Hains, 1980). In developing embryo, the synthesis of a large amount of proteins of different types is necessary to meet the demand of the fast rate of growth and differentiation. In most of the oviparous animals, therefore, a large amount of different RNAs are deposited during oogenesis. These RNAs remain quiescent till fertilization when they get activated to synthesize proteins by translation. Accumulation of excess RNA in the egg and its role in the protein synthesis during the early cleavage stages of the embryos have been shown in echinoderms, amphibians and some fishes (Aitkhozhin et al, 1964; Tyler, 1967; Denis, 1974; Paul, 1974b; Ballantine et al, 1979; Sarkar et al, 1979). The RNA concentration in the egg of scale carp, observed in the present study, is comparatively higher (about 3.8 μg) than rohu (about 2.6 μg). This might help the scale carp embryo in synthesizing new proteins, independent of transcription, for longer period during development. Participation of reserve RNA, in the protein synthesis and the lack of new RNA synthesis until gastrulation has been reported in the loach (Aitkhozhin et al, 1964). Electron microscopic study in the loach egg showed the presence of dense aggregations of ribosomes between the yolk granules. The developing embryo during cleavage and blastulation does not synthesize new ribosomes. Transfer of yolk RNA into embryonic RNA has also been shown electron microscopically in zebra fish, B. rerio (Neyfakh & Abramova, 1974) and cytochemically in trout (Hagenmaier, 1969). In loach (Timofeeva & Kafiani, 1964) and rainbow trout (Dontsova et al, 1970; Zeitoun et al, 1977), the total RNA content decreased from the time of fertilization to blastulation. However, in the present study, no significant change of RNA content was detected in scale carp until stage-10 (end of gastrulation) and in rohu until stage-5 (16-cells). In scale carp egg, the higher accumulation of RNA might have been enough to carry on the development to complete gastrulation without synthesizing new RNAs, whereas in rohu egg due to less amount of RNA deposited in the egg, synthesis of fresh RNA was required from the late cleavage stage (stage-7). Further, it could be that the degradation of RNA was faster in rohu, and the RNA molecules had longer half life in scale carp. Detailed studies on the varieties of RNA molecules and their half-life can only give a definite answer to this problem. Such species specific variations in the concentration and synthesis of this macromolecule are common in biological systems including fishes. In trout, S. gairdneri, the RNA content showed significant increase at gastrulation (Dontsova et al, 1970; Zeitoun et al, 1977) whereas in the Indian catfish the rise has been observed right from the time of fertilization with the peak concentration at blastulation (Sarkar et al, 1979).

In both the species, the RNA level went on increasing significantly in the later stages of development. However, the rate of increase was different at different stages of development, indicating that each developmental stage has a different requirement of RNA.

The high altitude had no effect on the RNA content of the embryo during the early developmental stages when it remained constant in the two species. The effect of altitude could only be seen after RNA level started increasing. At higher altitude the RNA level became significantly lower than that at lower altitude during the later stages of development. This difference became more and more apparent nearer to hatching. The lower level of RNA content might indicate a lower rate of RNA synthesis or higher rate of RNA degradation at higher altitude. Whatever be the reason, the protein synthesis must have been affected resulting in a lower level of protein concentration during the later stages of development at higher altitude. These stages of development showing deviations in RNA level also coincided with the stages showing higher rate of mortality at higher altitude.

The studies on the alterations in wet weight and different chemical constituents of the developing scale carp and rohu embryos indicated that scale carp egg with higher wet weight and higher amount of macromolecules, was capable of sustaining the development to a fairly later stage than rohu. The concentrations of important macromolecules such as DNA, RNA and protein started increasing much earlier, probably due to their high synthesis, causing an additional metabolic load on the embryos of rohu than scale carp. Therefore, at higher altitude with slight deviation in the environmental factors, rohu embryo showed greater mortality whereas scale carp embryo showed better adaptability. Besides, the developmental stages also showed two clear phases such as the early cleavage phase till blastula when the mitosis is very fast and the later development stages with differentiation and organogenesis. The former phase showed better adaptability, probably due to sufficient availability of nutrients and having comparatively less complex metabolic processes than the later phase which showed higher susceptibility to environmental variations.

CHAPTER III

METABOLIC CHANGES

INTRODUCTION

The living organism exists in a dynamic equilibrium state with regulated metatolic flux. The metabolic flux varies quantitatively and qualitatively in different animals, during different stages of development and in response to fluctuations in environmental conditions. In oviparous animals, the mature oocyte gets loaded with a large amount of different nutrients during vitellogenesis, needed for the embryonic development, but remains in a quiescent state with little metabolic activity till fertilization. Fertilization not only adds a set of haploid chromosomes into the oocyte, but also induces a sudden explosion of mitotic activity in the resultant diploid zygote. The fast rate of mitosis, in the early stages of development, requires a large amount of energy and the synthesis of several biomolecules in different concentrations. The fish egg being impermeable to most biomolecules, the nutrients deposited in it only gets utilized for these purposes till hatching, when the embryo comes out of the egg membrane and communicates with the external environment. Thus, the period from fertilization till hatching shows many important changes in metabolic processes for the repeated cleavage followed by morphogenetic movement of cells leading to differentiation and organogenesis. The total flux of metabolism during ontogenic development is genetically programmed and thus, maintains an efficient homeostasis of nutrient degradation, synthesis of new species of biomolecules during differentiation, and management of the waste products of metabolism.

The metabolic pathways are multistep processes, each step being catalyzed by an enzyme. Therefore, the activities of several enzymes control the functioning of a particular metabolic pathway and the regulation of a metabolic pathway takes place by regulation of the activities of its enzymes. Enzymes are a group of functional proteins which catalyze biochemical reactions in the biological systems. Each enzyme usually catalyze only a single reaction. But collectively, they maintain the homeostatis of the animal as a whole. The role of enzymes in bringing out the complex biochemical changes during embryonic development has been well recognised. The relationship between enzyme activities and development is so good that often development is considered as a state of differentiation of enzymes and metabolic pathways (Moog, 1965). Each cell type and tissue, contributing to a specific organ, contains a specific set of enzymes. This enables the various organs to maintain their specific metabolic role. Therefore, it is evident that differentiation, comprising of histo- and organogenesis, must be associated with the differentiation of enzymes in order to acquire the functional characteristics of the tissues and organs (Moog, 1971; Monroy, 1973). The specific appearance or increase in activity of some enzymes are so tightly coupled with the specific morphogenetic events that often, they are referred to as marker enzymes (Monroy, 1973). Glutamine synthetase is regarded as a marker enzyme for the functional differentiation of the neural retina (Chader, 1971; Moscona, 1972). AChE is also considered as a biochemical marker for the functional differentiation of typical neurons and muscle cells forming synapses and myoneural junctions (Brachet, 1974). The specific differentiation of enzymes with specific morphogenetic events during embryonic development has also been described with the help of isozymes (Markert, 1970, 1973; Whitt, 1981a,b).

Enzymes being proteins are the product of genes. Hence, appearance, disappearance and alterations of particular enzyme activity indicate the alterations in the activity of the corresponding gene during development. The differential gene expression during development and differentiation has been clearly shown (Markert, 1973; Davidson & Britten, 1979; Whitt, 1981a). However, this varies substantially in different animal species due to their different genetic programming, and under different environmental conditions due to their different regulatory mechanisms for expression. The in vivo regulation of expression of different genes by different modulator molecules, through the expression of specific enzyme activity during the developmental stages, has been extensively studied in higher vertebrates (Moog, 1971; Moscona, 1972). Such studies in fish are rare. However, Whitt (1981a) suggests that the mechanisms operating in higher vertebrates may also hold good in fish.

The metabolism of different organic or inorganic substances varies markedly in different developmental stages depending on the presence or absence or respective enzymes. The mouse oocyte in vivo undergoes maturation and the zygote is cleaved once, in the presence of pyruvate or oxaloacetate and not if lactate, phosphoenolpyruvate or glucose are present (Briggers et al, 1967). However, lactate and phosphoenolpyruvate supports the development from the 2-cell stage (Brinster, 1965a,b). Other intermediates of the glycolytic and Krebs cycle are ineffective at the 2-cell stage, but allow development to proceed from the 8-cell stage to the blastocyst (Brinster & Thompson, 1966). The rabbit embryo can use pyruvate, lactate and phosphoenolpyruvate to support development for 24 hours from the 2-cell stage, but apparently can not utilize glucose or TCA cycle intermediates until the beginning of blastulation (Fridhandler, 1961; Daniel, 1967). Similar shifting of the pattern of metabolism has been also reported in amphibians (Barth, 1964) and fishes (Love, 1970). In salmon, S. irideus, carbohydrate is the major energy source from the onset of blood circulation in the embryo till hatching but in the post-hatching period lipid takes over this task (Smith, 1952).

Changes in enzyme levels during fish development :

The unfertilized egg of fish contains a large store of most essential enzymes of the various metabolic pathways (Hishida & Nakano, 1954; Terner, 1968; Kusen' & Oleshko, 1974; Ermolaeva & Mil'man, 1975). These enzymes are kept ready for use at later stages of development. During the course of embryonic development, the levels of several enzymes alter as a result of the differentiation of cell types and altered level of metabolism (Shaklee & Whitt, 1977). The levels of some enzymes increase, some others decrease, whereas some others remain at a constant level. There is still another group of enzymes which show fluctuations in their level during development.

The activities of succinoxidase and cytochrome oxidase in medaka, O. latipes (Hishida & Nakano, 1954), fructose diphosphotase, creatine phosphokinase, glucose-6 phosphate dehydrogenase, phosphoenolpyruvate carboxylase, malate dehydrogenase in trout, S. gairdneri (Terner, 1968), lactate dehydrogenase in trout, loach (M. fossilis) and medaka (Nakano & Hasegawa, 1971; Philipp & Whitt, 1977), phosphofructokinase and pyruvate kinase in loach (Yurovitzky & Mil'man, 1971), acid ribonuclease in loach (Kusens & Oleshko, 1974), acid DNAase in loach (Oleshko & Kusen', 1974) are reported to show a constant increase with development.

The activities of NADH-cytochrome-c-reductase, fructose 1-6 diphosphatase, glucose-6-phosphate dehydrogenase and phosphoenol pyruvate dehydrogenase in loach are reported to decrease from the embryonic to the adult levels (Neyfakh & Abramova, 1974).

The levels of cytochrome oxidase and all enzymes of the HMP shunt pathway except glucose-6-phosphate dehydrogenase and fructose diphosphatase in loach remained more or less at a constant level throughout the course of embryonic development (Neyfakh & Abramova, 1974). In warmouth and green sunfish (*L. gulosus* & *L. cyanellus*), Shaklee and Whitt (1977) reported relatively constant levels of adenylate kinase, glucose-6-phosphate dehydrogenase, glucose phosphate isomerase, lactate dehydrogenase, malate dehydrogenase, pyruvate kinase and phosphofructokinase activities till hatching.

Fluctuating changes in activities have been reported for aldolase in loach (Kusakina *et al*, 1976) and creatine kinase in *B. rerio* (Pontier & Hart, 1979). The activity of phosphorylase kinase increased sharply, reaching the peak at blastulation following which it remained at a constant level (Neyfakh & Abramova, 1974) in loach. Alkaline ribonuclease activity decreased at the end of blastulation and gradually increased from the beginning of gastrulation reaching the highest level in the last hours of early development (Kusen' & Oleshko, 1974). The alkaline DNAase activity in loach gradually decreased till the onset of gastrulation following which the level sharply increased (Oleshko & Kusen', 1974). In warmouth and green sunfish, creatine kinase and fructose biphosphate aldolase activities showed decrease in the early embryogenesis followed by a marked increase at hatching (Shaklee & Whitt, 1977).

Differential expression of the isozymic forms of some enzymes have been studied during development of some species of fish. The most extensively studied is the isozymic forms of LDH. Besides, the isozymic forms of some other enzymes such as creatine kinase, MDH, esterase, aldolase, amylase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase have been studied during development of different fishes (Whitt et al, 1973; Champion et al, 1976; Philipp et al, 1979; Whitt, 1981a,b).

The activities of different enzymes and isoenzymes are located in different cells of the developing embryo depending on their functional requirement. This has been demonstrated histochemically for some enzymes and isozymes in different cell types of the developing embryo of a few species of fish (Kusakina et al, 1976; Roubaud et al, 1976; Boulekbache et al, 1977; Boulekbache, 1981).

Effect of environmental factors :

The metabolic process in fish is readily altered by any modification in the conditions of the ambient environment. These alterations, which take place through the alterations in the enzyme activities, enabled the fish to restore its physiological stability for survival (Hochachka & Somero, 1973; Smith, 1976). The changes in enzyme activities under variable environmental conditions might occur in three ways, either by changing the concentrations of the enzymes or by modulating their activities or by modifying the inherent catalytic efficiency by producing a "better catalyst" (Somero, 1975).

The regulation of the concentration of the enzymes as measured by their activities is most common in biochemical adaptation of fish to environment (Hazel & Prosser, 1974). It is seen in many species of fish that different enzymes, representing various metabolic pathways, change their activity due to the alterations in the environmental conditions such as temperature (Smith, 1976; Prosser, 1973a; Hochachka & Somero, 1973; Precht, 1973), dissolved gases (Jorgensen & Mustafa, 1980; Ratha & Bhagowati, 1981), pH (Hochachka & Somero, 1973), salinity (Bashamohideen & Parvatheswararao, 1979) or hydrostatic pressure (Low & Somero, 1976).

All enzymes are not regulatory in nature. A number of enzymes, playing key roles in energy metabolism, such as SDH which showed changes in activity with acclimation temperature in bitterling. However, in the same fish LDH did not show any change (Kruger, 1962). Many such non-adaptive enzymes have been reported in fish. The limit of alteration of enzymes to environmental variation is also genetically determined and may vary between species (Wilson *et al.*, 1975).

The studies on the effect of environment on fish enzymes have been mostly carried out in adult fishes. Not much is known about the same during their embryonic life except some sporadic reports such as inhibition of chorionase at low pH (Peterson _et al_, 1980). Metabolism in developing fishes is more labile and sensitive to environmental alterations as observed by the sharp alterations in the rate of differentiation and survival of embryos under variations in environmental conditions. It is therefore, thought that the enzymes controlling various metabolic pathways in the developing embryo would also be subjected to alterations with changes in the environmental conditions.

The present chapter deals with the normal pattern of changes and the effect of high altitude on some key enzymes and metabolities of three metabolic pathways during the early development of scale carp and rohu. The pathways studied were ammonia metabolism, neurotransmission and tyrosine oxidation. All these pathways are known to show developmental and adaptational changes in different animals and therefore,selected as models for the present study.

Ammonia metabolism :

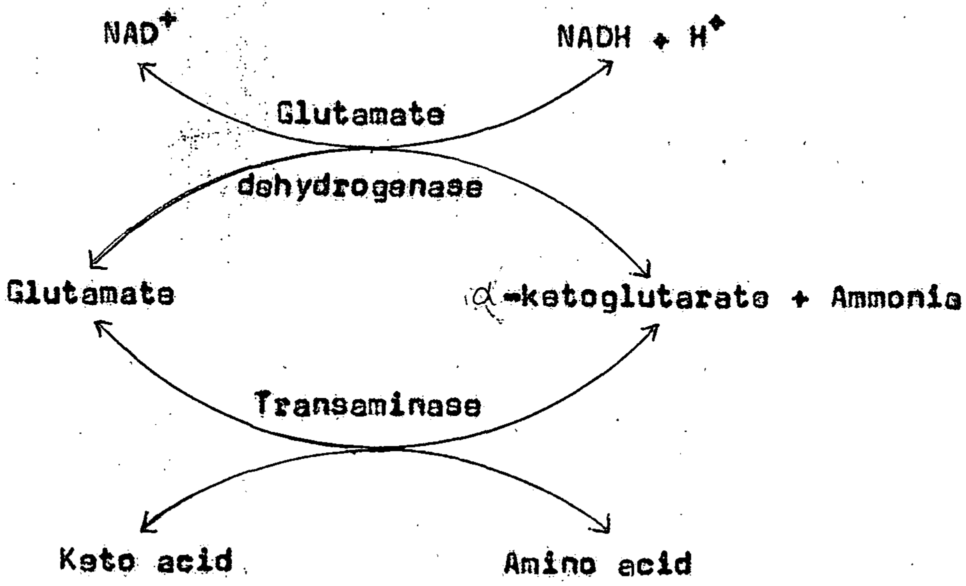
Ammonia is produced as a by-product of the catabolism of various nitrogenous biomolecules in the cell. Ammonia being highly toxic is either immediately excreted out of the body or gets converted to some less toxic products which are excreted out or used as a stored nitrogen (N) source. Three major N waste products excreted out of animals are ammonia, urea and uric acid. The nature of these waste products varies in different groups of animals depending mainly on the availability of water. Most of the freshwater fish are ammoniotelic, excreting ammonia as their major nitrogen waste product (Watts & Watts, 1974). In marine fishes, ammonia is converted to urea for osmoregulation. Excretion of N in the form of ammonia accounts for 55-80% of the total-N excreted in fish (Fromm, 1963). The major part of ammonia is produced in liver (Pequin & Serfaty, 1963; Vellas & Serfaty, 1974) and excreted primarily through the gills (Goldstein et al, 1964) in adult fish.

Ammonia has many advantages as an end product of nitrogen metabolism (Campbell, 1973). Some of the reactions involved in the production of ammonia, such as deamination of glutamate through GDH activity, ultimately produces energy (Bessman & Bessman, 1955). In addition, the small size of the molecule and its higher partition coefficient permits its easy elimination by diffusion (Forster & Goldstein, 1969). However, the disadvantage of ammonia, for which it cannot be stored in the body as such, is its high toxicity. This poses no problem to the fish which being aquatic readily dispose off the highly soluble ammonia into the surrounding water (Maetz, 1972).

Origin of ammonia :

In mammals, ammonia is produced by deamination of amino acids, amides, amines, purines, pyrimidines, nucleosides and nucleotides (Cohen & Brown, 1960). However, in fish, protein and amino acids are the major sources of excreted nitrogen (Walton & Cowey, 1977), although amides, nucleosides and nucleotides have also been identified as the precursors of ammonia in many species (Watts & Watts, 1974; Forster & Goldstein, 1969). Transdeamination and deamination are the main reactions producing ammonia in cells.

Transdeamination : The chief source of about 90% of the total N liberated is the α-amino-N of surplus amino acids (Baldwin, 1970). In some instances, as in case of serine and probably of histidine and cysteine, the amino group is directly deaminated to form ammonia (Watts & Watts, 1974). But in case of most of the amino acids, the amino group is transferred to another keto acid forming a new amino acid. In most animals, the dissociated amino group tends to be channeled, directly or indirectly, to the formation of glutamic acid, which undergoes an oxidative deamination catalyzed by the enzyme, glutamate dehydrogenase (GDH:EC 1.4.1.2), yielding ammonia. The overall reaction of liberation of ammonia from amino acids via glutamate formation is known as transdeamination (Braunstein, 1939) which may be summarized in the following reactions.



The reaction catalyzed by GDH is reversible and it functions in the reductive or oxidative direction depending upon the substrate being utilized (Chamalaun & Tager, 1970). Therefore, GDH has been considered to hold the regulatory link between energy production and nitrogen metabolism (Hochachka & Somero, 1973). The enzyme from mammalian sources has been shown to utilize NAD^+ or NADH equally well *in vitro*, although it has been claimed that the enzyme *in vivo* favours the glutamate (specific for NADH) formation (McGiven & Chappel, 1975). In some invertebrates also, GDH is shown to favour glutamate oxidation (Goldin & Frieden, 1971; Smith *et al*, 1975; Storey *et al*, 1978).

In fish, the activity of GDH has been detected in a variety of species (Forster & Goldstein, 1969; Watts & Watts, 1974; Walton & Coway, 1977; Arya, 1979) with liver showing the highest level of activity. It plays the important role for ammonia formation (Forster & Goldstein, 1969; Walton & Coway, 1977).

Deamination : Since the experiments of Van Slyke *et al* (1943), it is known that glutamine, an amide, acts for temporary storage and transport of ammonia. Glutamine is deaminated through hydrolytic removal of ammonia catalyzed by the enzyme glutaminase.

$$\text{Glutamine} \xrightarrow[\text{Glutaminase}]{+\ H_2O} \text{Glutamate} + NH_3$$

The activity of glutaminase exists in the form of two isoenzymes in various organs (Curthoys & Lowry, 1973; Kalra & Brosnan, 1973, 1974). One of these isoenzymes is phosphate dependent glutaminase (PDG or glutaminase-I) which requires phosphate for its activity. The other isoenzyme is phosphate independent glutaminase (PIG or glutaminase-II) which is activated by maleate. In most of the fish, so far studied for glutaminase activity, only PDG activity was detected. Walton and Cowey (1977) tried to assay PIG activity in different tissues of rainbow trout. They failed to detect the enzyme in any of the tissues examined (liver, kidney, gills & muscle).

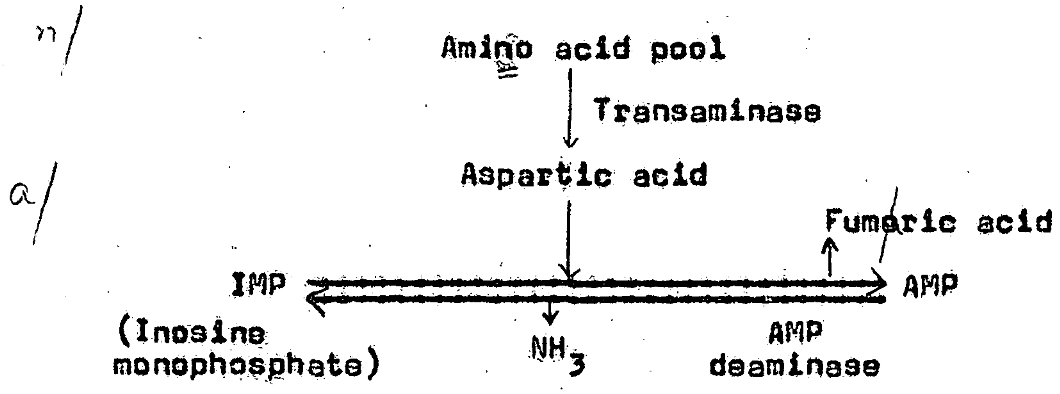
Direct deamination of aspartate, cysteine and histidine may also contribute to the production of ammonia. These deamination reactions are catalyzed by respective deaminases (Salvatore et al, 1965; Janicki & Lingis, 1970).

Nucleodeamination : Nucleodeaminases catalyze the deamination of nucleosides and nucleotides to liberate ammonia as follows (Cohen & Brown, 1960).

$$\text{Guanylic acid} \xrightarrow[\text{Guanylic acid deaminase}]{H_2O} \text{Xanthylic acid} + NH_3$$

$$\text{Adenosine} \xrightarrow[\text{Adenosine deaminase}]{} \text{Inosine} + NH_3$$

Hydrolysis of, particularly, adenosine monophosphate (AMP), may be ultimately utilized for deamination of amino acid as follows.



In some fishes, the role of AMP deaminase has been shown to be more important in ammonia production than glutaminase (Makarewicz & Zydowo, 1962; Makarewicz, 1963). However, in some fishes such as in rainbow trout, the activity of glutaminase is more than AMP deaminase in the major ammonia forming tissues (Walton & Cowey, 1977).

Detoxification of ammonia in fish tissues :

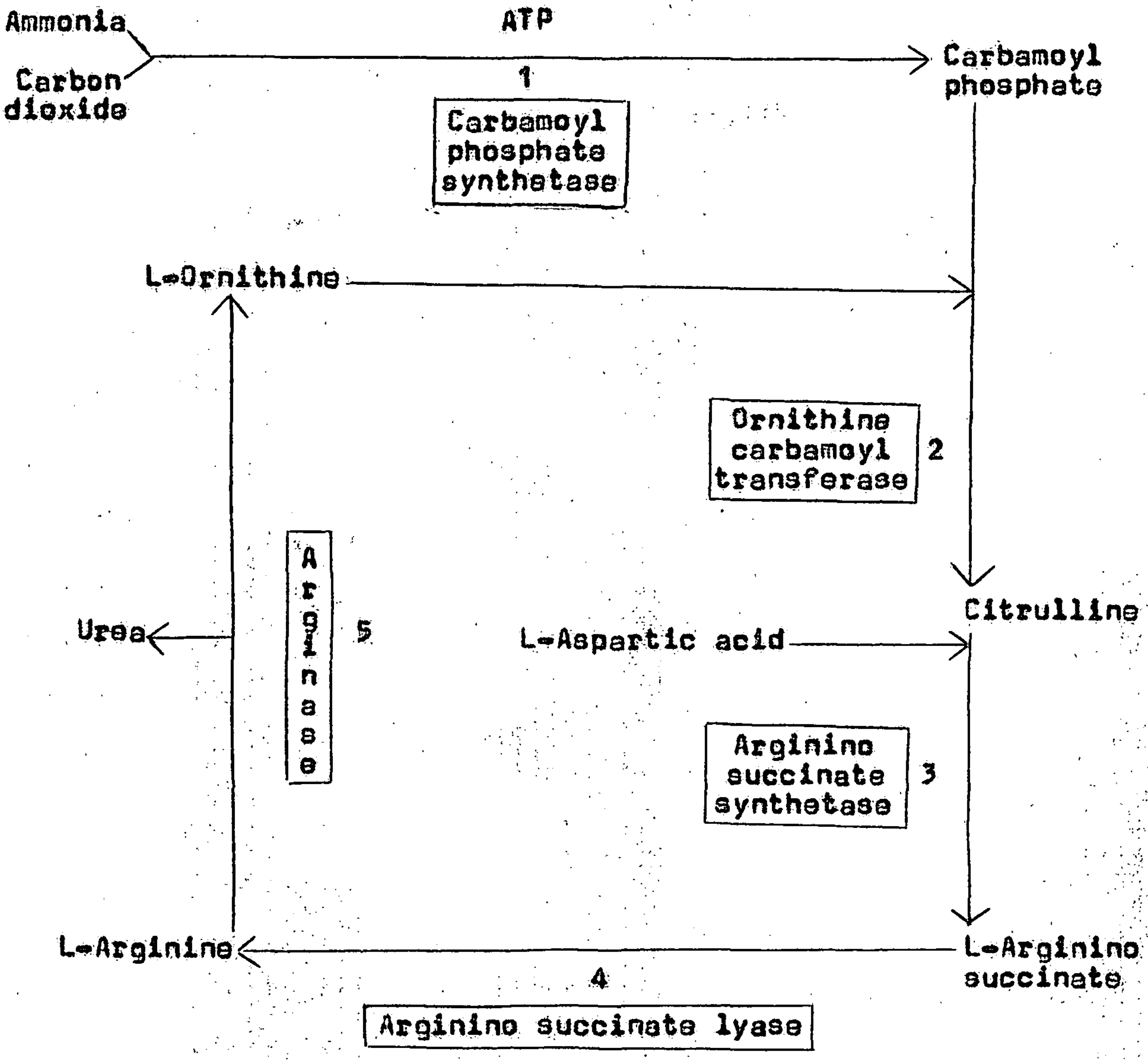
In fish, three major pathways have been known for detoxification of ammonia. They are glutamine synthesis, urea cycle, and uric acid cycle and uricolysis.

Glutamine synthesis : In this detoxification pathway, ammonia is converted to a non-toxic amide, glutamine, using the amino acid glutamate. The reaction is catalyzed by the enzyme glutamine synthetase (GS.EC.6.3.1.2).

$$\text{L-Glutamate} + \text{Ammonia} + \text{ATP} \xrightarrow[\text{GS}]{\text{Mg}^{2+}} \text{Glutamine} + \text{ADP} + \text{Pi}$$

The presence of glutamine synthetase has been detected in a wide variety of fish species (Forster & Goldstein, 1969; Watts & Watts, 1974; Webb & Brown Jr., 1976, 1980; Arya, 1979; Webb, 1980). The enzyme shows the highest activity in brain due to high sensitivity of neural tissue to ammonia toxicity. Glutamine is also used as a nitrogen source.

Urea cycle : In most of the terrestrial animals, ammonia is converted to a relatively less toxic form, urea, via ornithine-urea (O-U) cycle (Krebs & Henseleit, 1932). The O-U cycle involves five enzymatic steps, each step being catalyzed by a specific enzyme (Brown & Cohen, 1959a) and needs metabolic energy.



Amongst fish, the presence of a functional O-U cycle has been demonstrated in most of the marine fishes (Campbell, 1973). Although teleost fishes contain a significant amount of urea, which in some species may account for 20% or more of the total nitrogen excreted (Denis, 1913-14; Wood, 1958), they are reported to lack in the full complement of enzymes of the O-U cycle (Krebs & Henseleit, 1932; Brown & Cohen, 1960; Mayhall & Brown, 1967; Wilson, 1973). However, Huggins et al (1969) detected all the five enzymes of the O-U cycle in a number of freshwater teleostean fishes.

Although, the existence of a functional O-U cycle in teleosts has been controversial, the different tissues of these fishes are found to contain an appreciable activity of arginase (E.C.3.5.3.1) which is the terminal enzyme, catalyzing the conversion of arginine to urea and ornithine (Forster & Goldstein, 1969; Watts & Watts, 1974; Patnaik *et al*, 1976; Arya, 1979). In absence of a functional O-U cycle, the urea produced in these fishes might have come from the breakdown of dietary arginine by arginase.

Uric acid cycle and uricolysis : The uric acid pathway, in which ammonia is converted to purine via glutamine formation and subsequent release of uric acid, was first demonstrated by Brunel (1937). In teleost fishes and also in some amphibians, degradation of uric acid (uricolysis) has been proposed to be one of the sources for tissue urea (Brunel, 1937; Cvancara, 1969; Forster & Goldstein, 1969). Brunel (1937) proposed uricolysis as a three step enzymatic process.

$$\begin{tikzcd}
\text{Uric acid} \arrow[r, "\text{Uricase} + O_2"] & \text{Allantoin} \arrow[d, "H_2O"', "\text{Allantoinase}"] \\
& \text{Allantoic acid} \arrow[d, "2H_2O"', "\text{Allantoicase}"] \\
& \text{Urea}
\end{tikzcd}$$

He further suggested that in some teleosts allantoicase was lacking and hence, the end product of purine catabolism was allantoic acid instead of urea. This proposal was challenged by Goldstein & Forster (1965) who detected the activity of allantoicase in various groups of fishes by improved assay techniques.

The uricolytic pathway in teleosts has not yet received adequate attention and there is ample scope of exploring the role of uricolytic pathway in the production of urea in fishes.

Adaptive nature of ammonia metabolism :

Ammonia metabolism has been regarded as one of the most adaptive metabolic pathways during changes in environmental conditions (Gordon, 1970). In fish, the primary factors influencing the formation and excretion of various nitrogenous metabolities are availability of water, food and changes in ambient temperature.

The shift of ammoniotelism to ureo- or uricotelism, along with the transition of animals from water to land in course of evolution, is the best example of the adaptive nature of nitrogen metabolism to water availability (Brown & Cohen, 1959a,b). Further, short term deprivation of water as observed during the aestivation of lungfishes (Smith, 1930; Janssens, 1964; Janssens & Cohen, 1966, 1968; Goldstein *et al*, 1967) or transfer of some amphibious fishes from water to land (Morii *et al*, 1978), shifts the usual ammonotelic nature into ureotelism. Urea as well as glutamine are important osmoregulators in elasmobranchs, chondrichthyes and coelocanths (Goldstein *et al*, 1967; Webb, 1980; Webb & Brown Jr., 1980). The concentration of these organic metabolites decreased when these fishes are transferred to freshwater (Smith, 1931; Bittner & Lang, 1980).

The rate of nitrogen excretion in fishes has been shown to be correlated with the food intake (Jobling, 1981). Following feeding, rate of nitrogen excretion becomes high. However, direct measurements of ammonia (Guerin-Ancey, 1976b; Rychly & Marina, 1977) or urea (Guerin-Ancey, 1976b) in some fishes have shown that their excretion rate becomes higher during starvation, indicating a higher breakdown of amino acids to meet the energy demands. However, in catfish, *C. batrachus*, the level of urea has been shown to decrease during starvation (Tandon & Chandra, 1979). Changes in ammonia formation and excretion may be dependent on the amount and nature of the food intake. Cvancara (1969) reported that in teleosts the availability of arginine in food may contribute substantially to arginase activity and urea production.

The rates of ammonia and urea excretion in fish have been shown to be profoundly influenced by environmental temperature. Guerin-Ancey (1976a) reported a very high rate of ammonia and urea excretion is bass (*D. labrax*) at 24°C which decreased with the decrease in temperature. Similar direct correlation between ammonia and urea excretion and temperature, has been shown in many other fishes (Ray & Medda, 1976; Fauconneau & Luquet, 1979; Paulson, 1980). GDH activity in plasma of tench, *T. tinca*, increases with the increase in a thermal shock from 12-28°C (Perrier *et al*, 1980). In rainbow trout, GDH level showed a complex course of rise and fall with a rise in temperature from 3.5°C to 23°C (Perrier *et al*, 1980).

Enzymes of ammonia metabolism may also be modulated by sexual maturity of fish. Onishi *et al* (1974) reported GDH activity to increase in various species of salmonids during the maturation period. The hepatic arginase activity in them is shown to increase in male and decrease in female with maturation (Onishi & Murayama, 1969).

Developmental studies on ammonia metabolism in fish :

The ontogenic stages of elasmobranchs and the viviparous teleosts are ureotelic as their adults (Read, 1968; Depeche & Schoffeniels, 1975). The activity of arginase along with other enzymes of the O-U cycle are detected even in the early stages of development in elasmobranchs which slowly increased with development (Read, 1968). A very high activity of the O-U cycle enzymes have been found in embryos of the viviparous teleost, P. reticulata, which decreased with the progress of development (Depeche & Chiapello, 1977).

Ammonia metabolism during the development of oviparous freshwater teleosts may be considered as one of the most fascinating area for research in biochemical evolution, in view of the 'gene delation' theory put forward by Cohen and Brown (1960, 1963) and Cohen (1966). According to them, the ancestors of the teleosts possessed the functional O-U cycle and the structural genes for them might have been deleted in course of evolution. It has been suggested that in such a case of gene deletion, the developmental stages of the present day teleosts might possess traces of the urea-cycle (Huggins et al, 1969). However, there is little work done to assess such a possibility. Forster and Goldstein (1969) assumed that because the eggs of teleosts develop in water, the embryos should be able to dispose of ammonia by diffusion. However, Smith (1947, 1957) reported that the end products of nitrogen metabolism, ammonia, was unable to escape through the chorion. Rice and Stokes (1973) measured the ammonia, urea and uric acid concentrations in egg and alevins of rainbow trout and showed that all these components were permeable through the egg membrane. Ammonia and urea, besides being excreted out, were found to accumulate in the embryo but uric acid showed little accumulation. They further observed that out of the five enzymes of the O-U cycle, only two namely, ornithine transcarbamylase and arginase were present in the egg and embryos. Therefore, they presumed that the production of urea in the developmental stages might have taken place from the degradation of arginine present in the yolk. In a recent report in the same fish, using radioisotopic methods, Dépeche *et al* (1979) observed that at least in some developmental stages urea was produced via the O-U pathway.

Neurotransmission :

Acetylcholine (ACh) and acetylcholinesterase (AChE) : The impulse from one neuron to another neuron or from the neuron to the effector organ is conveyed by the release of chemical transmitters or mediators (Eccles, 1964). Acetylcholine (ACh) is one such important transmitter, which was first identified by Dale (1914). It is present in the cholinergic synapses in neromuscular junctions (Prosser, 1973b; Rubin *et al*, 1979) and nerve free endplates (Weinberg & Hall, 1979). The synthesis of ACh takes place from Acetyl-CoA and choline by the enzyme choline acetyltransferase (McIlwain & Bachelard, 1971). ACh may be removed by simple diffusion across the membrane but the most effective removal is achieved through the inactivation of ACh by hydrolysis into choline and acetate (Keele & Neil, 1972). The hydrolysis of ACh is catalyzed by the enzyme acetylcholinesterase (AChE, E.C: 3.1.1.7). This enzyme has been isolated from different tissues in a wide variety of species ranging from insects to humans (Rosenberry, 1925). In fish, it has a wide tissue distribution and show a higher activity in comparison to that of mammalian species (Close & Serfaty, 1957). Besides their important role in nerotransmission, ACh and AChE are a part of the basic excitation unit of regulating ion flow across the cell membrane (Kamemoto, 1961).

Adaptive nature of ACh and AChE : The nervous system is the coordinator of the different physiological processes in an animal. Therefore, in any type of environmental stress nervous system is the initial target to be affected. The level of ACh in brain has been shown to vary with different neural stimulation and inhibiting conditions in mammals (Saito, 1971). The level of AChE activity is correlated with the general state of activity of an animal (Vijayalakshmi *et al*, 1979). The enzyme is influenced by different physiological and physical factors like aestivation (Murali Mohan *et al*, 1977), photoperiod, light intensity and motor activity (Wood & Rose, 1979), hydrostatic pressure (Miller *et al*, 1974), temperature and pH (Ngo & Laidler, 1978), X-radiation (Valcana *et al*, 1974) and habitat (Ramanujam & Ratha, 1981). Different toxic substances also inhibit the AChE activity (Corbett, 1974; Olson & Christensen, 1980; Ramanujam, 1981). The enzyme exhibits compensatory changes in fish to adaptation temperature. Hause (1975) observed that in *R. amarus*, the activity of AChE is 60% higher at 10°C than compared with at 29°C. In gold-fish (*C. auratus*) brain, three forms of AChE exist of which the 12S form decreases with rising temperature (Guillon & Massoulie, 1976). Baldwin and Hochachka (1970) and Baldwin (1971) have examined the E-S affinity of AChE from the central nervous system of various species of fish. In all cases examined, the Km for ACh remained relatively constant at the optimal biological temperature range for each species. As the temperature dropped below this range, the Km increased.

Developmental studies in ACh and AChE in fish : The ontogeny of AChE in *S. gairdneri* has been reported by Uesugi and Yamazoe (1964). AChE activity was detected from the 10th day after fertilization reaching the peak in the eye stage which coincided with the development of excitable tissues. In the Salamander, *Ambystoma punctatum*, AChE activity could be detected in the mature~~d~~ oocytes which began to rise when the spinal cord and brain started developing. The activity doubled when the embryonic movement began and it progressively increased with development. The ontogeny of AChE in *Xenopus* is similar with the salamander (Gindi & Knowland, 1979). In chick, AChE in the optic tectum and forebrain hemispheres showed a significant increase apparently around the time of hatching (Marchand *et al*, 1977).

Tyrosine oxidation :

Tyrosine aminotransferase (TAT: E.C. 2.6.1.5) : Tyrosine is an aromatic amino acid with a wide variety of physiological functions. Besides being a precursor for protein synthesis, it is metabolized to produce thyroxine, dopamine, noradrenaline, adrenaline and melanin (West *et al*, 1974). Tyrosine is also oxidised to fumarate and acetoacetate involving a number of biochemical steps. First tyrosine is transaminated with ketoglutarate to yield p-hydroxyphenylpyruvate (pHPP) and glutamate. This is the rate limiting step of oxidation of tyrosine and is catalyzed by the enzyme tyrosine aminotransferase (TAT) (Knox & Le May-Knox, 1951; Lin & Knox, 1957). TAT plays an important role in gluconeogenesis (Feigelson & Feigelson, 1966). In mammals and fishes, the enzyme is primarily located in liver (Miller & Litwack, 1969a; Ratha & Bhagowati, 1981). In mammals, it is located in cytoplasmic, mitochondrial and nuclear fractions (Litwack et al, 1963; Miller & Litwack, 1969a,b; Ratha & Kanungo, 1977). These different sub-cellular TAT vary in some of their physico-chemical properties (Miller & Litwack, 1971) and regulations (Ratha & Kanungo, 1974), for which they are considered as different isoenzymes. The molecular properties of TAT has been considerably explored in mammals (Rosenberg & Litwack, 1970; Iwasaki et al, 1973) and in frogs (Ohisalo & Pispa, 1976; Ohisalo et al, 1977).

Adaptive nature of TAT : TAT is one of the most adaptive enzymes in mammals. In rat liver, TAT rapidly adjusts its activity through the pituitary-adrenal pathway in response to different physical and chemical stresses (Gelehrter, 1971; Ratha, 1975; Thompson, 1979). The enzyme has a definite circadian rhythm in rat which is dependent on the feeding cycle, dietary protein and photoperiod (Watanabe et al, 1968; Fuller & Snoddy, 1968; Zigmond et al, 1969). It has been shown that during any stress the level of the gluco-corticoid hormones enhance causing increase in TAT activity (Rosen & Milholland, 1971). The induction of TAT by glucocorticoids has been used as a model system for studying the gene regulation in eukaryotic cells (Gopalakrishnan & Thompson, 1977).

Studies on TAT in lower vertebrates are very limited. Whiting and Wiggs (1977) reported that prolonged starvation (40-days) induced hepatic TAT in brook trout (S. fontinalis). They have further shown a circadian rhythm of the enzyme, the peak activity being influenced by food intake analogus to that in rat. Ramanujam et al (1981) also observed a definite circadian rhythm of hepatic TAT in the catfish, H. fossilis, and noted that the pattern could be modulated by altering light conditions. Reports on glucocorticoid mediated inducation of TAT in fish and other lower vertebrates is controversial. Chan and Cohen (1964) suggested that glucocorticoid mediated inducation of TAT did not occur below the phylogenic level of reptiles. However, some reports have shown the induction of TAT by hydrocortisone in some fish (Whiting & Wiggs, 1976; Davis et al, 1980). In a report from this laboratory, we have shown the inhibition of hepatic TAT during hypoxic stress in C. carpio (Ratha & Bhagowati, 1981). Inhibition of TAT is not observed during any kind of stress in mammals. Hence, we have suggested that the regulation of TAT in fish might be different from that in mammals.

Developmental aspects of TAT : There is no information available on the ontogeny of TAT in fish. In mammalian liver, cytoplasmic TAT (c-TAT) activity was almost undetectable till the time of birth at which it induced significantly (Litwack & Nemeth, 1965). The mitochondrial TAT (m-TAT) activity was detectable in the foetal liver and was induced at about 2 days after birth (Koler et al, 1969; Holt & Oliver, 1970). In Xenopus laevis, TAT activity could not be detected until the age of about 1 day and it appeared after completion of neurulation reaching the peak at the age of 2-5 days (Ohisalo & Pispa, 1975). There has been no study on the activity of TAT during the ontogenic development of fish.

Plan of work :

In view of the fact that very little information is available on the metabolic changes in general and enzymatic studies in particular, during the development of freshwater fishes, the present work was planned to study the activities of some of the key enzymes and concentrations of some metabolites of three model metabolic pathways mentioned above and also to see the effect of high altitude on these parameters during the ontogenic development of scale carp (Cyprinus carpio) and an Indian major carp, rohu (Labeo rohita). The following estimations were done in the embryos starting from unfertilized egg till hatching in different developmental stages as mentioned in Chapter-I, both at lower and higher altitude.

1) The endogenous levels of ammonia and urea, and the activity levels of glutamate dehydrogenase (GDH), phosphate dependent glutaminase (PDG), glutamine synthetase (GS) and arginase from ammonia metabolic pathway were estimated.

2) The concentrations of acetylcholine (ACh) and acetylcholinesterase (AChE) activity level were determined.

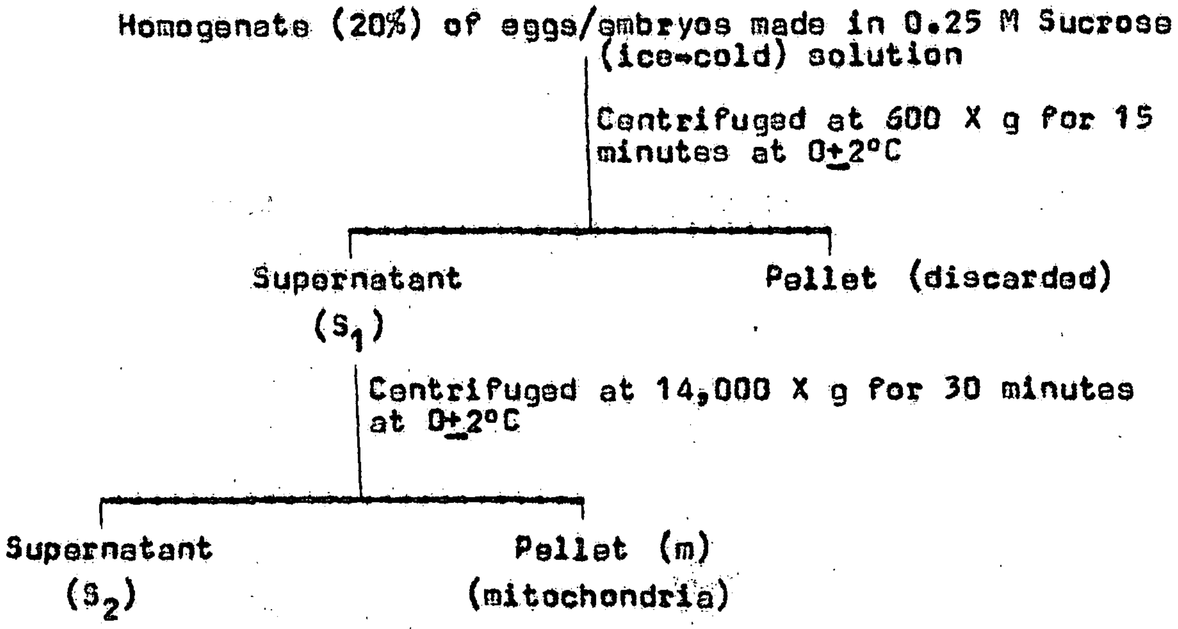
3) The activities of cytoplasmic (c-) and mitochondrial (m-) tyrosine aminotransferase (TAT) in different developmental stages were assayed.

MATERIALS AND METHODS

The developmental stages of scale carp (*Cyprinus carpio*) and rohu (*Labeo rohita*) used in the present investigation were the same as described in Chapter II. In all of them, the following estimations were done as described below.

Preparation of extracts :

Deep frozen eggs and embryos of each developmental stage were thawed on ice and the adhering water was slowly blotted with a filter paper. About 100 embryos of each stage were weighed in a single pan microbalance (K. Roy Model-K.16) and a 20% homogenate was prepared in ice-cold 0.25 M Sucrose using a pre-cooled all glass hand operated Potter Elvehjem type homogeniser in ice. The homogenate was then centrifuged (Remi Model K-24) at 600 X g at 0±2°C for 15 minutes to sediment nuclei. A part of this supernatant (S_1) was kept in ice for the estimation of ammonia and urea concentrations, and for assay of glutamate dehydrogenase (GDH), phosphate dependent glutaminase (PDG) and glutamine synthetase (GS) activities. The rest of the supernatant was centrifuged at 14,000 X g for 30 minutes at 0±2°C to sediment mitochondria. This supernatant (S_2) was kept in ice for assaying arginase, acetylcholinesterase (AChE), cytoplasmic tyrosine aminotransferase (c-TAT) activities and acetylcholine (ACh) concentration. The mitochondrial pellet (m) was suspended in 0.25 M Sucrose solution to make a 20% suspension using the homogeniser and was used for the assay of mitochondrial tyrosine aminotransferase (m-TAT) activity.



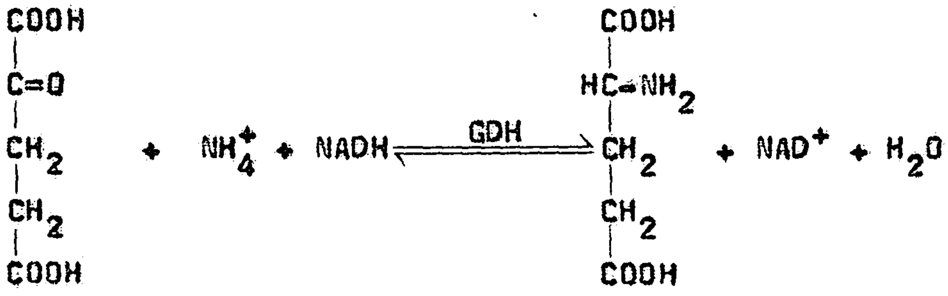
<u>Estimations :</u>

<u>Estimation of ammonia</u> : Ammonia was estimated following the method of Chaney and Marbach (1962) with 15 minutes incubation time. A portion of S_1 fraction was treated with 10% $ZnSO_4$ and 0.6 N $Ba(OH)_2$ in the proportion of 4:1:1 by volume and was centrifuged at 7,000 X g at 0±2°C for 15 minutes. This supernatant (S_3) was used for the estimation of ammonia and urea.

0.2 ml of the supernatant (S_3) was diluted to 1.0 ml with double distilled water. It was treated with 1.0 ml of alkaline-hypochlorite solution (320 ml of 1N NaOH and 21 ml of 5% Sodium hypochlorite diluted to 1 litre with double distilled water) followed by 1.0 ml phenate pentacyanitrosyloferrate solution (62 g phenol and 0.25 g of sodium nitroprusside diluted to 1 litre with double distilled water). The contents were mixed thoroughly and incubated at 40°C for 15 minutes. The optical density was then measured at 640 nm using a Spectrophotometer (Beckman Model-26). The concentration of ammonia was calculated using a standard curve prepared with ammonium chloride (NH_4Cl) and was linear between the concentrations of 0.2 to 2.0 µg.

Estimation of urea : The method of Archibald (1945) was employed to determine the urea level in the supernatant (S_3). To 1.0 ml of undiluted supernatant (S_3), 3.0 ml acid-mixture (90 ml H_2SO_4 and 270 ml H_3PO_4 diluted to 1 litre with double distilled water) and 0.1 ml of diacetyl monoxime reagent (3% diacetyl monoxime prepared in absolute alcohol) were added. The contents were thoroughly mixed, the tubes were covered with marbles and placed in a boiling water bath. After exactly boiling for 30 minutes the tubes were cooled in dark for 10 minutes and the optical density was measured at 540 nm in a Spectrophotometer (Beckman Model-26). The concentrations of urea were calculated from a standard graph prepared using different concentrations of urea within the range of 3.0 to 30.0 μg which was linear.

Assay of glutamate dehydrogenase (GDH) activity : GDH activity was assayed in the S_1 fraction following Olson and Anfinsen (1952). The assay was done spectrophotometrically by measuring the decrease in optical density at 340 nm due to the oxidation of NADH in the direction of glutamate formation in the following reaction.



$$\begin{array}{c} COOH \\ | \\ C{=}O \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array} + NH_4^+ + NADH \xrightleftharpoons{GDH} \begin{array}{c} COOH \\ | \\ HC{-}NH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array} + NAD^+ + H_2O$$

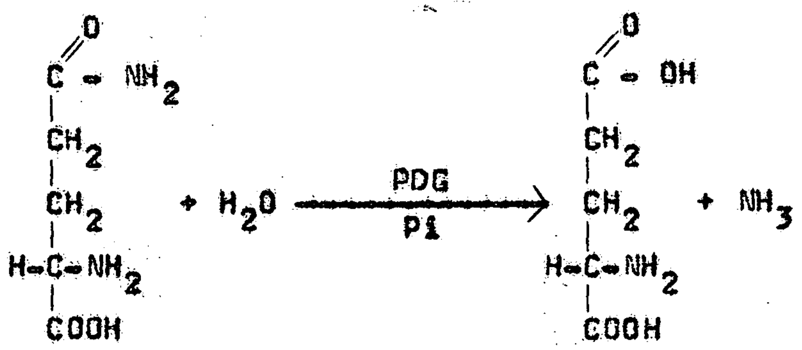
α - Ketoglutarate L - Glutamate

The assay mixture (pH 7.6) in a final volume of 3.0 ml contained:

Potassium phosphate buffer	370.0	µmoles
Ammonium chloride	450.0	µmoles
Sodium α-ketoglutarate	0.282	µmoles
NADH	33.4	µmoles
Enzyme source (S_1)	0.2	ml

The solution was added directly into the cuvette, mixed quickly and the decrease in optical density at 340 nm was recorded in a Spectrophotometer (Beckman Model-26) at 30 second intervals for 3 minutes or till the rate of change was linear. The activity of the enzyme was calculated considering the decrease in optical density of 6.2 equivalent to 1.0 µmole of NADH oxidised (Olson & Anfinsen, 1952). One unit of the enzyme was defined as that amount which catalyzed the oxidation of 1.0 µmole of NADH per hour under the assay conditions described.

Assay of phosphate dependent glutaminase (PDG) activity : The assay of phosphate dependant glutaminase (PDG) activity was done in S_1 fraction following the fixed time assay method described by Makarewicz and Żydowo (1962), but the final ammonia estimation was done following Chaney and Marbach (1962). To a portion of S_1 fraction an equal volume of KCl-Borate solution (0.1 N KCl containing 0.039 M Borate at pH 7.7) was added. The contents were mixed in chilled homogeniser and used as enzyme source for PDG.



L-Glutamine L-Glutamate ammonia

The final reaction mixture of 2.0 ml (pH 7.7) contained:

Sodium phosphate buffer	100.0 µmoles
Tris-HCl buffer	50.0 µmoles
Glutamine	20.0 µmoles
NaCl	30.0 µmoles
Enzyme source	0.2 ml

The reaction was carried out in stoppered glass centrifuge tubes. The reaction mixture was preincubated at 30°C for 10 minutes before adding the enzyme. The reaction was initiated by addition of the enzyme source and incubated at 30°C in a water bath for 15 minutes. 1 ml of 15% TCA was added to stop the reaction and the precipitated protein was removed by centrifugation at 2,000 X g for 10 minutes. The amount of ammonia formed during the reaction was estimated in 1.0 ml of the supernatant as described earlier. One unit of the enzyme was defined as that amount which catalyzed the formation of 1.0 µmole of ammonia per hour under the assay conditions described.

Assay of glutamine synthetase (GS) activity : A portion of S_1 was treated with 1% triton X-100 (1:1) and centrifuged at 14,000 X g for 30 minutes. This treatment was optimum to get maximum GS activity. The clear supernatant was used as the enzyme source for the assay of glutamine synthetase (GS).

Glutamine synthetase (GS) has been shown to catalyze the following three reactions (Webb & Brown Jr., 1976).

1. Glutamate + ammonia + ATP $\xrightarrow[GS]{Me^{++}}$ Glutamine + ADP + Pi

2. Glutamate + NH_2OH + ATP $\xrightarrow[GS]{Mg^{++} \text{ or } Mn^{+++}}$ γ-glutamylhydroxamate (GHA) + ADP + Pi

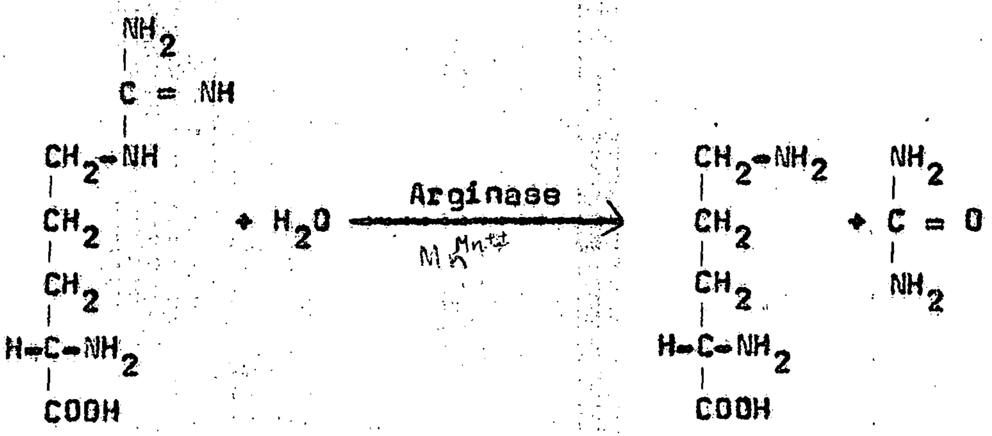
3. Glutamine + NH_2OH + ADP $\xrightarrow[GS]{Mn^{++} \quad AsO_4^{3-}}$ γ-glutamylhydroxamate (GHA) + ADP + ammonia

The assay of GS was done following the No.2 reaction mentioned above using a fixed time assay procedure by Wilson and Foulkes (1976). The reaction mixture of 1.0 ml (pH 7.2) contained :

Imidazole-HCl buffer	50.0 µmoles
$MgCl_2$	20.0 µmoles
2-mercaptoethanol	25.0 µmoles
Sodium L-glutamate	50.0 µmoles
Hydroxylamine hydrochloride	100.0 µmoles
Sodium ATP	10.0 µmoles
Enzyme source	0.1 ml

The reaction mixture without the enzyme was preincubated at 30°C for 10 minutes in 15 ml glass centrifuge tubes. The reaction was started by the addition of the enzyme source and was incubated at 30°C for 15 minutes. The reaction was stopped and the colour was developed by addition of 1.5 ml of Ferric Chloride reagent (0.37 M $FeCl_3$, 0.67 N HCl and 0.2 M TCA) to each tube with shaking. The precipitated protein was removed by centrifugation at 2,000 X g for 10 minutes. The optical density was read at 500 nm against a reagent blank in a Spectrophotometer (Beckman Model-26). One unit of the enzyme was defined as that amount which catalyzed the formation of 1.0 µmole of GHA per hour. The amount of GHA was calculated from a standard graph prepared using different concentrations of γ-glutamylhydroxamate (0.5-5.0 µmoles) which was linear.

Assay of arginase activity : Arginase was assayed following the fixed time assay of Schimke (1970) with incubation at 30°C. The urea produced by the catalysis of arginine by arginase was estimated colorimetrically (Archibald, 1945) as described earlier.



L - Arginine L - Ornithine Urea

The final reaction mixture of 2.0 ml (pH 7.6) contained :

L-arginine hydrochloride	250.0 µmole
$MnCl_2$	1.0 µmole
Enzyme source (S_2)	1.0 ml

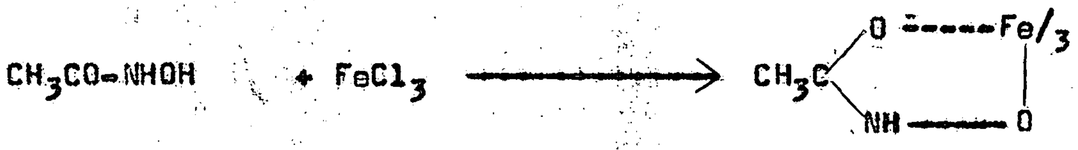
The substrate L-arginine hydrochloride and $MnCl_2$ were prepared in Tris-glycine buffer (0.125 M, pH 7.6). L-arginine and the supernatant with $MnCl_2$ were preincubated in two separate glass centrifuge tubes at 30°C in a water bath. After 10 minutes, the contents in the two tubes were mixed together and incubated for 15 minutes at 30°C. The reaction was stopped by adding 2.5 ml of $HClO_4$

(0.5 M) and the precipitated protein was removed by centrifugation at 2,000 X g for 10 minutes. The urea which was formed during the reaction was determined in 1.0 ml of the supernatant. The blank was prepared taking same amount of boiled S_2 instead of enzyme source. One unit of the enzyme was defined as that amount which catalyzed the formation of 1.0 µmole urea per hour.

Estimation of acetylcholine (ACh) : The concentration of acetylcholine (ACh) in S_2 was estimated following Augustinsson (1957). The method is based upon the reaction of ACh with hydroxylamine to form acethydroxamic acid. Acethydroxamic acid forms a soluble red-purple complex with ferric ions in acid solution. The colour intensity of which is proportional to the concentration of ACh present. This complex absorbs light maximally at 540 nm.

$$(CH_3)_3N^+{-}CH_2CH_2O{-}COCH_3 + NH_2OH \longrightarrow \underset{\text{(Choline}}{(CH_3)_3N^+{-}CH_2CH_2OH} + CH_3CO{-}NHOH$$

Acetylcholine Hydroxylamine Acethydroxamic acid



Acethydroxamic acid red-purple complex

2.0 ml of alkaline hydroxylamine reagent (2 M hydroxylamine-HCl + 3.5 N NaOH; mixed in equal volume just before use) was added to 1.0 ml of the supernatant (S_2). After 1 minute, 1.0 ml of 4 N HCl and 1.0 ml of $FeCl_3$ (0.37 M in 0.1 N HCl) were added to the above and the mixture was centrifuged for 5 minutes at 2,000 X g. The optical density of the supernatant was read at 540 nm in a

Spectrophotometer (Beckman Model-26). ACh concentration was calculated using a standard graph made with different concentrations (0.1-1.0 mmoles) of acetylcholine iodide which was linear.

Assay of acetylcholinesterase (AChE) activity : AChE was assayed following the spectrophotometric method of Ellman et al (1961) using acetylthiocholine iodide as the substrate. The enzyme activity was measured at 412 nm in a digital Spectrophotometer (Beckman Model-26) by following the rate of increase in optical density due to the yellow colour complex formed by the reaction of thiocholine, produced by the enzyme activity, with di-thio-bis-nitrobenzoate (DTNB).

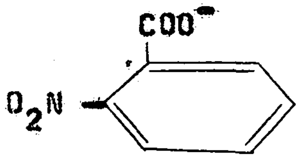
$$(CH_3)_3N^+CH_2CH_2SCOCH_3 + H_2O \xrightarrow{AChE} (CH_3)_3N^+CH_2CH_2S^-$$

Acetylthiocholine Thiocholine

$$+ CH_3COO^- + 2H^+$$

$$(CH_3)_3N^+CH_2CH_2S^- + RSSR \longrightarrow (CH_3)_3N^+CH_2CH_2SSR + RS$$

Thiocholine (DTNB) 5-thio-2-nitrobenzoic acid

R = 

The final reaction mixture of 3.12 ml (pH 8.0) contained :

Phosphate buffer	83.33 µmoles
Acetylthiocholine iodide	0.32 µmoles
DTNB	0.32 µmoles
Enzyme source (S_2)	0.1 ml

The blank contained all other reagents except the enzyme which was replaced by buffer. The enzyme was added to start the reaction and the increase in optical density was recorded at 30 second intervals for 3 minutes or till the rate of change was linear. The linear rate of increase in optical density, usually for the first 2 minutes, was used for calculating the enzyme activity. The calculation of activity was done following Ellman *et al.* (1961) using the following formula.

$$R = \frac{A}{1.36 \times 10^{4}} \times \frac{1}{(400/3120)\ CO}$$

$$R = 5.74 \times 10^{-4} \times \frac{AC}{CO}$$

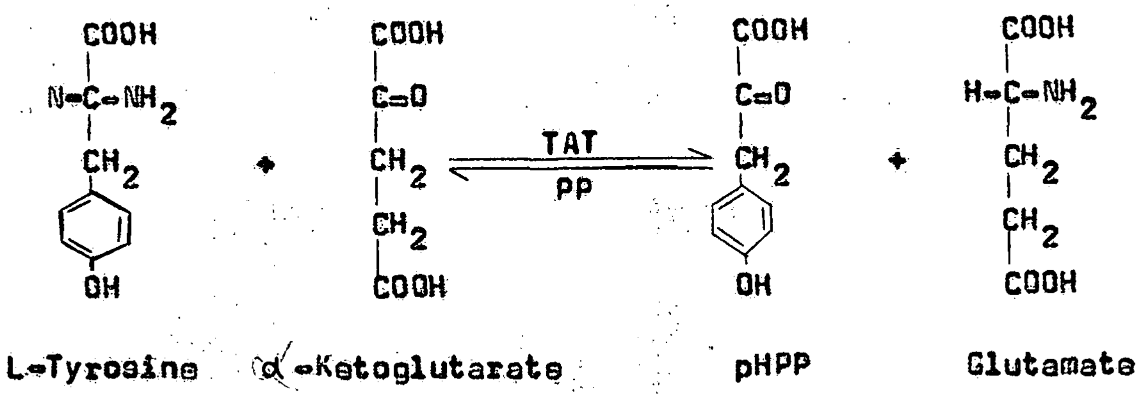
where R = moles of substrate hydrolyzed/min/g,

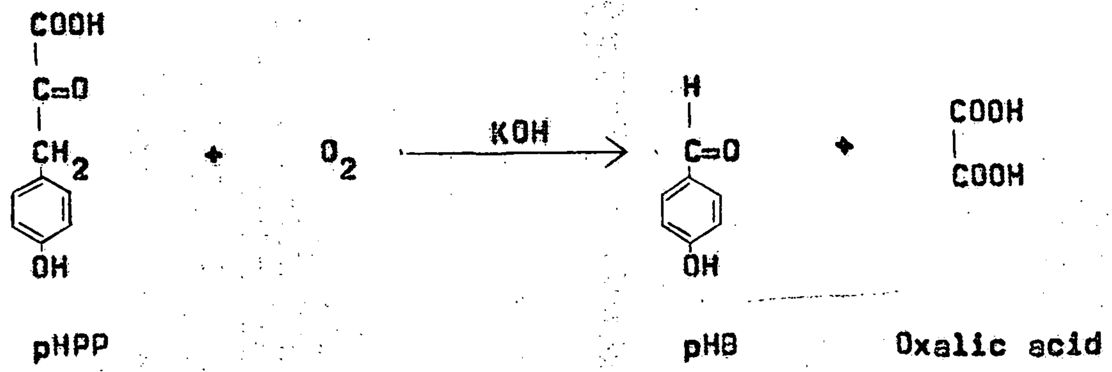
A = change in absorbance/min,

CO = original concentration of egg~~s~~/embryo homogenate (mg/ml).

One unit of enzyme activity was defined as µmoles of substrate hydrolyzed per hour.

Assay of cytoplasmic (c-) and mitochondrial (m-) tyrosine aminotransferase (TAT) activity : TAT activity in cytoplasmic (S_2) and mitochondrial (m) fractions were assayed following the methods of Ratha and Kanungo (1977). However, the incubation temperature used here was 30°C instead of 37°C used by them. This is a fixed time assay which depends on the oxidation of p-hydroxyphenylpyruvate (pHPP), one of the products of TAT catalyzed reaction, by molecular oxygen to p-hydroxybenzaldehyde (pHB) and oxalate. pHB absorbs light maximally at 331 nm in alkaline medium.





The final reaction mixture of 3.0 ml (pH 7.6) contained :

Triethanolamine buffer	240.0 µmoles
L-tyrosine	27.0 µmoles
α-ketoglutarate	27.0 µmoles
pyridoxal phosphate	0.25 µmoles
EDTA	3.0 µmoles
DTTA	3.0 µmoles
Enzyme source (S_2 or mitochondrial suspension)	0.2 ml

The enzyme was added to the reaction mixture after 10 minutes of pre-incubation to start the reaction at 30°C in a water bath. After 10 minutes of incubation, the reaction was stopped by adding 0.4 ml of 10.0 N KOH with immediate and vigorous mixing. The optical density was read at 331 nm against a reagent blank in a Spectrophotometer (Beckman Model-26) after 30 minutes of the addition of KOH. A standard curve was prepared by taking different concentrations of pHPP (0.05-0.5 µmoles) under the same conditions, as that for the assay of the enzyme, and was linear. One unit of the enzyme was taken as equal to that amount which catalyzed the formation of 1.0 µmole of pHPP per hour at 30°C.

Expression of data :

Data for all estimations and enzyme assays were obtained from 3-5 sets of experiments. The sources of chemicals and biochemicals, and the statistical analysis were same as described in Chapter II.

Ammonia and urea concentration have been expressed as µmoles per embryo. Acetylcholine concentration has been expressed as mmoles per embryo. The enzyme activities have been presented as total activity (units/embryo) and specific activity (units/mg protein).

RESULTS

Ammonia :

The alterations in ammonia concentrations during the ontogenic development of scale carp and rohu at lower and higher altitude have been presented in Table-23 and Fig. 14. Ammonia concentration in the egg of scale carp was about two times higher than that of rohu at both the altitudes. In all cases, there was a sudden increase in ammonia level after fertilization and the highest amount of ammonia per embryo was observed in pre-hatching (15a) stage. On hatching the ammonia concentration decreased significantly in all cases. Ammonia concentration was found to be much higher in scale carp than rohu.

In scale carp, the ammonia concentration per embryo went on increasing till the stage-3, at both the altitudes. At stage-6, the level decreased in both the places, but significantly only at Gauhati. The level again went up till stage-10 and remained more or less stable till stage-12 of development. A further increase in ammonia level took place from stage-13 till the peak level at pre-hatching (15a) stage. At higher altitude, the ammonia level increased from stage-7 to stage-12 which was maintained till stage-15a. It was significantly higher (65.36%) only at stage-6 and significantly lower (17.8%) only at stage-15a embryos of scale carp at the higher altitude.

In rohu, there was a significant decrease in ammonia level at both the altitudes from fertilized egg (stage-1) to stage-2. This decrease continued gradually to its lowest level at Gauhati at stage-5 and at Maupun at stage-4. Then the level decreased gradually till the highest level at the pre-hatching (15a) stage except a significant sudden fall at neurulation (stage-11) at Gauhati. At higher altitude the embryonic ammonia level in rohu was higher than those at lower altitude which was significant at stage-5 and from stage-11 till 15a.

Urea :

The urea concentrations of the embryos of scale carp and rohu observed at lower and higher altitudes have been presented in Table-24 and Fig. 14. There was not much variation in the urea concentration of the unfertilized egg of scale carp and rohu at both higher and lower altitudes. In the later stages of development, urea level increased from the unfertilized egg to its peak in the pre-hatching embryos by 4.0 and 3.5 times in scale carp and 2.5 and 1.5 times in rohu embryos in lower and higher altitude respectively. There was no significant altitudinal difference in the urea level of the embryos of the two species.

On fertilization the urea level increased in scale carp which was maintained at an insignificantly variable level till stage-8 at both the altitudes. There was an increase from stage-8 to stage-9 which was significant only at higher altitude. This increase continued slowly till its highest level in the pre-hatching (15a) stage.

In rohu, there was no increase in the urea level after fertilization till the stage-8 of development. A significant increase was observed from stage-8 to 9. There was a slow increase in the urea concentration after gastrulation in the developing embryos of rohu at both the altitudes to reach the peak at the pre-hatching (15a) stage.

In all cases there was decrease in urea level per embryo after hatching.

Glutamate dehydrogenase (GDH) activity :

The total activity (units/embryo) and specific activity (units/mg protein) of GDH in the embryonic stages of scale carp and rohu at lower and higher altitude have been presented in Tables-25 & 26 and Figs. 15 & 16. GDH activity could not be detected in the unfertilized egg and it appeared only after fertilization. The pattern of change in both total and specific activity of GDH were similar at both the altitudes. The total activity was lower in rohu and higher in scale carp whereas the specific activity was lower in scale carp and higher in rohu.

In scale carp, the activities did not alter significantly from fertilization till the stage-7. During blastulation (stage-8), there appeared a significant decrease in both the activities. They started increasing again from stage-9 onwards to reach the peak in the stage-15b. At Gauhati, the increase was continuous with the progress of development, resulting in 5 and 9 fold increase in total and specific activity respectively at stage-15b in comparison to stage-8. However, at higher altitude (Shillong) the fluctuations were more and the increase was in two steps- once from stage-8 till stage-11 and again from stage-14 till stage-15b. During the last 3 stages (stages-14, 15a & 15b) only, GDH activities were significantly lower at higher altitude than the lower altitude embryos.

Rohu depicted a different pattern of GDH activities than scale carp. The initial level detected after fertilization was maintained till stage-5 and stage-7 at lower and higher altitude respectively. After this, the pattern of changes in the total and specific activities showed variations. The total activity increased upto stage-9 in both the altitudes after which the level was maintained in the embryos at higher altitude only. At lower altitude the total activity started increasing after stage-12 till reaching its peak in stage-15b. The specific activity of GDH increased continuously from stage-7 till its peak in stage-15b in rohu embryos at higher altitude. However, at lower altitude the specific activity went up from stage-5 till stage-9. This was decreased significantly by stage-11, followed by a gradual increase to reach the peak in stage-15b. In the last stages of development (stage-14 & 15b), the specific activity was significantly lower at higher altitude in rohu embryos.

Phosphate dependent glutaminase (PDG) activity :

The total and specific activities of PDG have been presented in Tables-27 & 28 and Figs. 17 & 18. There were species specific and altitude related variations in the PDG activities during development of both scale carp and rohu embryos. PDG activities could be detected in all embryonic stages of scale carp starting from unfertilized egg upto stage-15b. However, in rohu, the enzyme activity could only be detected from stage-11 onwards. Even though it started late in rohu, the total activity of PDG became similar to that of carp in the pre-hatching (15a) stage, atleast at the higher altitude. The specific activity of PDG became similar to carps at lower altitude at hatching. However, it increased by about 100% in rohu at higher altitude in comparison to scale carp.

Both total and specific activity followed parallel pattern, almost throughout the developmental stages except at stages-1, 6, 7, 13 and 15b in scale carp. On fertilization, the specific activity of PDG significantly increased and then the activities did not alter significantly till the stage-8 at Gauhati. However, at higher altitude, there was a significant increase from stage-5 to 7 which returned back to the level of lower altitude at stage-8. There was again a significant increase at stage-9 which was followed by a significant decrease till stage-11 at both the altitudes. From stage-11 the PDG activities increased significantly till their peak at pre-hatching (15a) stage followed by a sudden and significant decrease at hatching (15b) at Gauhati. However, at higher altitude, the activities went on increasing from stage-11 till hatching except a significant decrease at stage-13. In general, from stage-9 till pre-hatching (15a) stage, PDG activities were lower at higher altitude than at Gauhati.

In rohu, PDG activities were detected only in the embryos from stage-11 onwards and the activities were significantly higher (about 3 times) at higher altitude than at lower altitude following a parallel developmental pattern.

Glutamine synthetase (GS) activity :

The total and specific activities of glutamine synthetase (GS) in different developmental stages of scale carp and rohu at lower and higher altitude have been presented in Tables-29 & 30 and Figs. 19 & 20. The enzyme activity was detected throughout the developmental stages studied, starting from the unfertilized eggs till hatching in both the species and at both the altitudes. The unfertilized egg had, in general, very low level of GS activity which increased significantly on fertilization. However, the GS activities were significantly higher, in general, in rohu than scale carp at both altitudes throughout their development.

In scale carp at Gauhati, both total and specific activity of GS slowly and gradually increased from stage-1 till stage-15a.

Though, the increases between individual stages were not significant, but the cumulative increase by the pre-hatching (15a) stage was more than twice the activity level at the zygote (1) stage. At higher altitude (Shillong), the GS activities were very similar to those at lower altitude till the stage-6 after which the activities decreased significantly to a lower level at stage-9. There was significant increase in the activities from stage-9 to stage-11 at higher altitude to reach the level of lower altitude which was maintained till stage-14. There was a significant increase in both total and specific activity of GS only during hatching at Gauhati. However, this increase started earlier from the pre-hatching stage (15a) at higher altitude and was also induced to a comparatively higher level than that at Gauhati.

In rohu, the GS activities were maintained at the level observed in the zygote (stage-1) till the stage-8 of development at both the altitudes. At Gauhati, the enzyme activities decreased significantly at stage-9, followed by a significant increase by stage-11. Then there was a very slow increasing trend till the pre-hatching (15a) stage. At the higher altitude (Mawpun), the specific activity of GS increased significantly from stage-8 till the pre-hatching stage (15a). The specific activity was almost similar at both the altitudes during developmental stages of rohu except at stage-9 where it was significantly higher and at stage-11 where it was significantly lower at higher altitude than at Gauhati. The total activity also increased significantly at stage-9 which was higher than the same at Gauhati. The level was almost maintained till stage-15a except that the last two stages showed a comparatively less significant increase. However, the total activity were significantly lower between stage-11 to 15a at higher altitude.

There was a sudden and sharp increase in the GS activities on hatching at Gauhati but this could not be verified at higher altitude due to lack of observations for stage-15b.

Arginase activity :

The total and specific activities of arginase during development of scale carp and rohu studied at lower and higher altitude have been presented in Tables-31 & 32 and Figs. 21 & 22. The enzyme activity could be detected in all embryos studied and the level of total activity, in general, was similar in both the species at both the altitudes. However, the specific activity of arginase was higher in rohu embryos at both the altitudes in comparison to those of scale carp. The pattern of ontogenic changes were species specific but was not affected much by variation in altitude. The unfertilized egg had a lower level of arginase activity and this was enhanced significantly after fertilization.

In scale carp, after the initial induction of the arginase activity at stage-1 (zygote), there was a gradual decrease in both total and specific activities till stage-7. After this, there was a sudden and significant increase in both the activities from stage-7 to 8. The total activity was maintained almost at that level with wide but insignificant variations upto the pre-hatching (15a) stage/at both the altitudes. However, the specific activity of arginase was decreased after stage-8 and then gradually picked up to reach the highest level at the pre-hatching (15a) stage at Gauhati. At higher altitude, the specific activity increased slowly from stage-8 onwards with a relatively higher level than those at lower altitude and reached the peak also in the pre-hatching stage. In all cases of scale carp, there was a significant decrease in the arginase activity on hatching.

In rohu, there were some altitudinal variations in arginase activities during the early developmental stages and at stage-14. The level of total activity did not change on fertilization but in the following two stages it went up significantly to a peak at stage-3. However, the specific activity increased on fertilization to a peak also at stage-3. In both the cases, the peak level at higher altitude was higher than the lower altitude. The peak level at stage-3 was decreased by stage-4. Both total and specific activity levels were maintained fairly constant from stage-4 till stage-11 after which they increased to another peak at stage-12. This was followed by significant decrease in the embryonic arginase activity till hatching.

Acetylcholine (ACh) concentrations :

The alterations in acetylcholine (ACh) concentration during the ontogenic development of scale carp and rohu studied at lower and higher altitude have been presented in Table-33 and Fig. 23. The ACh level in general, was higher in scale carp than in rohu embryos. There was not much altitudinal variations in ACh concentration in the two species except during the later stages of development in rohu.

In scale carp, the ACh concentration did not change from the unfertilized egg till stage-9. After that, the level significantly decreased to its lowest level at stage-12 followed by a significant and sharp increase to reach the peak at the pre-hatching (15a) stage. Hatching resulted in significant decrease in ACh concentration of the scale carp embryos.

In rohu, the ACh level was also maintained with insignificant fluctuations from unfertilized egg till stage-8 of development.

After that, the level decreased significantly to its lowest level at stage-12 at the lower altitude (Gauhati). This was increased again to a peak at the pre-hatching (15a) stage which significantly decreased on hatching. This pattern was almost similar to scale carp. However, at higher altitude the pattern was almost opposite with the ACh level, increasing from stage-8 till stage-11 and then decreasing gradually till the pre-hatching (15a) stage. The level was higher at stage-11 and lower at stage-14 and 15a of rohu development at higher altitude than those at lower altitude.

Acetylcholinesterase (AChE) activity :

The total and specific activities of AChE studied during the development of scale carp and rohu at two different altitudes have been presented in Tables-34 & 35 and Figs. 24 & 25. The enzyme activity could be detected only from stage-9 of development at both the altitudes in scale carp and at lower altitude (Gauhati) in rohu. At higher altitude, the enzyme activity in rohu embryos was detected from stage-11 only.

The total activity of AChE in scale carp increased gradually and significantly from stage-9 till the stage-15b without having any effect of altitude. However, the specific activity which followed almost a similar pattern as that of total activity, showed significantly higher level at higher altitude during the later stages of development.

In rohu, at stage-11, the total and specific activities of AChE was significantly lower at higher altitude. However, they increased sharply during the later stages to show a higher level at higher altitude and this was significant for specific activity.

The altitudinal difference in specific activity of AChE in both the species, indicating higher activity at higher altitude, became more pronounced with the progress of development. Both total and specific activity of AChE were higher in scale carp at both altitudes than those of rohu.

Cytoplasmic tyrosine aminotransferase (c-TAT) activity :

The total and specific activities of c-TAT studied during the development of scale carp and rohu at lower and higher altitude have been presented in Tables-36 & 37 and Figs. 26 & 27. The c-TAT activity could be detected throughout the development starting from the unfertilized egg. The enzyme activity, in general, was higher in scale carp than in rohu and did not show much variation with altitude except during a few stages before hatching.

In scale carp, the total activity of c-TAT decreased significantly on fertilization at both the altitudes and then it was maintained at a fairly constant level throughout the developmental stages studied at Gauhati. At Shillong, the activity was maintained at the same level as Gauhati till the stage-14 of development and then it increased significantly during hatching. The specific activity was maintained at both the altitudes at the same level as in unfertilized egg till the neurulation (11) stage. After this, the activity was maintained in a slightly higher level at lower altitude and increased gradually reaching the peak at a significantly higher level, at the higher altitude, at stage-15b.

In rohu, the pattern was different than scale carp. The total activity of c-TAT was higher in the unfertilized egg and decreased gradually to about half its activity by stage-8 and this was maintained at higher altitude. However, at lower altitude the total activity increased from stage-8 to 9 and was maintained in a significantly higher level from stage-12 onwards. The specific activity pattern was still different. The specific activity of c-TAT significantly increased on fertilization (stage-1) and then gradually decreased till stage-8 at both the altitudes. After this the specific activity showed an increasing trend with development. However, this increase was more pronounced at higher altitude resulting in significantly higher level of c-TAT specific activity at stage-14 and 15a in comparison to the same at lower altitude.

Mitochondrial tyrosine aminotransferase (m-TAT) activity :

The total and specific activities of m-TAT observed during the developmental stages of scale carp and rohu at both lower and higher altitude have been presented in Tables-38 & 39 and Figs. 28 & 29. It was a significant point to note that the enzyme activity could not be detected till the stage-11 in scale carp and stage-12 in rohu at both the altitudes. The m-TAT activity was comparatively much higher in scale carp than in rohu.

In scale carp, both total and specific activity showed a similar pattern of development at both the altitudes. They went on increasing parallely and showed no altitudinal variations except at stage-14 and 15b where the activities were higher at higher altitude. However, at stage-15b, the difference was more significant than stage-14, resulting in a total increase in activity of about 800% at higher altitude and 200% at lower altitude.

In rohu, the total activity was significantly lower at higher altitude with an insignificantly increasing trend. However, at lower altitude, the m-TAT total activity increased significantly from stage-12 till stage-15b. The specific activity of m-TAT of rohu embryos showed a decreasing trend at higher altitude and an increasing trend at lower altitude from stage-12 onwards. Neither these changes were significant nor there was any significant altitudinal variations.

DISCUSSION

The drastic transformation of a single celled zygote to diverse types of cells, tissues and organs, involves various metabolic changes. Metabolism of different biomolecules such as protein, carbohydrate, nucleic acids and lipid varies in different stages of development in different species of animals. Alterations in enzyme activities control the metabolic processes in an organism. Many enzymes have shown significant qualitative and quantitative changes during the development in various groups of animals (Boell, 1948; Wallace, 1961; Moog, 1971; Brachet, 1974; Neyfakh & Abramova, 1974). Such changes in the enzyme activity have been explained as alterations in gene expression (Davidson, 1976; Whitt, 1981a,b). Therefore, ontogenic variations in the activities of enzymes not only give an idea of the sequential metabolic alterations at different stages of development, but also they provide valuable informations on the regulation of gene expression during ontogenic development.

Ammonia :

The mature oocyte of scale carp and rohu contained a considerable amount of ammonia (Table-23; Fig. 14). The total amount of ammonia per embryo in scale carp was twice as much as in rohu in the unfertilized egg. However, when expressed per gram (g) wet weight, ammonia concentration in the egg of both the species were very much similar. The scale carp egg with bigger size and more protein might have contained more ammonia, but the concentration was the same in both. The ammonia concentration significantly increased at fertilization in both species and this coincides with the drastic decrease of the total protein in the fertilized egg. Both total ammonia per embryo and ammonia concentration per g wet weight gradually increased with development in scale carp. In general, this increase was showing direct correlation with decrease in protein concentration in the embryo. However, in scale carp, the increase was not very significant except the last few stages of development in which decrease in protein concentration was also significant. The higher rate of protein degradation in scale carp might have resulted into higher level of ammonia. It could also be that the ammonia utilization was higher in rohu than in scale carp. Removal of ammonia from the embryo through the egg membrane is still a controversial aspect. The permeability of chorionic membrane for ammonia has not yet been clearly established. Smith (1947, 1957) found that salmon embryo did not allow ammonia to pass through the chorionic membrane. Later, Rice and Stokes (1973) in *S. gairdneri* and Fedorov and Smirnova (1978) in pink salmon (*O. gorbuscha*) reported that about 90% of ammonia produced in the embryo was eliminated out. However, the situation is not very clear in carps. It might be that the diffusion of ammonia might be more efficient in rohu embryos than scale carp, resulting in its higher accumulation in the later species. The rate of ammonia excretion has also been shown to be different at different stages of development. In white fish, atlantic salmon and sea trout, the rate of ammonia excretion was found to be higher during the later stages of development (Nümann, 1953; Chistova & Shirokova, 1971) whereas in rainbow trout, the rate was higher during the early stages of development (Varzhombek & Maslennikova, 1971). Such a situation in the carps studied cannot be ruled out as the reason for the variable concentrations, though it cannot be confirmed with the present data. A sizable amount of total ammonia might be accumulated in the perivitelline space which being eliminated on hatching caused a loss of about 26-28% of the total ammonia.

The highest level of total ammonia in rohu embryos was near the lowest value for scale carp. This indicates that the tolerance for ammonia toxicity might be higher in scale carp embryos than rohu. Tolerance to toxicity varies not only with species but also with age of animal. In rainbow trout, *S. gairdneri*, the embryos were found to be more tolerant to ammonia toxicity than their adults (Rice & Stokes, 1973).

High altitude enhanced the ammonia level in scale carp embryos during the early stages but it stabilized during later stages of development, resulting in a significantly high level at stage-6 (32 cells) and significantly low level at the pre-hatching (15a) stage. In rohu, there was almost no effect of high altitude upto neurulation (11) stage in ammonia concentration except at stage-5 (16 cells) where the level was significantly higher at higher altitude. However, from neurulation (stage-11) onwards, there was a significantly high level of ammonia at higher altitude and this coincides with significantly lower level of protein in the corresponding embryos. The higher rate of protein degradation is also evident from the fact that amino acid level was also higher during these stages when protein level was low. Lower prevailing temperature at higher altitude, particularly during the later stages of rohu development, might have resulted into increased accumulation of ammonia. Decreased temperature has been shown to reduce the rate of ammonia excretion in adult bass, *D. labrax* (Guérin-Ancey, 1976a). Arya (1979) also reported that ammonia excretion in *C. punctatus* is influenced by season and thermal acclimation. At higher temperature, ammonia excretion rate became higher. Besides, due to lower availability of metabolic energy at low temperature, ammonia utilization for biosynthesis might have also been affected. The elevated level of ammonia from neurulation (stage-11) onwards at higher altitude in rohu embryos coincides with high mortality of the embryos as stated in Chapter-I. This might indicate that increased ammonia level is at least one of the causes of the mortality observed in rohu embryos at higher altitude. Besides, the higher level of endogenous ammonia at higher altitude in rohu embryos was only slightly higher than the lower level and about one third of the higher level of ammonia observed in the scale carp embryos. This again confirms a greater sensitivity of rohu embryos to ammonia toxicity than scale carp.

Urea :

In comparison to ammonia, urea is less toxic but still harmful to fish (Griffith *et al*, 1979) and is freely permeable across the egg membrane (Blaxter, 1969). Inspite of these, urea was found to accumulate in the embryos of both scale carp and rohu. Accumulation of urea during development has been reported in *S. gairdneri* (Rice & Stokes, 1973; Dépêche *et al*, 1979). It was suggested that this accumulated urea might be used for recycling of nitrogen by the enzyme urease for the synthesis of different biomolecules. Brookbank and Whiteley (1954) reported the presence of urease in starfish eggs and suggested its role in recycling of nitrogen for metabolic needs. The presence of urea might also be for the osmotic balance of the developing embryo to retain the required water level in the cytoplasm.

Urea was detected in all stages of development and its level was similar in the egg of both the species at both the altitudes. Its concentration increased on fertilization in all cases and this was correlated with an increase in arginase activity, increase in amino acids and also decrease in protein level. As the presence of urea cycle is not known for sure in the freshwater teleosts, this urea might have come from the breakdown of arginine by arginase. However, the correlation did not look very sound because the proportion of arginine was about two times more in rohu than scale carp, whereas urea was more in scale carp than rohu (Tables-17-20 & 24; Fig. 15). The level of total urea was comparatively higher in scale carp than rohu, even though the arginine level was much higher in the former species. However, the concentration of urea per g wet weight was not much different in the two species and in the two altitudes. It showed a gradual increase upto the gastrulation (stage-9) when the level showed a significant enhancement. The level again increased to its peak at the pre-hatching (15a) stage. Thus, there were again two different types of responses, one during the cleavage stages and the other during the differentiating stages as shown in the earlier chapter.

Glutamate dehydrogenase (GDH) activity :

The activity of GDH could not be detected in the unfertilized egg of both the carps and it appeared only after fertilization. Several enzymes are known to be deposited in the oocyte during oogenesis in an inactive state for utilization during the earlier stages of development (Hishida & Nakano, 1954; Terner, 1968; Ermolaeva & Mil'man, 1975; Shaklee & Whitt, 1977). Many of these enzymes get activated on the stimulus of fertilization. GDH might be one such enzyme to show such immediate activity after fertilization. GDH has been shown to be inhibited by ATP, GTP and GDP (Frieden, 1963, 1971) and activated by ADP and leucine (Goldin & Frieden, 1971) in animal tissues. Alterations in the concentrations of these regulatory molecules in the egg might have taken place to activate the inactive GDH molecules on fertilization. However, no such regulation has yet been shown in fish eggs. It could also be that the mRNAs for GDH might have been deposited alongwith the high RNA deposits during vitellogenesis which would have started translating immediately after fertilization. Presence of a large amount of such masked mRNAs which translate during the early stages of development has been reported earlier in the egg of several oviparous animals (Tyler, 1967; Denis, 1974; Neyfakh & Abramova, 1974; Sarkar et al, 1979). It has also been shown that no new mRNAs are synthesized during the early phases of development of oviparous animals (Davidson, 1976; Denis, 1974). Therefore, Shaklee and Whitt (1977) have suggested that the enzyme activity till the gastrulation stages might not be due to the direct activity of their genes. Hence, activation of the GDH gene at this early stage might not be a possible reason for the appearance of GDH activity.

The total activity of GDH was comparatively higher in scale carp than rohu, whereas the specific activity was higher in the later species. This, besides being a species specific variation, is also due to lower protein concentration in rohu embryos. The enzyme activity was maintained at the same level till the stage-7 in scale carp at both the altitudes and only in higher altitude in rohu, whereas at lower altitude the activity was much increased from stage-5 to 7 in rohu. This variation could also be due to the longer half life of the maternal GDH molecules or its mRNA, which possibly has contributed to the enzyme activity during this period. In rohu, the synthesis of new GDH molecules might have started little earlier than scale carp due to earlier gene activation resulting in enhancement in the activity. These stages also coincide with the time of enhancement of proteins in the embryos of both the species. Such differential gene activation in different species and under different conditions are known in animals (Davidson & Britten, 1979; Scandalios, 1979).

The fall of GDH activity in scale carp embryo at blastulation (stage-8) might be due to a selective degradation of the enzyme molecules. Shaklee and Whitt (1977) have reported such abrupt decrease of creatine kinase and aldolase activities during the development of sunfish. In case, the initial observed activity of GDH was the result of maternal contribution in stored form, degradation of the original molecules without a concurrent new synthesis might be responsible for the drop in the enzyme activity at the blastulation (8) stage. It is also known that high endogenous levels of ammonia may act as an inhibitor of GDH activity (McBean et al, 1966). However, the levels of endogenous ammonia during blastulation or earlier stages in scale carp was not as high as in the later stages. Therefore, such feedback inhibition might not be involved in the observed drop in GDH activity.

In the subsequent developmental stages, the activity of GDH gradually increased in scale carp, in the opposite pattern of the general fate of the soluble protein. This might be due to continuous activation of the GDH gene. However, in rohu, the increase was in two phases, once from stage-5 to stage-9 and then from stage-12 till hatching (stage-15b). Between stage-9 and 12, the specific activity remained constant whereas the total activity decreased. These variations could be due to the demand of the embryo at a particular stage for specific metabolism.

The reaction catalyzed by GDH is reversible and an equilibrium was considered to arise in vivo in mammals (Krebs & Veech, 1969). However, the transdeamination scheme in the direction of ammonia formation in mammals has been later challenged (McGiven & Chappel, 1975) and it was advocated that GDH favours glutamate formation for the storage of nitrogen. So far, such evidence is lacking in fish. Walton and Coway (1977) have reported in rainbow trout that the GDH favoured more the formation of ammonia. A direct correlation between the endogenous ammonia level and GDH activity was not found in most of the stages in the present study. Such lack of correlation could also be due to the complicated ammonia metabolism pathway for ammonia synthesis and utilization, and the rate of its elimination.

The higher altitude did not show any effect on the total and specific activity of GDH in both the species for most part of development. In rohu, the induction of the activity was delayed from stage-7 to stage-8 and the specific activity was significantly higher during stages-10-11 instead of the decrease seen at lower altitude. In scale carp, though the total activity was lower during the last 3 stages before hatching (stages-13-15a), the specific activity was not significantly lower. The exact mechanism of variations in GDH activity due to the higher altitude is difficult to explain, whether they are due to modulation of the gene function or allosteric regulation of the enzyme activity.

<u>Phosphate dependent glutaminase (PDG) activity</u> :

The appearance of glutaminase activity has been reported in other animals to vary during ontogenic development. In rats, PDG level was quite low in foetal liver but suddenly increased on the 1st day after birth and rapidly reached the adult level (Katunuma *et al*, 1973). The enzyme activity in chick increased by 27% between 10th hr and 4th day after hatching which was followed by a decrease by 30 days (Nehlig & Lehr, 1978). Similarly, in the present study, PDG activity was detected in all the developmental stages including the unfertilized egg in scale carp, but the activity started only at neurulation (stage-11) in rohu. This is an interesting finding of species specific difference, that in scale carp the PDG gene has remained active throughout or there was sufficient accumulation of PDG molecules and/or its mRNA in the egg which maintained its activity during the early developmental stages till the embryonic gene started functioning. In rohu, there was probably no accumulation of the enzyme molecules and its activity started only when the embryonic gene was activated. This stage (11) coincides with the protein enhancement stage indicating that fresh protein synthesis might have resulted the beginning of PDG activity in rohu.

PDG catalyzes the reaction converting glutamine to glutamate and ammonia (Kalra & Brosnan, 1973, 1974) and thus regulates the balance between glutamate and glutamine in the cell (Berl et al, 1975). Glutamate is a major neurotransmitter in fish (Watts & Watts, 1974). Therefore, during and after neurulation (stage-11), PDG activity was found to enhance, possibly to produce more glutamate. Increase in glutamate and ammonia concentration has been reported during the later stages of development in the present study (Tables-17-20 & 23; Fig. 14). Some of the fluctuations observed at some particular stages could only be due to the variations in the rate of synthesis and degradation depending on the requirements of these stages.

High altitude did not have much effect on the general ontogenic pattern of PDG activity. However, in scale carp, there was a significant induction of the total and specific activity in the late morula (7) stage which decreased significantly at blastula (8) and gastrula (9) stages. It was followed by fluctuations in PDG activity at higher altitude. In rohu, the activity of PDG was induced to a significantly higher level from the beginning at neurula (11) stage and continued to increase till hatching (stage-15b). These variations are the effect of high altitude factors, more likely of the lower temperature. Lower temperature has been known to influence the level of enzyme activity (Hochachka & Somero, 1973; Precht et al, 1973). The higher level of activity at higher altitude correlates with higher ammonia level. This might have caused the lethal stress on the developing embryo. The response of high altitude was more apparent in terms of enhancement in PDG activity in rohu than in scale carp embryos indicating the higher sensitivity of rohu to environmental fluctuations.

Glutamine synthetase (GS) activity :

The presence of glutamine synthetase activity has been associated mainly with ammonia detoxification by glutamine formation (Wu, 1963; Lund & Goldstein, 1969; Webb, 1980). This enzyme was found in all stages of development starting from the unfertilized egg in both the species and at both the altitudes. The enzyme activity was higher in rohu than scale carp and this might have resulted in a higher level of ammonia in embryos of the later species. The increase in activity was very slow but steady with progress of development in scale carp. However, in rohu, there were specific stages of induction. At fertilization (stage-1), there was the first significant increase in GS activity and the level was maintained till stage-9. The activity was again induced significantly by neurulation (stage-11) which was maintained till the pre-hatching (15a) stage. On hatching (stage-15b) the GS activity was induced significantly in all cases.

These two patterns of species specific variation in the two carps indicate that (i) ammonia tolerance is more in scale carp embryos and (ii) GS might not be playing a major role in ammonia detoxification. However, in rohu there are some correlation of the GS activity change with ammonia and also with ammonia producing enzyme levels. PDG was induced to a very high level at neurulation (stage-11) when ammonia level was enhanced. Increased ammonia level might have caused an inducing influence on the GS gene, to produce more enzymes to meet the demand of detoxification. Besides, ammonia diffusion might have been an easier process in early developmental stages when the cell are less in number and also arranged in a spherical fashion. With morphogenetic movements and differentiation, the diffusion might be difficult from the internal tissues or cells. Therefore, after gastrulation (stage-9), GS gene might have been activated to take up the ammonia detoxification process which it continues throughout the life of the fish. However, the behaviour of GS gene in the two species are quite different during the ontogenic development.

High altitude had almost no effect during the early stages of development. In scale carp, the GS activity decreased from stage-7 till stage-9, possibly due to the degradation of the pre-existing molecules before the synthesis of new enzyme molecules. Fresh enzyme synthesis possibly took place from stage-10 to recover the activity level by stage-11. The pre-hatching induction also took place from stage-14 instead of stage-15a. This could be due to a continuously higher ammonia level in high altitude scale carp embryos. However, in rohu, the induction at stage-11 was at a low key than normal and the total activity remained at a low level resulting in the higher amount of total ammonia. This might be due to degradation of GS molecules with cytoplasmic protein which also decreased simultaneously. Thus, there was not much variation in specific activity. Higher altitude, therefore, affected GS activity only by random degradation of the protein.

Arginase activity :

The detection of arginase activity in the unfertilized egg indicates the activity of arginase gene or accumulation of pre-formed enzyme molecules in the egg of the two carps. The total activity was mostly similar during different developmental stages in the two species. However, the specific activity was higher in rohu, probably due to the less amount of proteins. The significant induction on fertilization (stage-1) of both total and specific activity was in contrast to the significant decrease in both total and cytoplasmic protein concentration during these stages. This would mean a selective retention of arginase molecules, besides their synthesis, if at all any. The pattern of alteration was also species specific. In scale carp, the increase on fertilization (stage-1) was followed by a slow decrease till the lower level at stage-7 which might be due to gradual loss of existing molecules. It was followed by a sudden induction at stage-8 which was more or less maintained till the pre-hatching (15a) stage. In rohu, the increase in activity after fertilization (stage-1) continued till the 2nd cleavage (3) stage after which it remained stable till the stage-12. The arginase activity decreased only at hatching (stage-15a) in scale carp and after stage-12 in rohu. Both total activity and specific activity was also much lower in rohu than scale carp in the newly hatched larva (15b). The decreased levels in rohu coincide with the increase in protein level indicating selective breakdown of the arginase molecules. These different patterns indicate different ontogenic regulation of arginase in the two species.

In amphibians, the activity of the enzyme remained at a low level throughout the development (till hatching) and then sharply increased during metamorphosis (Brown & Cohen, 1959b). In elasmobranchs, Read (1968) observed the enzyme at a low level in the embryo but as development proceeded, it gradually increased in activity. In S. gairdneri, it has been shown that the activity of arginase is not detectable until sometime before hatching following which it gradually increased in activity (Rice & Stokes, 1973).

This is in sharp contrast to our observations in the embryos of scale carp and rohu. The activity of arginase observed in the present study during the developmental stages of scale carp and rohu were several times higher than the specific activity of hepatic arginase (14.6±0.5) reported in adult scale carp by Cvancara (1969). In one of our unpublished observations, it was also found that the arginase activity in the liver of the 3-month old rohu was also very low (sp. activity, 1.28±0.24) in comparison to the embryonic stages. The ancestors of the teleost fishes were capable of urea formation through the O-U pathway (Brown & Cohen, 1960; Cohen & Brown, 1960) which was supposed to be lost during evolution of these fishes. Huggins et al (1969) suggested that, in such case, the O-U cycle enzyme activities should be detectable during the ontogeny of the present day teleosts. The present results from the two carps reveal that, at least for arginase, there is a clear tendency of the transition from the high level of the enzyme activity in embryos towards a lower level in the adult. However, it does not necessarily mean the presence of O-U cycle and the "gene deletion theory" in these fishes.

The high altitude again did not show much effect on the arginase activity in the two species. In the later stages of development only the specific activity of arginase was comparatively higher but the values were not significant. Only some variations in the level of the enzyme activity in some stages of development could be seen. This could be due to the local effect of the environment regulating the synthesis and degradation rate of the enzyme molecules. However, during the pre-gastrulation stages, scale carp showed better tolerance than rohu.

Acetylcholine (ACh) concentration and acetylcholinesterase (AChE) activity :

ACh was detected in the unfertilized egg of both scale carp and rohu. Existance of ACh in mature oocyte has been reported in amphibians (Buznikov et al, 1964). It was suggested that besides being involved in neurotransmission, which commences at a later stages of development (alongwith the differentiation of neural and muscle tissue), ACh might perform certain other important metabolic functions during the earlier stages of development. These include its participation in cell division and morphogenetic movement of cells as well as in growth and maturation of neurons (Loh, 1976). Studies on adult animals have shown that ACh alongwith cholinesterases has been associated with the ionic conductance of the cell membrane by regulating Na^+ transport (Fleming et al, 1962). ACh has been reported to be present in a remarkable concentration in mammalian placenta (Rama Sastry et al, 1976). There, it has been suggested to control the permeability of placental membrane which separates the maternal and foetal blood streams (Harbison et al, 1975). The presence of ACh in the unfertilized egg might be the ready stock to perform similar functions during the early development.

The pattern of ontogenic changes of ACh and AChE were apparently similar in scale carp and rohu. The level of ACh in both scale carp and rohu remained without any significant alterations during the cleavage and blastulation (8) stages. This might be due to the lack of its synthesis, as its degradation was not possible in absence of the hydrolysing enzyme, AChE, during these stages. It is very likely that the synthesis of ACh started around gastrulation (stage-9) stage showing a slight increase in

ACh level. This coincided with the sudden appearance of AChE. The level of ACh decreased after stage-9 reaching the lower level by stage-12 in both the carps. The activity of AChE rapidly increased during these stages and probably the rate of degradation of ACh was higher than the rate of its synthesis for which ACh level declined in the embryos. However, inspite of a continuous rise in the AChE till the pre-hatching (15a) stage, the level of ACh increased after stage-12 in embryos of both the carps. This indicates a higher rate of synthesis of the neurotransmitter. The presence of choline acetyltransferase (CAT), the enzyme associated with the synthesis of ACh and its dramatic changes alongwith the changes in AChE and ACh have been reported in frogs (Loh, 1976) and insects (Prescott et al, 1977). However, the synthesis rate of ACh was perhaps lower over the hydrolysis at the onset of hatching (stage-15a) which might have resulted in a significant decline of ACh level in the newly hatched embryos (stage-15b) of both the species studied.

The pattern of ontogenic development of AChE in the scale carp and rohu was found to be different than that reported in S. gairdneri (Uesugi & Yamazoe, 1964) and in X. laevis (Gindi & Knowland, 1979). A low activity of the enzyme was detected in mature oocytes of the later two species. However, in the present study no activity could be detected from the egg till gastrulation. Further, the sharp rise of the enzyme activity, immediately after its first appearance at the beginning of gastrulation (stage-9), was in contrast with the reports on salmon (S. gairdneri) and amphibians. In salmon, the enzyme activity was significantly induced only by the eye stage, at about the 10th day after fertilization (Uesugi & Yamazoe, 1964), whereas in salamander (*Ambystoma punctatus*), the activity of the enzyme began to rise at stage-22, when the spinal cord and brain started to develop (Sawyer, 1942, 1943). A similar pattern of AChE ontogeny was also reported in *X. laevis* by Gindi & Knowland (1979). However, in the same frog, Atherton and Lee (1975) found a high activity of AChE during early and late gastrula stages. The absence of AChE activity during the pre-gastrula stages in the present study might be due to the repression of its gene or due to the presence of any specific inhibitor. In *S. gairdneri*, the interference of an inhibitor, for the low level of AChE during the early developmental stages, has been ruled out because addition of the newly fertilized eggs to the eye stage embryos (with a high level of AChE) did not reduce the enzyme activity (Uesugi & Yamazoe, 1964). Such situation in carps is not known. However, the appearance and the sharp increase of the enzyme activity during the observed stages might be due to the specific gene activation for neural differentiation. Studies on chick (McGeer *et al*, 1974), mammals (Valcana *et al*, 1974) and amphibians (Gindi & Knowland, 1979) have shown that AChE is a marker enzyme for nerve and muscle tissue differentiation. Its increase during development is connected with the differentiation of neuron and neuromuscular junction. Histochemical studies in ascidian, *Ciona intestinalis*, showed the location of AChE in the presumptive muscle cells of the neurula (Whittaker, 1973). The present study in scale carp and rohu indicates that AChE appearance is related with the beginning of neural and muscular differentiation at cellular level.

The response of ACh and AChE of scale carp and rohu embryos to the high altitudinal conditions was found to be different in the two carp species. Scale carp showed almost no effect except a little enhancement of AChE level in developmental stages at higher altitude. Such changes might be compensatory alteration as there were not much visible effect on the embryos. Rohu embryos showed a significant response to the high altitude from gastrulation stage onwards. The levels of ACh and AChE are altered in order to co-ordinate and adjust the different physiological or behavioural process, of the animal under any environmental stress (Prosser, 1973b; Lagerspetz, 1977). Therefore, the levels of both these components serve as sensitive indicators of the intensity of physiological stress, the animal is undergoing. Hence, the alterations in the ACh and AChE levels in the rohu embryos at higher altitude are indicative of physiological stress which was perhaps not encountered by the scale carp embryos. This supports our earlier assumption that rohu embryos were more susceptible than scale carp to higher altitude (Chapter-I).

The alterations of ACh concentration in the rohu embryos at higher altitude was first observed only at the neurula (11) stage, when its level was significantly higher. This was probably due to the late beginning of AChE activity at high altitude (at stage-11) and also due to low enzyme activity. At the 8-somite (stage-12) stage, the level of AChE returned to its normal level alongwith the level of ACh. However, in the two subsequent stages (stage-14 & 15a), though the AChE activity per embryo was similar at both altitudes, the level of ACh dropped considerably at the higher altitude. The specific activity of AChE was significantly high at higher altitude in all these stages of rohu embryo. ACh synthesis was perhaps below the normal level during this period.

Synthesis of ACh requires two common metabolites, choline and acetyl-CoA. The later is a most necessary compound for energy metabolism being at the confluence of the metabolism of carbohydrate, fat and amino acids and it is connected to TCA cycle. Therefore, it might be possible that during the stressed condition at the higher altitude, Acetyl-CoA might have been more vigorously fed into the TCA cycle to meet the immediate energy demand, rather than the synthesis of ACh in rohu. This might have resulted in the failure of neural integration of physiological processes leading to high scale mortality of the embryos in these stages of development at higher altitude.

Tyrosine aminotransferase (c-TAT & m-TAT) activities :

The developmental pattern of cytoplasmic TAT (c-TAT) and mitochondrial TAT (m-TAT) in scale carp and rohu was observed to be quite different. c-TAT activity was detected in the mature oocyte and in all developmental stages studied in the two species. In contrast, m-TAT activity was detected only from stage-11 in scale carp and stage-12 in rohu. In scale carp, total c-TAT activity, after a significant decrease at fertilization, did not change during the later stages of development. However, there was no change in specific activity of c-TAT even after fertilization. In rohu, c-TAT level gradually decreased from the oocyte stage till stage-8 and then showed slow enhancement till hatching. m-TAT level was higher in scale carp than rohu, and in both cases, the activity increased linearly till hatching. Subcellular localization of TAT has been reported in the adult catfish, *H. fossilis* (Ratha &,Bhagowati, 1980) and also in mammals (Litwack *et al*, 1963; Miller & Litwack, 1969a,b, 1971). It has been shown in mammalian tissues that the c-TAT and m-TAT differ from each other in their physico-chemical characteristics (Miller & Litwack, 1969a,b) and having different physiological significance (Fellman *et al*, 1969). Thus, it has been suggested that c-TAT and m-TAT might be two different iso-enzymes being controlled by different regulatory mechanisms. The observed ontogenic differences on the activity pattern of c-TAT and m-TAT suggests that the regulation of their appearance and level of the activity are under different regulatory mechanisms in the two fishes studied. The sequential appearance of c-TAT and m-TAT may be indicative of the differential gene regulation.

Our observations on c-TAT and m-TAT during the early development of the two carps is in sharp contrast to the observations earlier reported in mammals and in frogs. In mammals c-TAT activity was reported to be absent or negligible during the foetal life and reached a significantly high level within hours after birth (Sereni *et al*, 1959; Greengard & Dewey, 1969). m-TAT activity was at a low, but detectable, level till birth and then it slowly increased to reach the adult level in about two days postnatal life (Koller *et al*, 1969; Holt & Oliver, 1971). In the toad, *Xenopus*, Ohisalo and Pispa (1976) reported that c-TAT activity was not detectable till the neurulation stage. The enzyme activity which was present in a very low level was shown to increase considerably only after hatching. The present results on a highly detectable level of c-TAT activity and its significant alterations during the pre-hatching developmental stages might suggest its role in the embryonic differentiation in these two fishes.

Total c-TAT level was maintained, in general, in a stable state during the developmental stages studied. However, the fall in total activity on fertilization could not be detected when the specific activity was considered. This could be due to a proportionate degradation of c-TAT molecules alongwith the cytoplasmic protein loss during fertilization. The appearance of m-TAT activity in both the species of carp was observed after their enhancement of mitochondrial proteins at stage-9. The mitochondrial protein which was maintained, almost at egg level, till stage-8 started increasing at stage-9 indicating synthesis of new mitochondria. After this, the m-TAT activity was detected which might have come from the newly synthesized mitochondria.

These differences of the ontogenic variation of TAT in the two fishes and also between different development stages might reflect a differential need of tyrosine oxidation. TAT has been shown to be a gluconeogenic enzyme and is one of the best studied enzymes to understand the mechanism of gene regulation in eukaryotes (Thompson, 1979). In *Xenopus*, a sharp increase of the TAT activity has been linked with the sudden increase in its substrate, tyrosine, during development (Ohisalo & Pispa, 1976). However, in the present study no such substrate correlation could be seen with the alterations in the enzyme activity. TAT activity is also inversely modulated by glucose level (Ohisalo & Pispa, 1975). It is known that during the development of fish, glucose level of the embryo shows periodic fluctuations (Blaxter, 1969) and such fluctuations would have also contributed to the fluctuations of TAT activities in the carp embryos.

The effect of high altitude on c-TAT and m-TAT activity was different in different species and also at different stages of development. m-TAT activity was not detected during the cleavage stages in both the species and c-TAT activity showed no significant effect of high altitude. In the later stages of development in *C. carpio*, the specific activity of c-TAT increased at higher altitude from the stage-12 and total activity at the pre-hatching and post-hatching stages. In rohu, the total c-TAT activity decreased from stage-12 whereas the specific activity increased at stages-14 & 15a. In case of m-TAT, the activity was increased in *C. carpio* whereas it decreased in *L. rohita* at higher altitude.

Induction and inhibition of TAT activity with response to different environmental stresses at different stages of development have been shown in different animals. TAT activity is induced by different physical and chemical stresses in mammals (Gelehrter, 1971). The induction has been shown to be brought about through the release of glucocorticoid hormones which, in turn, causes the transcription of specific TAT gene (Gopalakrishnan & Thompson, 1977). In the lower vertebrates, glucorticoid has been suggested not to have any effect on TAT induction (Chan & Cohen, 1964; Fellman *et al*, 1971; Ohisalo & Pispa, 1975). However, in the larval stages of the frog, *Philautus cherrapunjiae*, TAT activity was induced by hydrocortisone with a similar dose of the hormone as used for mammals (Ratha *et al*, 1980). Ratha and Bhagowati (1981) also observed in adult scale carp that TAT activity was inhibited by a severe hypoxic stress. Inhibition of TAT activity by any stress was not known in animals. Therefore, it was suggested that the regulation of TAT gene has been different not only in different animal groups but also during different stages of development. Such phenomena might have been the cause of the different effects observed in the present study.

Observations on the metabolites and enzymes of ammonia metabolism, neurotransmission and tyrosine oxidation pathways indicate that the egg has accumulated provisions for some enzymes, necessary during the early development and the production of new enzymes starts at different stages of development depending on the level of differentiation. It also gives another indication that the early cleavage stages are comparatively independent, perhaps being maintained by the reserve materials in the egg and the morphogenetic movements of the cells needs the synthesis of new molecules. The high altitude also, generally, did not show any effect during the cleavage stages and its effects were clearly seen in the post-gastrulation stages. It might be that undifferentiated embryo is less susceptible to the environmental factors than the differentiated stages. Similar effects were also seen in the survival and chemical changes, as discussed in the earlier chapters.

GENERAL DISCUSSION

GENERAL DISCUSSION

The continuation of an animal group with time and their diversification in space, depend primarily on their having proper adaptations for successful reproduction and development, under the existing environmental conditions. The adaptations are brought about through regulations of different physiological and biochemical processes in response to the environmental changes. The reproductive period and the early developmental stages are most sensitive to the intrinsic and extrinsic stresses, particularly in oviparous animals. In these animals reproduction involves an active process of accumulation of nutrients in the maturing oocyte called oogenesis, for the early development, and the development being external, the embryos are subjected to onslaughts of environmental hazards.

Fish, is the largest group of oviparous aquatic vertebrates, with a varieties of adaptations to different types of aquatic environments at different stages of their life. Different species of fish respond differently to different environmental factors, depending on their range of tolerance and elasticity of adaptation. Reproduction and development in fish are regulated by both intrinsic and extrinsic control mechanisms. The intrinsic control mechanism includes a chain of biochemical and physiological processes leading to the deposition of necessary substances in the egg during oogenesis, catabolism of these substances to meet the energy demand of the embryos and to supply precursors for synthesis of new molecules during embryogenesis. Studies on different groups of animals (Barth, 1964; Moog, 1965, 1971) including some species of fish (Love, 1970) reveal that the pattern and rate of metabolism of biomolecules vary in different stages of development. The chorionic membrane of fish embryo is impermeable to most of the biomolecules (Blaxter, 1969). Therefore, the alterations in the concentrations of various biomolecules such as protein, amino acids, DNA, RNA, carbohydrate or fat give a gross idea about the utilization of the deposited substances in the egg during embryogenesis. The changes in the type of metabolism of various substances occur due to the changes in corresponding enzyme activities. This has been reported in various groups of animals (Barth, 1964; Moog, 1965, 1971) including some species of fish (Hishida & Nakano, 1954; Terner, 1968; Shaklee & Whitt, 1977). Some enzymes are deposited in the egg in inactive state and some others in form of messenger RNA molecules for their translation during early developmental stages. Besides, many new enzymes and proteins are synthesized to give the functional entity to the specific kinds of differentiating cells (Brachet, 1974).

The external factors profusely influence the intrinsic control mechanisms. The variations in the environmental factors influence the reproduction and ontogenic development in fish. The optimum conditions vary in different species (Blaxter, 1969; Hoar, 1969; Braum, 1978; Peter & Crim, 1979). The alterations in environmental factors beyond the 'tolerance level' cause adverse effect on the rate of development and survival of the embryo. These are due to the changes taking place at cellular and sub-cellular levels. Such studies on embryonic stages of fish are limited (Lindroth, 1942; Hayes, 1949; Holliday <u>et al</u>, 1964;

Harmor & Garside, 1975; Marr, 1966; Blaxter & Hempel, 1966; May, 1975a,b; Howell, 1980; Johns & Howell, 1980; Johns *et al*, 1981).

Differential response to environmental conditions for reproduction and development of various species of fish are presumably associated with the differences in their adaptive characteristics. It is, therefore, expected that these differences to environmental factors may be reflected in their physiological and biochemical processes.

Some species of fish show wider adaptations for reproduction and development, thus efficiently growing at different environmental conditions. However, some other species show very narrow adaptations to live in a limited environment. *C. carpio*, the common carp, is a better adapted species which is capable of breeding and growing both at tropical warm water and also in colder climates, whereas, *L. rohita*, an Indian major carp, breeds, and grows only in the tropical or sub-tropical warm waters. The success of scale carp and failure of rohu to live in higher altitude water bodies must be related to their sub-cellular regulatory mechanisms. In the present study a comparison of the response to hypophysation, rate of development, rate of survival and some biochemical changes during the early development of scale carp and rohu at both lower and higher altitude was done. The results indicate that the normal pattern of breeding, development and the biochemical changes during the early development of each species were fairly specific and different from the other species. It further revealed that scale carp which is commonly known as an 'eurythermal species' is better adapted to the environmental conditions at the higher altitude than rohu. Rohu breeders, embryos and juveniles showed a clear susceptibility to the high altitude conditions and correlated with a higher intensity of alterations in various biochemical parameters studied in the developmental stages.

Normal pattern of breeding and development of *C. carpio* and *L. rohita* at lower altitude under optimal conditions :

The induced breeding experiments conducted during 1979 and 1980 showed successful spawning of both scale carp and rohu upon hypophysation at the low altitude (Gauhati). However, the rate of fertilization and development were related to the prevailing temperature in the ponds. The optimum temperature range for successful breeding and development of scale carp and rohu has been reported to be 18 to 35°C and 24 to 31°C respectively. In scale carp, the incubation period in 1980 at the temperature range of 18.5-26°C was lower (58-60 hrs.) than in 1979 (80-83 hrs.) at a temperature range of 17-23.5°C. Incubation period in rohu, under a temperature range of 25-31.5°C was lower (12-14 hrs.) in 1979 than that in 1980 (12.5-14.05 hrs.) during which period the temperature ranged between 24-31°C. Besides shorter incubation period, the rate of development was also faster in rohu than in scale carp (Tables-6 & 7; Fig.). This might be the inherent character of this species needing higher water temperature during the incubation period. It is, however, evident that the optimum temperature requirement of the two carps are different, rohu with a higher and narrow range and scale carp with a wider range. This has resulted in two breeding periods in scale carp (monsoon & winter) and only one in rohu (monsoon).

Survival studies conducted at some stages of development (Tables-2, 3 & 8; Fig. 5) and final yield of fry of scale carp and rohu, showed an appreciable success of embryos developed under two different periods of the annual cycle. Due to the high survival of embryos and young ones at Gauhati, this low altitude was considered as the normal environment for development of the two carp species studied.

The difference in the environmental conditions and in the rate of development indicated different patterns of metabolism of yolk substances during ontogenesis of the two species. The quantitative analysis of the wet weight, water content, dry matter content (DMC), protein (total, soluble, cytoplasmic and mitochondrial), free amino acids (FAA), DNA and RNA and qualitative analysis of FAA during the early development (till hatching) were found to be different in the two species.

The relative size and total weight of the unfertilized egg was larger in scale carp than in rohu. The level of water was 68.39% in scale carp and 56.9% in rohu and the rest belonged to DMC. At fertilization, the water content of the eggs increased due to its imbibition for swelling the chorionic membrane and increasing metabolism (Blaxter, 1969). Water as a metabolic indicator has been shown in salmon (Penaz et al, 1976). The DMC which indicated the amount of deposited nutrients (Johns & Howell, 1980), gradually decreased with development. Such decrease was stage specific and varied between scale carp and rohu. Therefore, reduction in DMC during the development indicating catabolism of the yolk substances for maintenance and growth of the embryo was both species and stage specific. The decrease in DMC was higher in rohu (40.32%) than in scale carp (31.45%). This might be due to a comparatively higher catabolism of inert substances and higher energy requirement of rohu embryos for their faster rate of development.

Protein is known to be a major source of energy for fish development (Hayes, 1949; Smith, 1958; Harmor & Garside, 1977). The yolk protein is degraded during the course of development to provide energy and raw materials for synthesis of new proteins. It was observed in the present study that the pattern of changes in the total, soluble (s-) and cytoplasmic (c-) protein were different in scale carp and rohu. Except for a significant rise at the blastulation of embryos, the protein contents (total, s- & c-) showed a constant decreasing trend during scale carp development. However, in rohu, after an initial decrease till the morula stage, the total, s- and c- protein contents exhibited a steady increase with development. The increase in protein synthesis during later stages of development and lower amount of yolk protein in rohu might be the cause of the specific pattern of changes. In scale carp, probably the neutral yolk proteins are much more than the proteins synthesized fresh for which there was a gradual decrease in protein content with development. The mitochondrial (m-) protein content in both the species increased right from the time of fertilization till hatching. However, there was a conspicuous increase in its level in post-gastrulation stages. The formation of new mitochondria with the progress of ontogenic development and particularly with the commencement of morphogenetic movements, might have taken place to meet the energy requirements of the embryo.

Amino acids are the building blocks of protein. Therefore, changes in the rate of synthesis of protein in the developing embryo is expected to alter the amino acid (FAA) pool. Quantitative analysis of FAA in the early development of the two carps revealed that the total FAA generally showed an inverse relationship with the total protein content. However, in scale carp embryos, the FAA pool exhibited an increase with the increase in protein content at blastulation and also showed a decrease, parallel to the decrease of protein at the 8-cell stage, early gastrulation and in the freshly hatched larva. The increase of FAA with the increase in protein content is suggestive of fresh synthesis of amino acids. The parallel decrease of FAA with protein may be attributed to a high catabolism of amino acids for energy production.

Qualitative analysis of FAA in the mature egg showed that its composition and relative proportion of individual amino acids varied in embryos of the two species. In scale carp, and in rohu, the number of free amino acids identified were fifteen and fourteen respectively. The mature oocyte of the later species lacked in threonine - which was detected in scale carp. Rohu egg contained cysteine and cystine in addition to other amino acids observed in scale carp. The relative proportion of essential and non-essential amino acids was found to be 47.62:52.38 in scale carp but in rohu it was 59.4:40.6. Such differences in the egg of the two carps must be due to their differential deposition during oogenesis, possibly for the specific requirements during the course of early embryogenesis. Qualitative variation in the deposition of FAA has been reported in other species of fish (Krishnamoorthi, 1958). It has been observed in the present study, that the individual FAA exhibited qualitative and quantitative variations with development, some appearing newly, some increasing in proportion, some decreasing and some others remaining unchanged in their proportion during development. The individual changes of FAA during development, as pointed out by Zeitoun et al (1977) is difficult to explain precisely. It may only be speculated that the variations in the individual FAA during the early development of the two carps, as observed, may be a net result of their liberation through yolk degradation, incorporation into new proteins, their new synthesis, conversion to other products and their degradation for energy purposes.

Protein synthesis is accomplished through the genetic informations located in the nucleotide sequence in the DNA molecule. Amount of DNA per nucleus of a somatic cell of a given species is a constant. In fish, the diploid DNA content is known to range between 0.8 to 8.88 pg per cell and the value range between 3.04 to 3.83 pg in carp (White, 1973). However, the egg DNA content of scale carp and rohu were found to exceed these values by several thousand times. Such high DNA values have been also reported in eggs of echinoderms (Tyler, 1967), amphibians (Denis, 1974) and also in some fishes (Neyfakh & Abramova, 1974). In echinoderms and amphibians, the high DNA content in the egg has been shown to be due to amplification of specific genes and chromosomes, and a heavy deposition of mitochondria during

The amount of DNA in the developing embryo was found to increase only by gastrulation in scale carp and at the late morula stage in rohu. Even though the number of cells and the nuclei started increasing from the first cleavage stage, involving synthesis of nuclear DNA, the quantity of DNA did not increase proportionately, probably due to the minute increase of the nuclear DNA in comparison to total DNA. Also, there might be some amount of degradation of the cytoplasmic DNA during the early developmental stages. The rise of DNA in the above mentioned stages and its quick increase with development might be due to complete degradation of the deposited DNA resulting in the dominance of the nuclear DNA of the fast growing cells of the embryo. The late rise of DNA content in scale carp embryo than in rohu might be due to greater synthesis and slower rate of degradation of DNA in scale carp. DNA as an energy source has been also reported in *S. gairdneri* (Zeitoun *et al*, 1977).

Similar to DNA, the unfertilized egg of scale carp and rohu were found to contain a large amount of RNA. This reserve RNA was perhaps accumulated during oogenesis. A large amount of mRNA molecules are known to be deposited in the egg which remains in masked condition till fertilization and starts translating the required proteins in early stages of development. The high egg RNA values and its deposition during oogenesis have been shown in other animals (Tyler, 1967; Paul, 1974b). The RNA content of the scale carp embryos increased only at neurulation and in rohu at the late morula stage of the embryo. RNA is the organizer of protein synthesis. Therefore, it may be assumed that the protein synthesis in the earlier stages of development took place with the help of the accumulated RNAs in the egg. The new RNA synthesis started significantly during the later stages when the egg RNA got degraded and new protein synthesis was necessary for morphogenetic movements and differentiation. The RNA synthesis, if at all was there, might be at a very low key during early developmental stages. Lack of new protein synthesis during early developmental stages has been shown in other animals (Tyler, 1967; Denis, 1974; Ballantine et al, 1979). The early rise of RNA content in rohu embryo indicated an earlier beginning of the protein synthesis, which correlated well with the earlier rise in the protein content in this species.

Catabolism of protein and nucleic acids release ammonia as a by-product. Ammonia is extremely toxic and cannot be retained byond a certain level in the cell. It was observed in the present study that the unfertilized egg of scale carp and rohu contained a significant amount of ammonia. The level increased in the embryo with development and at the pre-hatching stage its concentration was higher or near the values in their adult tissues. Similar high accumulation of ammonia has been also reported in salmon (Rice & Stokes, 1973). It has been shown that eggs and embryos of salmon are more resistant to ammonia toxicity than their adults. The high concentration of endogenous ammonia in carp embryo might indicate their better resistance capacity to ammonia toxicity than adults. The functional significance of the accumulation of ammonia might be for recycling of nitrogen in the embryo because of the impermeable nature of the egg membrane to amino acids and nucleotides (Blaxter, 1969; Neyfakh & Abramova, 1974). The overall accumulation of ammonia was found to be higher in scale carp than in rohu. This was parallel to the higher rate of catabolism of protein in the former species. It was further observed that though, in general, the ammonia level of embryos increased with development, but at certain stages of development, it decreased or remained constant, in comparison to their immediate previous stages of development. Such fluctuations may be associated with its differential rate of production, its conversion to other products and diffusion out of the embryo.

Studies on some enzymes associated with ammonia metabolism (GDH, PDG, GS and arginase) showed different patterns. Some of the enzymes such as arginase and GS in both the species and PDG in scale carp were present in all stages 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 relatively maintained during the cleavage stages and showed the enhancement of their activity in some stages after the morphogenetic movement had started. In a few cases, the enzyme activity decreased at particular stages. The accumulation of some enzymes in active state and some in inactive stage during oogenesis has been known (Brachet, 1974). 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 was maintained for sometime followed by a decrease 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. Similar observations have been reported by Shaklee and Whitt (1977) for the enzymes of some other metabolic pathways during the development of sunfish. The activation or repression of enzyme activity has been studied as models for gene activation and repression in different animals both in adults and embryos (Davidson, 1976; Scandalios, 1979).

The fluctuations in the endogenous ammonia and urea levels during the development of the scale carp and rohu embryo and lack of a definite correlation with the 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.

Functional differentiation during development has been coupled with differentiation of specific enzymes. AChE is one of such enzymes associated with the functional differentiation of nerve and muscle cells (Brachet, 1974). In the present study, AChE activity in the embryo was detected at the onset of gastrulation in both the species. This is similar to earlier report in

Xenopus showing a high induction of the enzyme activity at gastrulation (Atherton & Lee, 1975). Although it is difficult to explain a definite functional significance of the appearance of AChE at gastraulation, 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 is also occurred at a large scale, probably 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 directly differentiation related gene activation.

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, studied in scale carp and rohu. In the mitochondrial fraction, TAT activity (m-TAT) was detected only at neurulation in scale carp and at 8-somites 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 (Sereni et al, 1959; Koler et al, 1969; Holt & Oliver, 1971). The difference 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, may suggest a different way of regulation of the two isoenzymes in the two carp species.

The developmental pattern of all enzymes of 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 enzymes activities were either not affected or increased. Similar contradictory 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 also were different, possibly, due to their difference in genetically controlled biochemical regulations.

Effect of higher altitude :

Analysis of physico-chemical factors in identically managed fish ponds at Gauhati (100 m ASL) and at a moderately higher altitude (1500 m ASL) at Shillong revealed that temperature and pH, in general, were lower and free CO_2 was higher at the higher altitude. Dissolved oxygen content did not show much difference between the two altitudes. The low water temperature at higher altitude was the result of low atmospheric temperature which is the general character of the higher altitude. The low pH of pond water at higher altitude was due to inflow of acidic water from a stream to the experimental pond to maintain the water level. The acidic nature of the stream water might be due to the general acidic nature of the soil and falling of pine needles which are known to be acidic in nature. The high CO_2 content of Shillong water was attributed to an independent or combined effect of low algal production, higher rainfall or higher production of CO_2 at low pH.

Attempts to breed rohu at Shillong was not successful because none of the breeders brought from the Gauhati survived. However, when tried at a comparatively lower altitude at Mawpun (1000m ASL), there was some success. All breeders survived only once in 1979. However, the female breeders brought from Gauhati failed to survive in all other attempts. In contrast to rohu, the scale carp breeders brought from Gauhati under identical conditions of transportation, survived even at Shillong. Thus, it was evident that the capacity to adapt to higher altitude water was present in scale carp breeders and was lacking in rohu. The susceptibility of rohu breeders increased with increase in altitude. The tolerance capacity to withstand the high altitude stress was less in the females. The weakness of the females and their physical exhaustion due to large energy expenditure in maturation of eggs have been reported to make the female breeders highly sensitive (Love, 1970).

The response to hypophysation, rate of fertilization, rate of development and survival of both scale carp and rohu were found to be different from the normal pattern at higher altitude. The intensity of such effect was more profound in rohu than in scale carp, though the former was bred at about 500 m below the high altitude level for scale carp (1500 m ASL). The spawning of the two carps at higher altitude was generally partial except for once in scale carp in 1979. This might be due to the existing lower temperature of the water which was either below or near the lower limit of the optimal range. The requirement of optimum temperature for successful spawning by hypophysation has been stressed by several workers (Alikunhi *et al*, 1964; Anonymous, 1967; Chaudhuri, 1976). Besides spawning, the rate of fertilization and development of the two carps at higher altitude were also low. This might be the combined effect of low water temperature and low pH at higher altitude. It has been shown that low pH imparis the fertilization of fish egg (Saha *et al*, 1957; Craig & Baksi, 1977). Blaxter (1969) has reported that low temperature and low pH retards the rate of development in fish embryo. Therefore, the adverse effect of high altitude on the two carps must be the cumulative effect of more than one factor which were different at higher altitude.

Survival of both scale carp and rohu were considerably lower at higher altitude. The effect was more pronounced in rohu particularly during the later stages of development including the production of hatchlings and frys. Susceptibility of embryos to high altitude conditions in both the carps was stage specific. In scale carp, mortality of embryo started only from the stage with the onset of blood circulation, whereas all stages of development in rohu were vulnerable to higher altitude. However, maximum mortality occurred at neurulation and before hatching in rohu. Difference in the tolerance capacity of embryos to environmental stress has been reported in various species of fish (Gottwald, 1965; Combs, 1965; Peterson *et al*, 1977; Brooke & Colby, 1980). Non-availability of an optimum temperature and pH were probably the obvious leading conditions for mortality. In scale carp, during a particular breeding in 1979 at Shillong, the higher mortality of embryos occurred due to the attack of *Saprolegnia*, which is known to cause catastrophic infection in fish embryos (Bauer *et al*, 1973). Low temperature and low pH, as observed at higher altitude, are shown to favour the growth of this fungi (Suzuki, 1976). In general, scale carp had lesser problems than rohu at higher altitude. In rohu, the rate of survival showed a clear-cut relationship with water temperature with the embryos showing better survival at or above 24°C. It is known that a drastic change in environmental condition deprives the organism from compensating so efficiently its metabolism, and that becomes dreadful for survival (Braum, 1978; Smit, 1980). Thus, during the development of rohu, a sudden reduction of water temperature from 25°C to 15°C had been found to kill virtually all the embryos at pre-hatching stage at higher altitude.

Survival studies of young rohu at higher altitude showed that 1- and 2- month old juveniles failed to adapt at Shillong water and 3- month old rohu showed better (60%) survival. This indicates a gradual development of adaptive capacity in rohu with increasing age. Higher susceptibility of younger fishes to stress than their adults has been reported in some species (Cairns & Scheir, 1958; Smith & Oseid, 1974).

The changes, observed in the rate of development and survival of scale carp and rohu at higher altitude, may be reflected at biochemical and physiological levels. The present study on the biochemical components during the early development of the scale carp and rohu revealed several variations from the normal pattern at higher altitude. The magnitude of such changes were more in those stages which showed a higher rate of mortality and was generally more significant in rohu.

The low water content and DMC of the embryos at higher altitude were indicative of a low rate of metabolism and a low rate of conversion of yolk substances. This was reflected by a low rate of development of embryos of scale carp at higher altitude. In rohu, at neurulation and pre-hatching stages/the embryos showed a sudden rise in water content and a simultaneous fall in DMC. This might be suggestive of sudden acceleration in degradation of inert substances for energy production without any considerable increase in the rate of synthesis. As a result, more water was probably imbibed from outside to avoid a shrinkage in the egg. The rise in water and decline in DMC at neurulation and pre-hatching stage of rohu indicated a physiological stress which might have resulted into the higher mortality during these stages.

Studies on the protein contents showed no appreciable alterations in scale carp embryos. The changes in rohu embryos at the higher altitude was more significant. The total, s-, c- and m- protein exhibited a lower level than normal from neurulation onwards. In scale carp, m-protein showed no alteration at higher altitude. This may be suggestive of a more physiological vulnerability of the rohu embryos than that of scale carp. The lowering of protein contents of embryos of the two carps may be attributed to a higher rate of degradation of yolk protein and/or a concurrent lower rate of synthesis of new proteins under the effect of higher altitude. This has been supported by the findings on FAA and RNA content of the embryos.

The level of FAA is known to be a sensitive indicator for assessing physiological stress in fish (Atherton & Aitken, 1970; Knapp & Wieser, 1980). Therefore, the observed fluctuations in FAA level might indicate a physiological disorder of the carp embryos developed at higher altitude. During these stages either FAA might have been catabolized extensively to meet the high energy demand of the embryos or protein synthesis might have been inhibited. Studies on individual FAA also showed that their relative proportion in the FAA pool was considerably changed at some developmental stages at the higher altitude. All these indicated that the normal rate of yolk degradation, synthesis of new proteins and amino acids, and catabolism of FAA were different at higher altitude.

The level of RNA became significantly lower during the later stages of development in both the species at higher altitude. The level of RNA indicates the rate of protein synthesis and growth (Bulow, 1970; Hains, 1980). Therefore, the low level of RNA in the differentiating embryos could be interpreted as their low rate of protein synthesis and growth in the two species at higher altitude. Besides RNA, DNA content has also been known to be an index of growth rate (Bulow, 1970; Hains, 1980). DNA content also became significantly lower at the stages nearer hatching in both the species at higher altitude. This might have resulted due to less number of cells indicating slow growth of the two carps at higher altitude. In S. gairdneri, Zeitoun et al (1977) reported a large decline of DNA content during early development which they explained to be due to its catabolism to meet the energy demand of embryos. Catabolism of DNA for procurement of energy might have been one of the reasons for the decline in DNA level in the embryos at the higher altitude. However, this conclusion look to be little far fetched.

The changes in the protein and FAA content in the embryos at higher altitude have indicated a possible alteration in the ammonia metabolism. Estimations of ammonia and urea concentrations in scale carp and rohu embryos revealed that the endogenous level of ammonia 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, even 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 ammonia metabolising enzymes studied.

Activity of ammonia metabolising enzymes studied (GDH, PDG, 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.

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 stage-9 to stage-11 in rohu indicating the delay in functional differentiation of neural and muscular tissues. In c-TAT and m-TAT activities, there was no alteration in the appearance of the enzyme at higher altitude. However, during the later stages of development c-TAT activity increased in scale carp and m-TAT activities decreased in rohu at higher altitude.

One basic information which seems apparent from the observations stated in the foregoing chapters that during the ontogenic development of carps studied, the early stages of development, dominated by fast rate of cell multiplication, is less sensitive to environmental fluctuations than the later stages of development, dominated by differentiation and morphogenesis. This could be possibly due to the fact that besides supplying energy, the deposited yolk in the egg contains necessary precursors and enzymes for completing the cleavage, and due to less number of cells and their simpler organization, the gaseous exchange and metabolic waste diffusion take place efficiently. However, morphogenetic movements started from gastrulation followed by differentiation and organogenesis involving 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 <u>in vivo</u> becomes so complicated 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 nutrients 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, 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.

TABLES

Table 1 : Seasonal variation of physico-chemical factors of the managed nursery ponds at two different altitudes (Gauhati, 100m and Shillong, 1500m).

Month	Gauhati (100m)					Shillong (1500m)				
	Air temp. (°C)	Water Temp. (°C)	Dissolved Oxygen (mg/l)	Free CO_2 (mg/l)	pH	Air temp. (°C)	Water temp. (°C)	Dissolved Oxygen (mg/l)	Free CO_2 (mg/l)	pH
1979										
Jan.	18	19.5	7.9	2.2	7.1	12	12.5	6.8	4.9	7.0
Feb.	17.5	17	8.5	0.4	7.8	15	13	6.08	5.0	6.3
Mar.	29	25	10.0	0.2	8.2	21	19	7.2	5.0	6.6
Apr.	26	25	9.6	0.6	7.6	21.5	20	9.6	4.0	7.0
May	29	27.5	9.6	0.5	7.4	22	21	8.6	4.0	7.0
Jun.	34	35	7.2	0.9	7.6	22.5	21.5	12.8	6.4	7.0
Jul.	32	34.5	11.6	0.4	7.7	22	20	11.6	6.0	7.1
Aug.	31	29	7.5	3.2	7.0	22	24	8.8	3.9	6.1
Sep.	28	29	7.6	1.1	7.6	23	22	6.08	3.4	5.9
Oct.	27	28	7.2	0.9	7.4	22	19	6.0	3.0	6.0
Nov.	24.5	24	8.8	2.1	7.3	17	15	6.8	4.6	5.8
Dec.	25	24	9.4	1.8	7.4	16	16.5	7.2	5.6	6.6
1980										
Jan.	17	15	7.8	2.1	7.6	15	15.5	6.8	4.6	6.8
Feb.	25	24	10.4	1.2	7.5	14.5	15	5.2	5.2	6.7
Mar.	28	26	11.2	0.8	7.8	20	19.5	9.6	6.0	6.9
Apr.	29	27	11.6	1.0	7.8	21	22	9.6	6.0	6.4
May	31	29	10.8	0.6	7.8	21	19	8.8	4.9	5.9
Jun.	32	31	7.2	0.8	8.0	22	23	6.2	5.0	5.8
Jul.	32	33	8.8	0.4	7.5	24	22	7.2	5.6	5.8

Table 2 : Results of induced breeding of C. carpio at two different altitudes (Gauhati, 100m and Shillong, 1500m).

Location	Gauhati (100m)		Shillong (1500m)	
Year-month	1979, Jan.	1980, Mar.	1979, May	1980, May
Temperature (°C) range	17-23.5	19-28	16-20	17-22
Sets of breeders used & wt. of the females	3 250g, 225g & 390g	3 225g, 230g & 280g	3 220g, 230g & 270g	3 250g, 270g & 270g
Percentage success	100	100	100	100
No. of eggs released	20,000	19,000	10,000	20,000
Percentage fertilization	90	94.28	55	74
Percentage hatching	60	73.68	20	34.5
Percentage fry obtained	52.5	61.05	6	20

(% fertilization, % hatching and % fry obtained were calculated taking total eggs released as 100%).

Table 3 : Results of induced breeding of *L. rohita* at three different altitudes (Gauhati, 100m; Mawpun, 1000m & Shillong, 1500m).

Location	Gauhati (100m)		Mawpun (1000m)			Shillong (1500m)	
Year, month	1979, July	1980, June	1979, Aug.	1980, July	1980, Aug.	1979, Aug.	1980, June
Temperature (°C) range	25-31.5	24-31	15-28	*25	*27	*23	*21
Sets of breeders used & wt. of the females	2 450g & 500g	3 450g, 455g & 500g	2 500g & 520g	2 450g & 500g	2 600g & 690g	2 600g & 700g	2 500g & 700g
Percentage success	100	100	100	**0	**0	***0	***0
No. of eggs released	20,000	24,000	15,000	-	-	-	-
Percentage fertilization.	70	55	50	-	-	-	-
Percentage hatching	50	52	0.67	-	-	-	-
Percentage fry obtained	40	38.5	0.24	-	-	-	-

* Temperature at the time of introducing the breeders into the pond.

** All female breeders died.

*** All breeders died.
(% fertilization, % hatching and % fry obtained were calculated taking total eggs released as 100%).

Table 4 : Time required for hatching of *C. carpio* and *L. rohita* at two different altitudes in relation to water temperature.

Species	*C. carpio*				*L. rohita*		
Location	Gauhati(100m)		Shillong (1500m)		Gauhati (100m)		Mawpun (1000m)
Year, month	January, 1979	March, 1980	May, 1979	May, 1980	July, 1979	June, 1980	August, 1979
Water temp. (°C)	17-23.5	18.5-26	16-20	17-21	25-31.5	24-31	15-25
Time required for hatching (hours)	80-83	58-60	126-131	82-85	12-14	12.30-14.05	18-21

Table 5 : The developmental stages of C. carpio and L. rohita

Stage No.	Description
0	Unfertilized egg
1	Fertilized egg
2	Two cells
3	Four cells
4	Eight cells
5	Sixteen cells
*6	Thirty two cells
7	Late morula
8	Blastula
9	Early gastrula
*10	Late gastrula
11	Neurula
12	Embryo with eight-somites
*13	Beginning of eye pigmentation
14	Onset of blood circulation
15a	Pre-hatched embryo
15b	Freshly hatched larva
16	Fry

* could not be observed in L. rohita.

Table 6 : Rate of development of *C. carpio* and the corresponding water temperature at two different altitudes.

Dev. stages	Gauhati (100m) (March, 1980)					Shillong (1500m) (May, 1980)				
	Water temperature (°C)	Time taken for commencement of each stage after fertilization hr.	min.	Duration of each stage hr.	min.	Water temperature (°C)	Time taken for commencement of each stage after fertilization hr.	min.	Duration of each stage hr.	min.
0	20	00	00	00	00	17.5	00	00	00	00
1	20	00	00	00	44	17.5	00	00	00	50
2	19	00	44	00	21	17	00	50	00	26
3	19	01	05	00	20	17	01	16	00	24
4	19	01	25	00	25	17	01	40	00	25
5	18.5	01	50	00	10	17	02	05	00	25
6	19	02	00	00	45	17	02	30	00	40
7	19	02	45	00	45	17	04	10	01	00
8	19	03	30	00	30	17	05	10	00	45
9	19	04	00	06	00	17.5	05	55	04	55
10	23	10	00	02	00	20	10	50	00	45
11	27	12	00	06	00	22	11	30	05	35
12	21.5	18	00	10	00	20	17	05	13	55
13	19	28	00	02	10	21	31	00	02	05
14	19.5	30	10	27	50	18	33	05	48	55
15a	26	58	00	00	00	20	82	00	00	00

Table 7 : Rate of development of *L. rohita* and the corresponding water temperature at two different altitudes.

Dev. stages	Gauhati (100m) (June, 1980)					Mawpun (1000m) (August, 1979)				
	Water temperature (°C)	Time taken for commencement of each stage after fertilization hr.	min.	Duration of each stage hr.	min.	Water temperature (°C)	Time taken for commencement of each stage after fertilization hr.	min.	Duration of each stage hr.	min.
0	25	00	00	00	00	23.5	00	00	00	00
1	24	00	00	00	50	23.5	00	00	00	50
2	24	00	50	00	10	23.5	00	50	00	20
3	24	01	00	00	12	23	1	10	00	10
4	24	1	12	00	08	21	1	20	00	10
5	24	1	20	00	30	22	1	30	00	15
6 ?										
7	24	1	50	00	22	22.5	1	45	00	31
8	24.5	2	12	00	05	24.5	2	16	00	06
9	24.5	2	17	00	33	24	2	22	00	33
10 ?										
11	24.5	2	50	01	10	24	2	55	01	18
12	25	4	00	00	55	25	4	13	00	47
13 ?										
14	26	4	55	07	35	25	5	00	13	00
15a	31	12	30	00	00	15	18	00	00	00

? stage could not be identified.

Table 8 : Rate of survival of the embryos at some developmental stages of *C. carpio* and *L. rohita* at different altitudes in relation to water temperature.

Dev. stages	*C. carpio*				*L. rohita*			
	Gauhati (100m)		Shillong (1500m)		Gauhati (100m)		Mawpun (1500m)	
	WT	Survival percentage	WT	Survival percentage	WT	Survival percentage	WT	Survival percentage
1	20	96.5	17.5	99	25	98	24	85
4	19	99.5	17	99	24	100	21	78
7	19	99.7	17	98	24	100	22.5	93.5
9	19	99.5	17.5	98	24.5	99.5	24	94
11	27	99	22	99	24.5	99	24	51
14	19.5	99.5	18	81.5	26	100	25	95.5
15a	26	95.8	21	61.5	31	97.5	15	7
15b	28	85	21	84	31	98.5	16	24
16	27	82	20	52	28	74	24	36

Survival percentage of a stage has been calculated as follows :

$$\text{Survival percentage} = \frac{(N - N_1) \times 100}{N}$$

where N = known number of embryo of a particular stage

N_1 = number of embryos which failed to proceed to the next stage

WT = water temperature in °C

Table 9 : Changes in wet wt. (mg) of the embryo during early development of C. carpio and L. rohita at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	1.68±0.11	1.62±0.1	N.S.	0.875±0.015	0.876±0.004	N.S.
1	2.1±0.13 (+25.0)***	1.91±0.17 (+17.9)*	N.S.	0.913±0.009 (+4.34)***	0.912±0.006 (+4.11)***	N.S.
2	2.28±0.14 N.S.	1.94±0.21 N.S.	(-14.91) *	0.933±0.007 (+2.19)*	0.931±0.008 (+2.08)**	N.S.
3	2.41±0.17 N.S.	1.96±0.16 N.S.	(-14.78) *	0.951±0.009 (+1.93)**	0.949±0.005 (+1.93)***	N.S.
4	2.4±0.01 N.S.	1.97±0.11 N.S.	(-17.92) ***	0.951±0.009 N.S.	0.95±0.003 N.S.	N.S.
5	2.4±0.02 N.S.	1.99±0.06 N.S.	(-17.08) ***	0.96±0.005 (+0.95)*	0.954±0.006 N.S.	N.S.
6	2.4±0.02 N.S.	1.99±0.04 N.S.	(-17.08) ***	?	?	
7	2.4±0.003 N.S.	2.01±0.04 N.S.	(-16.25) ***	0.959±0.009 N.S.	0.954±0.006 N.S.	N.S.
8	2.46±0.2 N.S.	2.01±0.02 N.S.	(-18.29) **	0.963±0.01 N.S.	0.958±0.004 N.S.	N.S.
9	2.39±0.06 N.S.	2.02±0.06 N.S.	(-15.48) ***	0.966±0.003 N.S.	0.961±0.003 N.S.	N.S.
10	2.41±0.1 N.S.	2.03±0.004 N.S.	(-15.77) ***	?	?	
11	2.4±0.07 N.S.	2.04±0.03 N.S.	(-15.0) ***	0.969±0.008 N.S.	0.963±0.004 N.S.	N.S.
12	2.41±0.02 N.S.	2.12±0.01 (+3.92)**	(-12.03) ***	0.977±0.01 N.S.	0.962±0.002	(-1.54) *
13	2.41±0.01 N.S.	2.25±0.03 (+6.13)***	(-6.64) ***	?	?	
14	2.41±0.21 N.S.	2.27±0.22 N.S.	N.S.	0.978±0.006 N.S.	0.965±0.002 N.S.	(-1.33) **
15a	2.42±0.11 N.S.	2.36±0.12 N.S.	N.S.	0.981±0.011 N.S.	0.961±0.009 N.S.	(-2.04) *
15b	1.85±0.1 (-23.55)***	1.73±0.08 (-26.7)***	N.S.	0.885±0.005 (-9.79)***	-	

? stage could not be observed; - embryos were not enough for estimation; * p<0.05; ** p<0.01; *** p<0.001; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 10 : Changes in dry matter content (mg/embryo) during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	0.531±0.015	0.535±0.016	N.S.	0.377±0.012	0.377±0.003	N.S.
1	0.494±0.016 (-6.97)**	0.501±0.021 (-6.36)*	N.S.	0.343±0.007 (-9.02)***	0.345±0.004 (-8.49)***	N.S.
2	0.493±0.015 N.S.	0.461±0.082 N.S.	N.S.	0.335±0.001 (-2.33)***	0.332±0.009 (-3.77)***	N.S.
3	0.491±0.005 N.S.	0.468±0.044 N.S.	N.S.	0.339±0.013 N.S.	0.342±0.014 N.S.	N.S.
4	0.494±0.005 N.S.	0.469±0.07 N.S.	N.S.	0.33±0.007 (-2.65)*	0.322±0.005 (-5.85)***	N.S.
5	0.498±0.024 N.S.	0.438±0.066 N.S.	N.S.	0.31±0.012 (-6.06)*	0.317±0.012 N.S.	N.S.
6	0.501±0.018 N.S.	0.453±0.038 N.S.	N.S.	?	?	
7	0.473±0.011 (-5.59)**	0.454±0.031 N.S.	N.S.	0.286±0.003 (-7.74)*	0.29±0.03 N.S.	N.S.
8	0.51±0.017 N.S.	0.425±0.075 N.S.	N.S.	0.278±0.005 (-2.8)**	0.294±0.028 N.S.	N.S.
9	0.48±0.101	0.415±0.096 N.S.	N.S.	0.261±0.002 (-6.12)***	0.273±0.018	N.S.
10	0.5±0.02 N.S.	0.419±0.086 N.S.	N.S.	?	?	
11	0.436±0.009 (-12.8)***	0.423±0.025 N.S.	N.S.	0.246±0.003 (-5.75)***	0.203±0.017 (-25.64)***	(-17.5) ***
12	0.432±0.008 N.S.	0.403±0.026 N.S.	N.S.	0.242±0.002 (-1.63)***	0.205±0.018	(-15.29) **
13	0.421±0.003 (-2.55)***	0.429±0.065 N.S.	N.S.	?	?	
14	0.405±0.013 (-3.8)*	0.375±0.055 N.S.	N.S.	0.24±0.008 N.S.	0.199±0.007 N.S.	(-17.08) ***
15a	0.386±0.014 (-4.69)***	0.399±0.013 N.S.	N.S.	0.235±0.002 N.S.	0.175±0.009 (-12.06)**	(-25.53) ***
15b	0.364±0.006 (-5.7)*	0.376±0.001 (-5.76)*	N.S.	0.225±0.008 (-8.51)*	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 11 : Changes in percent water content of the embryo during early development of *C. carpio* and *L. rohita* at different altitudes (Values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	68.39±0.52	66.98±1.42	N.S.	56.91±0.72	56.96±0.62	N.S.
1	76.48±1.72 (+11.83)***	73.77±1.43 (+10.14)***	N.S.	62.43±1.14 (+9.7)***	62.17±1.09 (+9.15)***	N.S.
2	78.38±1.05 N.S.	76.24±4.29 N.S.	N.S.	64.09±0.56 (+2.66)*	64.34±0.52 (+3.49)*	N.S.
3	79.62±0.35 N.S.	76.12±3.88 N.S.	N.S.	64.36±1.44 N.S.	63.96±0.65 N.S.	N.S.
4	79.42±0.39 N.S.	76.2±4.05 N.S.	N.S.	65.34±1.75 N.S.	66.11±1.66 N.S.	N.S.
5	79.25±0.58 N.S.	77.99±4.44 N.S.	N.S.	67.71±1.85 N.S.	66.77±1.61 N.S.	N.S.
6	79.13±0.42 N.S.	77.24±2.46 N.S.	N.S.	?	?	
7	80.2±0.56 (+1.35)*	77.41±3.05 N.S.	N.S.	70.18±1.95 N.S.	69.61±1.53 (+4.25)*	N.S.
8	79.27±0.72 N.S.	78.86±4.59 N.S.	N.S.	71.13±1.44 N.S.	69.31±1.87	N.S.
9	79.92±6.14 N.S.	79.46±4.62 N.S.	N.S.	72.98±0.59 N.S.	71.59±2.21 N.S.	N.S.
10	79.25±0.54 N.S.	79.36±0.59 N.S.	N.S.	?	?	
11	81.53±0.48 (+3.26)**	79.27±3.56 N.S.	N.S.	74.61±0.56 (+2.23)*	78.92±1.05 (+10.24)***	(+5.78) ***
12	82.08±0.48 N.S.	80.99±2.59 (+2.17)*	N.S.	75.23±0.42 N.S.	78.69±0.65 N.S.	(+4.59) ***
13	82.53±0.29 N.S.	80.93±4.02 N.S.	N.S.	?	?	
14	83.2±0.69 N.S.	83.48±3.11 (+2.67)*	N.S.	75.46±0.49 N.S.	79.38±0.69 N.S.	(+5.2) ***
15a	84.05±0.78 N.S.	83.09±1.28 N.S.	N.S.	76.05±0.14 N.S.	81.79±0.62 (+3.04)**	(+7.55) ***
15b	79.78±0.59 (-5.08)***	77.69±1.55 (-6.94)***	N.S.	75.71±0.24 (-0.45)*	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 12 : Changes in Total (TCA ppt.) protein content (mg/embryo) x 10^2 during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	30.83±0.34	30.6±1.72	N.S.	13.67±1.5	13.65±0.57	N.S.
1	20.31±0.24 (-34.12)***	20.64±1.78 (-32.55)***	N.S.	8.89±1.42 (-34.97)***	9.1±1.85 (-33.33)**	N.S.
2	18.23±0.34 (-10.24)***	20.11±1.23 N.S.	N.S.	8.82±0.23 N.S.	8.83±0.23 N.S.	N.S.
3	18.14±0.34 N.S.	19.48±1.03 N.S.	N.S.	8.89±0.13 N.S.	8.79±0.42 N.S.	N.S.
4	18.14±0.39 N.S.	18.88±0.75 N.S.	N.S.	8.76±0.08 (-1.46)*	8.68±0.47 N.S.	N.S.
5	18.02±0.4 N.S.	18.52±1.73 N.S.	N.S.	8.32±0.16 (-5.02)**	8.31±0.41 N.S.	N.S.
6	17.73±0.44 N.S.	17.41±1.37 N.S.	N.S.	?	?	
7	16.37±0.23 (-7.67)**	16.53±0.59 N.S.	N.S.	8.49±0.32 N.S.	8.41±0.48 N.S.	N.S.
8	19.69±0.65 (+20.28)***	20.45±0.41 (+23.71)***	N.S.	8.94±0.13 (+5.3)*	8.91±0.14 N.S.	N.S.
9	18.28±0.26 (-7.16)**	19.67±0.7 N.S.	N.S.	9.45±0.2 (+5.7)**	9.42±0.18 (+5.72)**	N.S.
10	18.52±0.34 N.S.	18.99±0.96 N.S.	N.S.	?	?	
11	18.78±0.32 N.S.	18.27±0.35 N.S.	N.S.	10.11±0.24 (+6.98)**	8.44±0.36 (-10.4)***	(-16.52) ***
12	18.82±0.44 N.S.	17.77±1.34 N.S.	N.S.	11.19±0.31 (+10.68)***	8.76±0.27 N.S.	(-21.72) ***
13	18.43±0.74 N.S.	18.26±0.51 N.S.	N.S.	?	?	
14	17.33±0.35 (-5.97)*	16.5±0.95 (-9.64)*	N.S.	12.68±0.37 (+13.32)**	9.55±0.13 (+9.02)**	(-24.69) ***
15a	17.27±0.22 N.S.	14.37±0.78 N.S.	(-16.89) ***	13.36±0.16 (+5.36)***	9.61±0.66 N.S.	(-28.07) ***
15b	15.68±0.61 (-9.31)**	12.72±0.97 (-11.48)*	(-18.88) ***	12.87±0.24 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M in parentheses show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 13 : Changes in Total (soluble) protein content (mg/embryo) x 10^2 during early development of C. carpio and L. rohita at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	C. carpio			L. rohita		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	30.09±2.59	30.13±1.66	N.S.	13.42±1.24	13.18±0.97	N.S.
1	19.78±2.0 (-35.26)***	20.54±1.19 (-31.83)***	N.S.	8.73±0.94 (-34.95)***	8.37±0.36 (-36.5)***	N.S.
2	17.34±1.25 N.S.	19.88±2.63 N.S.	N.S.	8.67±1.22 N.S.	8.86±1.65 N.S.	N.S.
3	18.03±1.49 N.S.	19.4±1.35 N.S.	N.S.	8.67±0.62 N.S.	8.57±0.97 N.S.	N.S.
4	18.06±0.59 N.S.	18.19±1.93 N.S.	N.S.	8.48±1.06 N.S.	8.48±1.42 N.S.	N.S.
5	18.06±1.91 N.S.	18.34±1.5 N.S.	N.S.	7.82±2.43 N.S.	7.54±0.79 N.S.	N.S.
6	17.82±0.96 N.S.	17.54±1.82 N.S.	N.S.	?	?	
7	16.81±0.21 N.S.	16.74±1.07 N.S.	N.S.	7.54±0.32 N.S.	7.54±0.23 N.S.	N.S.
8	19.62±1.53 (+16.72)*	20.63±1.43 (+23.24)**	N.S.	7.83±0.38 N.S.	8.67±0.77 (+14.99)*	N.S.
9	18.2±2.52 N.S.	19.48±2.21 N.S.	N.S.	8.51±0.21 (+8.7)*	8.9±0.34 N.S.	N.S.
10	18.06±1.93 N.S.	18.04±0.97 N.S.	N.S.	?	?	
11	18.29±1.52 N.S.	18.04±0.67 N.S.	N.S.	9.2±0.39 (+8.11)*	8.02±0.26 (-9.9)**	(-12.83) **
12	18.32±0.38 N.S.	16.63±1.3 N.S.	(-9.22)	9.49±0.34 N.S.	6.56±0.39 (-17.84)***	(-30.88) **
13	17.52±0.24 (4.37)**	16.02±0.86 N.S.	(-8.56) *	?	?	
14	16.14±1.03 (-7.88)*	14.03±0.35 (-12.44)**	(-13.07) *	9.98±0.81 N.S.	7.73±0.89 N.S.	(-22.55) **
15a	15.79±1.46 N.S.	12.87±0.66 (-8.27)*	(-18.4) *	9.73±0.15 N.S.	6.26±0.81 N.S.	(-35.67) ***
15b	13.83±0.59 (-12.41)*	10.98±0.53 (-14.69)***	(-20.61) *	9.65±0.36	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M in parentheses show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 14 : Changes in cytoplasmic protein content (mg/embryo) $\times 10^2$ during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	28.67±0.03	29.65±0.603	N.S.	12.88±0.24	13.03±0.71	N.S.
1	19.79±0.69 (-30.97)***	19.78±0.99 (-33.29)***	N.S.	(-36.64)***	(-38.37)***	N.S.
2	16.98±0.93 (-14.2)**	19.12±0.94 N.S.	N.S.	8.1±0.68 N.S.	8.13±0.66 N.S.	N.S.
3	17.52±0.92 N.S.	19.2±1.22 N.S.	N.S.	8.15±0.54 (-7.48)*	7.98±0.58 N.S.	N.S.
4	16.74±0.74 N.S.	18.22±0.77 N.S.	N.S.	7.54±0.44 (-7.48)*	7.49±0.61 N.S.	N.S.
5	16.86±0.43 N.S.	17.48±0.99 N.S.	N.S.	7.02±0.54 N.S.	7.1±0.62 N.S.	N.S.
6	16.68±0.85 N.S.	16.34±0.85 N.S.	N.S.	?	?	
7	15.66±0.39 N.S.	15.36±1.03 N.S.	N.S.	7.02±0.54 N.S.	6.57±0.28 N.S.	N.S.
8	18.3±0.99 (+16.86)**	19.27±0.99 (+25.46)***	N.S.	7.11±0.75 N.S.	7.46±0.47 (+13.55)*	N.S.
9	16.8±1.15 N.S.	18.44±0.68 N.S.	N.S.	7.54±0.55 N.S.	7.38±0.59 N.S.	N.S.
10	17.04±0.79 N.S.	17.64±1.17 N.S.	N.S.	?	?	
11	17.1±1.19 N.S.	16.84±0.98 N.S.	N.S.	8.04±0.69 N.S.	7.01±0.24 N.S.	(-12.81) *
12	16.74±0.48 N.S.	15.53±0.51 N.S.	(-7.23) *	8.22±0.28 N.S.	5.58±0.31 (-20.39)*	(-32.12)
13	15.79±0.57 (-5.68)*	14.15±1.1 (-8.89)*	(-10.39) *	?	?	
14	13.63±1.29 (-30.68)*	11.94±0.39 (-15.62)***	(-12.4) *	8.12±0.35 N.S.	5.39±0.61 N.S.	(-33.62) ***
15a	13.39±1.08 N.S.	10.32±1.08 (-13.57)*	(-22.99) **	7.68±1.25 N.S.	4.88±0.55 N.S.	(-28.17) **
15b	10.63±1.26 (-20.61)*	8.03±0.74 (-22.1)***	(-24.46) *	7.44±1.17 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M in parentheses show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 15 : Changes in mitochondrial protein content (mg/embryo) $\times 10^2$ during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	0.62±0.15	0.78±0.07	N.S.	0.56±0.15	0.56±0.05	N.S.
1	0.79±0.05 N.S.	0.77±0.09 N.S.	N.S.	0.57±0.06 N.S.	0.56±0.12 N.S.	N.S.
2	0.73±0.08 N.S.	0.74±0.18 N.S.	N.S.	0.6±0.05 N.S.	0.58±0.07 N.S.	N.S.
3	0.72±0.06 N.S.	0.81±0.08 N.S.	N.S.	0.55±0.06 N.S.	0.69±0.12 N.S.	N.S.
4	0.78±0.09 N.S.	0.79±0.11 N.S.	N.S.	0.6±0.13 N.S.	0.61±0.07 N.S.	N.S.
5	0.8±0.12 N.S.	0.75±0.06 N.S.	N.S.	0.61±0.18 N.S.	0.61±0.07 N.S.	N.S.
6	0.77±0.38 N.S.	0.78±0.16 N.S.	N.S.	?	?	
7	0.89±0.11 N.S.	0.88±0.11 N.S.	N.S.	0.66±0.25 N.S.	0.6±0.08 N.S.	N.S.
8	0.89±0.11 N.S.	0.9±0.19 N.S.	N.S.	0.66±0.03 N.S.	0.63±0.08 N.S.	N.S.
9	1.05±0.17 N.S.	0.96±0.21 N.S.	N.S.	0.83±0.04 (+25.76)***	0.8±0.11 (+24.11)*	N.S.
10	1.14±0.12 N.S.	1.12±0.16 N.S.	N.S.	?	?	
11	1.23±0.03 N.S.	1.14±0.22 N.S.	N.S.	0.89±0.04 (+7.23)*	0.76±0.05 N.S.	(-14.61) *
12	1.54±0.19 (+15.2)*	1.13±0.37 N.S.	N.S.	1.06±0.08 (+19.2)**	0.91±0.08 (+19.74)**	(-14.15) **
13	1.62±0.19 N.S.	1.81±0.23 (+12.71)*	N.S.	?	?	
14	1.9±0.27 N.S.	2.04±0.17 N.S.	N.S.	1.73±0.09 (+63.2)***	1.06±0.05 (+16.48)**	(-38.73) ***
15a	2.12±0.13 (+11.58)*	2.39±0.27 N.S.	N.S.	2.0±0.11 (+15.61)**	1.57±0.15 (+48.11)***	(-21.5) **
15b	3.01±0.31 (+41.98)**	2.59±0.34 N.S.	N.S.	2.2±0.07 (+10.0)*	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M in parentheses show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 16 : Changes in the total free amino-acid content (mg/embryo) x 10^3 during early development of C. carpio and L. rohita at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	19.57±0.29	20.04±0.34	N.S.	11.5±1.1	10.8±1.3	N.S.
1	20.72±0.27 (+5.88)**	21.81±0.38 (+8.83)***	N.S.	19.2±1.1 (+66.96)***	14.2±1.1 (+31.4)*	(-26.04) ***
2	21.28±0.34 N.S.	22.21±0.85 N.S.	N.S.	17.5±1.1 N.S.	14.8±1.8 N.S.	(-15.43) *
3	22.68±0.39 (+6.58)*	21.14±1.06 N.S.	N.S.	16.6±1.7 N.S.	15.5±1.4 N.S.	N.S.
4	21.63±1.05 (-4.63)*	22.04±1.15 N.S.	N.S.	18.4±0.9 N.S.	16.8±1.9 N.S.	N.S.
5	23.95±0.72 (+10.73)*	22.31±1.19 N.S.	N.S.	19.9±0.7 N.S.	18.5±0.7 N.S.	N.S.
6	24.03±0.34 N.S.	21.78±1.7 N.S.	(-9.36) *	?	?	
7	24.69±0.29 N.S.	23.68±0.52 N.S.	(-4.09) *	20.5±1.0 N.S.	20.1±0.9 (+8.65)*	N.S.
8	27.8±0.35 (+12.6)***	24.16±1.36 N.S.	(-13.09) **	19.6±0.8 N.S.	17.2±1.2 (-14.43)*	(-12.25) *
9	22.48±0.61 (-19.14)***	24.48±0.1 N.S.	(+8.9) ***	14.3±1.1 (-27.04)***	15.2±4.7 N.S.	N.S.
10	22.44±0.42 N.S.	24.12±0.87 N.S.	(+7.58) *	?	?	
11	21.7±0.73 N.S.	25.6±1.57 N.S.	(+17.97) **	16.0±1.3 N.S.	18.5±1.5 N.S.	(+15.63) *
12	20.99±0.89 N.S.	28.56±0.99 (+11.56)***	(+36.07) ***	13.6±1.2 (-15.0)*	16.3±1.2 N.S.	(+19.85) *
13	22.6±0.44 (+7.67)***	30.46±1.71 N.S.	(+34.78) ***	?	?	
14	24.94±0.46 (+10.35)***	31.17±0.99 N.S.	(+24.98) ***	12.4±2.4 N.S.	16.3±2.1 N.S.	(+31.45) *
15a	24.66±1.59 N.S.	32.15±1.17 N.S.	(+30.37) **	9.3±1.3 (-25.0)*	14.6±1.5 N.S.	(+56.99) *
15b	17.8±0.45 (-27.82)***	24.63±1.59 (-23.39)***	(+38.37) ***	8.97±0.24 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M in parentheses show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 17 : Qualitative and quantitative % individual free amino-acid and total free amino-acid (mg/embryo x 10^3) changes of free amino acids during early development of <u>C. carpio</u> at Gauhati (100m).

Amino acid	DEVELOPMENTAL STAGES																
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15a	15b
Glutamic acid	13.9	14.1	13.6	13.9	14.1	14.6	14.6	14.1	14.4	14.7	15.9	16.8	17.2	17.2	17.3	18.7	17.2
Isoleucine	4.9	5.1	5.0	4.8	4.9	5.3	5.3	5.2	6.4	8.0	7.8	7.6	6.4	5.8	5.8	6.4	6.9
Aspartic acid	10.7	8.2	8.7	8.5	8.5	8.3	8.2	8.1	9.5	9.6	10.4	10.6	10.0	9.6	9.2	8.9	9.4
Arginine	6.5	6.3	6.2	6.6	6.7	6.6	6.6	6.5	5.7	6.5	5.7	5.4	5.1	5.0	4.9	4.7	3.9
Serine	5.6	5.9	5.6	5.6	5.6	5.3	5.6	5.5	5.4	5.5	5.7	5.0	4.7	4.6	4.3	3.6	3.7
Glycine	2.1	2.2	2.0	2.1	2.2	2.1	2.1	2.1	1.8	2.1	4.0	3.2	4.5	4.8	5.0	5.1	5.5
Threonine	5.3	5.2	5.0	4.9	5.0	4.8	4.9	4.8	5.8	5.1	5.1	5.2	5.1	5.1	5.0	3.9	3.1
Alanine	8.6	8.6	8.6	8.4	8.4	8.4	8.3	8.1	7.1	8.7	8.8	9.0	8.7	8.8	5.1	6.5	7.2
Proline	8.3	8.3	8.1	8.0	8.0	7.9	7.8	7.5	6.6	7.8	6.2	4.9	4.6	4.5	4.4	10.1	12.6
Valine	4.3	3.8	4.1	4.1	4.1	4.0	4.0	3.9	3.7	3.8	3.8	3.9	4.2	4.1	4.0	2.0	1.0
Methionine	3.3	3.3	3.6	3.5	3.5	3.5	3.5	3.3	3.5	3.7	3.6	3.6	3.4	3.3	3.2	4.3	4.3
Lysine	8.9	9.1	8.7	8.4	8.4	8.4	8.4	8.2	7.1	3.4	3.9	4.8	5.5	8.1	8.4	10.6	11.2
Leucine	8.6	8.5	8.5	8.6	8.3	8.2	8.3	8.0	7.3	6.0	5.9	5.8	5.5	5.3	5.3	5.0	3.5
Phenylalanine	5.8	5.8	5.8	5.8	5.6	5.9	6.0	5.9	6.0	5.3	5.1	4.9	5.6	4.5	4.4	5.1	5.8
Tyrosine	3.1	3.1	3.0	3.0	2.9	3.0	2.7	2.2	2.2	2.0	2.2	2.2	2.6	2.5	2.5	2.2	1.2
Histidine	N.D.	0.6	1.1	1.0	1.1	1.1	1.4	2.0	2.1	2.4	2.4	2.5	2.7	2.8	3.0	1.8	1.8
Tryptophan	N.D.	1.1	2.1	2.2	2.2	2.2	2.2	2.8	3.1	3.8	3.7	3.8	3.6	3.7	4.6	1.0	0.9
Cysteine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cystine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total amino acid (mg/embryo) x 10^3	19.6 ±0.3	20.7 ±0.3	21.3 ±0.3	22.7 ±0.4	21.6 ±1.1	24.0 ±0.6	24.0 ±0.3	24.7 ±0.3	27.8 ±0.4	22.5 ±0.6	22.4 ±0.4	21.8 ±0.7	21.0 ±0.9	22.6 ±0.4	25.0 ±0.5	24.7 ±1.6	17.8 ±0.5

N.D. = not detected

Table 18 : Qualitative and quantitative % individual free amino acid and total free amino acid (mg/embryo x 10^3) changes of free amino acids during early development of *C. carpio* at Shillong (1500m).

Amino acid	DEVELOPMENTAL STAGES																
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15a	15b
Glutamic acid	13.8	13.0	13.4	13.9	14.1	14.3	14.0	14.1	14.2	14.6	15.9	16.0	17.0	17.6	18.9	19.0	18.5
Isoleucine	4.9	4.9	5.0	5.0	5.0	5.0	4.8	5.0	5.0	5.3	5.3	5.4	5.5	5.1	5.1	5.2	6.2
Aspartic acid	9.9	10.0	10.0	10.1	10.1	10.2	10.0	10.0	10.1	10.1	9.9	9.9	9.8	9.8	9.7	10.6	9.0
Arginine	6.5	6.4	6.3	6.5	6.8	6.7	6.8	6.8	6.0	6.0	5.6	5.4	5.2	4.6	4.8	5.0	3.8
Serine	6.2	6.1	6.0	6.0	6.0	6.0	5.9	5.9	5.9	5.8	5.7	5.6	5.6	5.5	4.3	4.1	3.9
Glycine	2.2	2.1	2.1	2.1	2.1	3.0	3.1	3.1	3.2	3.2	3.2	3.3	3.5	5.0	5.1	5.9	6.8
Threonine	5.1	5.0	5.0	4.9	5.0	4.8	4.9	4.8	5.5	5.1	5.1	5.1	5.2	5.1	5.1	5.2	4.0
Alanine	8.7	8.6	8.5	8.4	8.5	8.4	8.4	8.4	8.5	8.8	9.9	9.8	9.8	8.9	8.9	6.2	6.9
Proline	8.3	8.3	8.1	7.9	7.9	7.5	7.8	8.0	7.9	7.7	8.9	8.5	7.0	6.7	4.7	6.5	8.6
Valine	4.0	4.1	4.1	4.2	4.1	4.0	4.1	4.1	4.2	4.5	5.9	5.0	5.2	4.1	4.1	4.2	2.5
Methionine	3.9	3.5	3.5	3.4	3.4	3.5	3.4	3.3	3.4	4.5	4.2	4.6	4.6	4.4	4.0	3.1	4.2
Lysine	8.9	9.1	8.3	8.4	8.4	8.5	8.5	7.0	7.7	2.8	2.5	3.5	3.9	3.6	5.0	5.0	5.2
Leucine	8.9	8.5	8.6	8.4	8.4	8.6	8.4	8.2	7.8	6.9	5.5	5.2	5.0	5.1	6.0	4.4	4.9
Phenylalanine	5.3	5.7	5.8	5.6	5.7	5.6	5.7	5.6	5.6	5.8	5.1	5.0	4.8	4.5	4.3	4.2	5.9
Tyrosine	3.2	3.2	3.0	3.0	3.1	2.9	3.0	2.9	2.8	3.9	2.9	3.0	3.7	4.0	4.1	4.2	0.5
Histidine	N.D.	0.5	1.0	1.1	1.1	1.0	1.1	1.0	1.0	1.6	1.6	2.2	2.1	2.2	2.4	2.8	2.9
Tryptophan	N.D.	1.1	1.1	1.1	1.0	1.1	1.1	1.0	1.0	2.1	2.1	2.1	2.2	2.3	3.3	3.5	2.1
Cysteine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cystine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total amino acid (mg/embryo) x 10^3	20.0 ±0.3	21.8 ±0.4	22.2 ±0.9	21.1 ±1.1	22.0 ±1.1	22.3 ±1.2	21.8 ±1.7	23.7 ±0.5	24.2 ±1.4	24.5 ±0.1	24.1 ±0.9	25.6 ±1.6	28.6 ±1.0	30.5 ±1.7	31.2 ±1.0	32.2 ±1.2	24.6 ±1.6

N.D. = not detected

Table 19 : Qualitative and quantitative % individual free amino-acid and total free amino-acid (mg/ embryo x 10^3) changes of free amino acids during early development of *L. rohita* at Gauhati (100m).

Amino acid	DEVELOPMENTAL STAGES																
	0	1	2	3	4	5	6?	7	8	9	10?	11	12	13?	14	15a	15b
Glutamic acid	18.6	18.2	18.1	18.0	16.2	15.8		14.8	13.4	14.5		15.4	16.6		18.5	18.9	19.2
Isoleucine	13.2	15.3	15.9	15.6	15.0	15.2		15.2	16.5	16.9		16.5	15.2		14.5	13.1	10.5
Aspartic acid	4.6	4.0	3.5	3.1	3.8	3.6		2.9	3.6	3.7		3.6	3.5		3.1	3.1	3.0
Arginine	19.5	23.2	23.8	23.9	23.8	24.3		24.4	21.2	20.1		19.5	20.9		20.9	21.5	23.2
Serine	2.9	1.8	1.6	1.5	1.8	1.2		1.6	1.9	1.8		1.6	1.4		1.2	1.5	1.1
Glycine	4.8	3.5	3.4	3.3	3.2	3.2		3.2	3.2	2.2		2.1	2.0		2.0	1.2	1.2
Threonine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		N.D.	N.D.	N.D.		N.D.	N.D.		N.D.	N.D.	N.D.
Alanine	N.D.	N.D.	0.5	0.6	0.6	0.6		0.6	1.0	1.3		1.5	1.4		1.9	1.5	1.9
Proline	N.D.	0.6	0.8	0.8	0.9	1.0		1.0	1.0	1.0		1.3	1.2		1.1	5.9	6.2
Valine	5.2	5.2	4.6	4.4	4.9	4.6		4.7	5.6	5.5		5.6	5.7		5.8	3.0	2.2
Methionine	5.5	5.4	5.2	4.9	5.2	5.1		4.9	4.8	4.5		3.5	3.3		3.1	3.2	3.2
Lysine	3.9	2.2	2.5	2.4	2.5	2.4		2.3	2.5	2.8		2.6	2.4		2.6	2.1	2.9
Leucine	5.6	5.9	5.8	5.7	5.6	5.5		5.4	5.3	5.6		5.2	5.1		5.5	13.1	10.5
Phenylalanine	6.5	6.5	6.4	6.4	6.4	6.1		5.8	5.7	5.5		5.6	5.1		3.6	3.9	0.3
Tyrosine	4.2	4.2	4.1	4.5	4.6	4.6		6.0	5.5	5.9		5.1	5.2		5.9	6.2	6.9
Histidine	N.D.	0.2	0.2	1.0	1.1	2.1		2.3	2.5	2.5		2.4	2.3		2.5	2.4	2.6
Tryptophan	N.D.	N.D.	0.3	0.3	0.4	0.5		0.6	0.9	1.0		1.6	1.9		2.1	2.4	2.8
Cysteine	2.5	2.4	2.5	2.6	2.7	2.7		3.2	3.3	3.2		3.3	3.3		3.1	2.9	3.5
Cystine	2.5	1.1	1.0	1.0	1.0	1.0		1.1	1.6	1.4		1.5	1.9		2.4	2.9	2.7
Total free amino acid (mg/ embryo) x 10^3	11.5 ±1.1	19.2 ±1.1	17.5 ±1.1	16.6 ±1.7	18.4 ±0.9	19.9 ±0.7		20.5 ±1.0	19.6 ±0.8	14.3 ±1.1		16.0 ±1.3	13.6 ±1.2		12.4 ±2.4	9.3 ±1.3	9.0 ±0.2

N.D. = not detected
? = stage could not be observed

Table 20 : Qualitative and quantitative % individual free amino-acid and total free amino-acid (mg/embryo x 10^3) changes of free amino acids during early development of *L. rohita* at Mawpun (1000m).

Amino acid	DEVELOPMENTAL STAGES																
	0	1	2	3	4	5	6?	7	8	9	10?	11	12	13?	14	15a	15b
Glutamic acid	18.4	18.0	17.9	17.6	17.6	18.3		18.5	18.9	19.2		19.3	20.2		18.6	18.5	-
Isoleucine	13.9	12.5	12.1	12.0	10.6	10.0		9.5	10.2	10.7		11.6	10.0		12.8	12.8	
Aspartic acid	4.3	4.2	4.1	4.2	4.1	4.6		4.5	4.2	4.5		4.1	4.2		4.2	3.9	
Arginine	19.1	22.5	23.5	23.5	23.6	24.6		24.2	22.2	20.6		19.1	20.3		19.5	19.7	
Serine	2.5	2.4	2.2	2.1	2.1	2.0		1.9	1.9	1.9		1.8	1.8		2.0	0.9	
Glycine	4.9	4.8	3.5	3.2	3.2	3.3		3.2	3.2	3.1		3.0	3.0		2.8	2.8	
Threonine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.		N.D.	N.D.	N.D.		N.D.	N.D.		N.D.	N.D.	
Alanine	N.D.	N.D.	0.7	0.7	1.5	1.2		1.5	1.8	1.6		1.9	1.8		1.9	2.0	
Proline	N.D.	0.45	0.5	0.5	0.6	0.7		0.9	0.9	1.0		2.2	2.9		2.9	3.2	
Valine	5.6	5.2	5.2	5.6	5.9	5.7		5.8	5.7	6.2		6.2	5.7		5.5	6.5	
Methionine	5.9	5.6	5.7	5.5	5.0	4.2		4.6	4.7	4.3		3.2	3.1		2.2	1.9	
Lysine	4.2	3.8	3.5	3.2	3.1	3.2		3.1	3.2	3.5		3.2	3.8		3.5	4.2	
Leucine	5.3	5.4	5.5	5.7	5.7	5.6		5.5	5.6	5.8		5.7	5.8		5.6	4.6	
Phenylalanine	6.9	6.5	6.7	6.6	6.9	6.5		5.2	4.6	4.9		4.5	3.2		2.7	2.0	
Tyrosine	3.9	3.5	3.9	4.4	4.6	4.9		5.2	5.3	5.7		5.9	6.2		6.5	6.9	
Histidine	N.D.	0.2	0.2	1.0	1.2	1.3		1.5	1.5	1.6		1.6	1.7		1.6	1.7	
Tryptophan	N.D.	N.D.	0.4	0.3	0.3	0.4		0.4	0.2	0.8		0.9	1.0		1.1	1.3	
Cysteine	2.4	2.3	2.2	2.4	2.6	2.5		2.9	3.0	3.2		3.3	3.5		4.2	4.5	
Cystine	2.5	1.9	1.7	1.0	1.0	1.0		1.0	1.0	1.0		1.5	1.6		1.8	2.2	
Total free amino acid (mg/embryo) x 10^3	10.8 ±1.3	14.2 ±1.1	14.8 ±1.8	15.5 ±1.4	16.8 ±1.9	18.5 ±0.7		20.1 ±0.9	17.2 ±1.2	15.2 ±4.7		18.5 ±1.5	16.3 ±1.2		16.3 ±2.1	14.6 ±1.5	

N.D. = not detected
? = stage could not be observed
- = embryos were not enough for estimation

Table 21 : Changes in DNA content (µg/embryo) x 10 during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	4.62±0.83	4.77±0.88	N.S.	5.93±0.72	6.13±1.43	N.S.
1	4.64±0.68 N.S.	4.52±1.98 N.S.	N.S.	5.81±0.98 N.S.	5.69±0.72 N.S.	N.S.
2	4.8±0.85 N.S.	5.05±0.46 N.S.	N.S.	5.54±1.26 N.S.	5.77±1.34 N.S.	N.S.
3	5.05±0.78 N.S.	5.04±0.46 N.S.	N.S.	5.63±0.43 N.S.	5.72±1.02 N.S.	N.S.
4	5.0±0.89 N.S.	4.73±0.91 N.S.	N.S.	5.81±0.97 N.S.	5.86±1.11 N.S.	N.S.
5	5.0±0.5 N.S.	4.88±0.73 N.S.	N.S.	5.71±0.34 N.S.	5.91±0.42 N.S.	N.S.
6	5.3±0.83 N.S.	4.82±1.08 N.S.	N.S.	?	?	
7	6.3±0.78 N.S.	6.73±0.48 N.S.	N.S.	11.58±0.49 (+102.8)***	11.2±1.09 (+89.51)***	N.S.
8	7.29±2.07 N.S.	7.04±1.04 N.S.	N.S.	20.63±0.75 (+78.15)***	19.66±0.96 (+75.54)***	N.S.
9	8.2±0.85 N.S.	7.7±1.29 N.S.	N.S.	25.2±1.88 (+22.15)***	24.99±1.75 (+17.11)***	N.S.
10	9.77±0.82 (+19.15)*	9.7±0.33 (+25.97)*	N.S.	?	?	
11	17.86±2.17 (+82.81)***	17.66±2.09 (+82.06)***	N.S.	29.29±2.03 (+16.23)*	28.31±1.55 (+13.29)**	N.S.
12	32.24±2.03 (+80.62)***	32.61±2.58 (+84.66)***	N.S.	55.54±3.38 (+89.62)***	55.27±2.23 (+95.23)***	N.S.
13	58.39±4.76 (+81.11)***	52.39±4.13 (+60.66)***	N.S.	?	?	
14	83.89±1.39 (+43.67)***	62.98±1.57 (+20.21)**	(-24.93) ***	70.29±1.94 (+26.56)***	61.33±2.14 (+10.96)***	(-12.75) **
15a	128.75±8.81 (+53.48)***	98.78±9.2 (+56.84)***	(-23.28) *	140.9±3.87 (+100.5)***	111.3±6.41 (+81.48)***	(-21.01) **
15b	148.61±2.67 (+15.43)**	110.82±2.41 (+12.19)*	(-25.43) ***	188.58±5.61 (+33.84)***	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M in parentheses show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 22 : Changes in RNA content (µg/embryo) x 10 during the early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	38.79±0.68	38.52±0.76	N.S.	25.74±0.58	25.86±1.5	N.S.
1	38.5±0.99 N.S.	38.69±1.03 N.S.	N.S.	25.91±0.75 N.S.	25.69±1.68 N.S.	N.S.
2	38.63±0.79 N.S.	37.29±3.54 N.S.	N.S.	25.82±0.82 N.S.	25.82±1.65 N.S.	N.S.
3	38.35±1.18 N.S.	38.7±1.28 N.S.	N.S.	25.77±0.95 N.S.	25.79±1.2 N.S.	N.S.
4	38.24±1.57 N.S.	38.35±2.12 N.S.	N.S.	26.35±0.49 N.S.	26.17±1.08 N.S.	N.S.
5	38.54±1.27 N.S.	38.64±3.15 N.S.	N.S.	26.63±0.36 N.S.	26.56±0.84 N.S.	N.S.
6	38.96±0.903 N.S.	38.69±3.26 N.S.	N.S.	?	?	
7	39.1±0.67 N.S.	39.29±3.21 N.S.	N.S.	44.2±0.63 (+65.98)***	43.2±2.07 (+62.65)***	N.S.
8	39.65±1.48 N.S.	38.25±0.63 N.S.	N.S.	65.51±0.54 (+48.21)***	63.27±3.87 (+46.46)***	N.S.
9	40.15±1.25 N.S.	41.42±2.82 N.S.	N.S.	91.88±2.05 (+40.25)***	88.54±18.22 (+39.94)*	N.S.
10	41.15±5.73 N.S.	41.56±1.29 N.S.	N.S.	?	?	
11	49.45±1.67 (+20.17)*	49.38±4.03 (+18.82)*	N.S.	128.75±3.43 (+40.12)***	108.57±4.41 N.S.	(-15.67) ***
12	76.52±6.02 (+54.74)***	69.31±7.09 (+40.36)**	N.S.	199.15±19.15 (+54.68)***	179.74±2.21 (+65.55)***	N.S.
13	86.21±2.31 (+12.66)*	71.13±7.1 N.S.	(-17.49) **	?	?	
14	132.77±4.32 (+54.01)***	89.51±5.92 (+25.84)*	(-32.58) ***	281.44±10.09 (+41.32)***	231.41±12.62 (+28.75)***	(-17.78) ***
15a	183.63±7.74 (+38.31)***	134.51±12.32 (+50.27)***	(-26.75) ***	434.52±23.92 (+54.39)***	334.2±14.6 (+44.42)***	(-23.09) ***
15b	355.01±13.05 (+93.33)***	179.37±7.46 (+33.35)***	(-49.48) ***	511.17±9.32 (+17.64)***	-	

? Stage could not be observed; - embryos were not enough for estimation.
* p<0.05; ** p<0.01; *** p<0.001; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table : 23 : Changes in ammonia content (μ moles/embryo) x 10^3 during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	3.55±1.18	3.68±0.45	N.S.	1.88±0.16	1.89±0.34	N.S.
1	6.72±1.0 (+89.3)**	5.94±0.32 (+61.41)***	N.S.	2.9±0.23 (+54.26)***	2.83±0.17 (+49.74)***	N.S.
2	6.99±1.61 N.S.	6.59±0.32 (+10.94)*	N.S.	2.13±0.12 (-26.55)**	2.12±0.34 (-25.09)**	N.S.
3	8.46±1.22 N.S.	8.08±0.63 (+22.61)**	N.S.	2.19±0.11 N.S.	2.11±0.25 N.S.	
4	8.96±1.76 N.S.	9.86±1.0 (+22.03)*	N.S.	2.15±0.07 N.S.	1.98±0.12 N.S.	
5	11.48±0.95 (+28.13)*	11.11±1.38 N.S.	N.S.	1.67±0.22 (-22.33)**	2.11±0.05 N.S.	(+26.35) *
6	6.61±1.05 (-42.42)***	10.93±0.84 N.S.	(+65.36) ***	?	?	
7	7.28±0.84 N.S.	10.29±4.47 N.S.	N.S.	2.15±0.29 N.S.	2.36±0.25 N.S.	N.S.
8	8.82±1.26 N.S.	11.51±3.15 N.S.	N.S.	2.31±1.42 N.S.	2.35±0.27 N.S.	N.S.
9	12.6±2.26 (+42.86)*	14.67±1.49 N.S.	N.S.	2.59±0.33 N.S.	2.89±0.26 (+22.98)*	N.S.
10	16.95±1.82 (+34.52)*	17.24±1.95 N.S.	N.S.	?	?	
11	15.95±2.92 N.S.	18.2±0.95 N.S.	N.S.	1.88±0.19 (-27.41)**	2.83±0.09 N.S.	(+50.53) ***
12	16.77±3.23 N.S.	19.29±1.48 N.S.	N.S.	2.89±0.19 (+53.72)**	4.37±0.17 (+54.42)***	(+51.21) ***
13	18.06±4.91 N.S.	19.17±1.41 N.S.	N.S.	?	?	
14	25.05±4.6 N.S.	19.31±0.63 N.S.	N.S.	3.76±0.36 (+30.1)**	4.76±0.14 (+8.92)**	(+26.6) ***
15a	29.68±2.98 N.S.	19.51±1.1 N.S.	(-17.81) ***	4.12±0.41 N.S.	7.13±0.602 (+49.79)***	(+73.06) ***
15b	21.11±3.33 (-28.88)**	17.35±1.18 (-11.07)*	N.S.	3.02±0.07 (-26.7)***	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 24 : Changes in urea content (μmoles/embryo) x 10^3 during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.)

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	1.56±0.55	1.87±0.84	N.S.	1.13±0.45	1.68±0.55	N.S.
1	2.74±1.22 N.S.	2.21±0.78 N.S.	N.S.	1.55±0.63 N.S.	1.47±0.71 N.S.	N.S.
2	2.57±0.65 N.S.	2.21±1.0 N.S.	N.S.	1.95±0.63 N.S.	1.56±0.32 N.S.	N.S.
3	2.47±0.72 N.S.	2.48±0.71 N.S.	N.S.	1.32±0.45 N.S.	1.55±0.63 N.S.	N.S.
4	1.97±0.95 N.S.	2.7±0.55 N.S.	N.S.	1.33±0.45 N.S.	1.11±0.32 N.S.	N.S.
5	3.11±0.45 N.S.	2.7±0.55 N.S.	N.S.	1.33±0.45 N.S.	1.55±0.63 N.S.	N.S.
6	2.54±0.95 N.S.	3.15±0.57 N.S.	N.S.	?	?	
7	3.39±0.78 N.S.	2.7±1.05 N.S.	N.S.	1.33±0.71 N.S.	1.77±0.55 N.S.	N.S.
8	3.1±0.95 N.S.	3.15±0.45 N.S.	N.S.	1.38±0.18 N.S.	1.55±0.12 N.S.	N.S.
9	4.35±0.71 N.S.	4.68±0.45 (+48.57)**	N.S.	1.84±0.13 (+33.33)**	1.82±0.15 (+17.42)*	N.S.
10	4.5±0.78 N.S.	4.45±0.67 N.S.	N.S.	?	?	
11	4.88±1.34 N.S.	4.72±0.84 N.S.	N.S.	1.85±0.11 N.S.	1.84±0.63 N.S.	N.S.
12	5.89±0.89 N.S.	5.66±0.49 N.S.	N.S.	2.3±0.78 N.S.	1.84±0.63 N.S.	N.S.
13	6.17±0.55 N.S.	6.07±0.63 N.S.	N.S.	?	?	
14	5.89±0.32 N.S.	5.81±0.56 N.S.	N.S.	2.3±0.1 N.S.	2.3±0.45 N.S.	N.S.
15a	6.17±0.95 N.S.	6.47±0.84 N.S.	N.S.	2.75±0.19 N.S.	2.53±0.45 N.S.	N.S.
15b	4.9±1.0	4.65±0.97 N.S.	N.S.	2.45±0.63 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant; G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 25 : Changes in the total activity (units/embryo) x 10^3 of Glutamate dehydrogenase (GDH) during the early development of <u>C. carpio</u> and <u>L. rohita</u> at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	<u>C. carpio</u>			<u>L. rohita</u>		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Maupun (1000m)	G/M
0	N.D.	N.D.		N.D.	N.D.	
1	17.04±3.5	15.97±2.39	N.S.	13.46±1.3	13.7±2.07	N.S.
2	19.18±3.98 N.S.	20.14±4.35 N.S.	N.S.	14.45±2.05 N.S.	13.3±1.34 N.S.	N.S.
3	18.58±3.15 N.S.	21.39±3.91 N.S.	N.S.	13.71±1.18 N.S.	15.1±4.62 N.S.	N.S.
4	21.09±3.76 N.S.	19.24±2.86 N.S.	N.S.	15.05±1.76 N.S.	11.35±9.26 N.S.	N.S.
5	21.58±4.74 N.S.	19.23±3.95 N.S.	N.S.	15.05±1.76 N.S.	17.61±2.17 N.S.	N.S.
6	19.19±5.99 N.S.	20.9±4.16 N.S.	N.S.	?	?	
7	20.92±2.61 N.S.	18.29±3.24 N.S.	N.S.	27.65±1.76 (+83.73)***	16.98±4.46 N.S.	(-38.59) ***
8	15.98±2.32 (-23.61)*	13.07±2.03 (-28.54)*	N.S.	44.4±4.11 (+60.58)***	42.8±5.37 (+152.06)***	N.S.
9	30.99±5.76 (+93.93)**	34.6±8.19 (+164.73)**	N.S.	58.0±7.82 (+30.63)*	54.9±7.14 (+28.27)*	N.S.
10	35.2±5.06 N.S.	37.7±7.57 N.S.	N.S.	?	?	
11	38.4±3.68 N.S.	47.9±11.46 N.S.	N.S.	53.61±1.48 N.S.	53.6±9.04 N.S.	N.S.
12	51.15±8.12 N.S.	40.65±6.12 N.S.	N.S.	51.4±4.89 N.S.	55.08±14.07 N.S.	N.S.
13	57.54±9.83 N.S.	53.14±10.82 N.S.	N.S.	?	?	
14	68.7±15.04 N.S.	44.28±9.42 N.S.	(-35.55) *	70.6±7.12 (+37.35)**	60.36±14.37 N.S.	N.S.
15a	79.9±6.14 N.S.	55.76±4.25 N.S.	(-30.21) ***	75.8±9.73 N.S.	60.37±16.43 N.S.	N.S.
15b	90.61±5.22 (+13.4)*	63.71±4.67 (+14.26)	(-24.38) ***	76.6±7.16 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.D. not detected; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Maupun respectively for each developmental stage.

Table 26 : Changes in the specific activity (units/mg protein) x 10^2 of Glutamate dehydrogenase (GDH) during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. sta-ges	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	N.D.	N.D.		N.D.	N.D.	
1	8.78±1.25	7.17±1.43	N.S.	17.55±6.29	16.76±4.21	N.S.
2	10.99±1.57 N.S.	10.78±3.76 N.S.	N.S.	17.05±3.48 N.S.	15.03±1.4 N.S.	N.S.
3	10.41±2.07 N.S.	10.95±2.2 N.S.	N.S.	16.08±2.01 N.S.	17.74±5.16 N.S.	N.S.
4	11.69±1.99 N.S.	10.74±2.3 N.S.	N.S.	17.95±2.67 N.S.	17.87±5.39 N.S.	N.S.
5	11.91±2.18 N.S.	10.69±2.72 N.S.	N.S.	21.65±7.91 N.S.	23.59±2.83 N.S.	N.S.
6	10.89±4.72 N.S.	12.29±3.66 N.S.	N.S.	?	?	
7	12.79±2.0 N.S.	11.06±2.32 N.S.	N.S.	39.83±10.35 (+83.97)*	23.11±7.28 N.S.	(-41.98) *
8	9.04±2.04 (-29.32)*	6.31±2.49 (-42.95)*	N.S.	56.89±2.49 (+42.83)*	49.6±6.58 (+114.63)***	N.S.
9	16.9±3.71 (+68.33)**	18.19±5.62 (+188.27)**	N.S.	73.18±2.56 (+28.63)*	62.99±11.5 N.S.	N.S.
10	19.75±3.52 N.S.	20.99±4.59 N.S.	N.S.	?	?	
11	21.17±3.0 N.S.	26.49±6.0 N.S.	N.S.	59.57±10.48 (-18.6)*	69.68±10.86 N.S.	N.S.
12	28.76±3.31 (+35.85)*	24.48±3.61 N.S.	N.S.	59.85±7.71 N.S.	88.17±9.89 (+26.54)*	(+47.32) **
13	33.69±8.45 N.S.	32.93±3.64 N.S.	N.S.	?	?	
14	42.95±6.48 N.S.	32.02±4.29 N.S.	(-24.21) *	71.99±7.69 N.S.	79.22±12.07 N.S.	N.S.
15a	50.89±2.38 (+20.45)*	45.28±3.6 N.S.	(-11.02) *	80.01±11.48 N.S.	98.87±10.15 (+24.8)*	(+23.57) *
15b	65.45±3.31 (+28.61)***	59.48±2.7 (+31.36)**	(-9.12) *	85.29±14.81 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.D. not detected; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 27 : Changes in the total activity (units/[illegible] embryo) x 10^3 of phosphate dependent Glutaminase (PDG) during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	27.45±5.43	27.67±6.38	N.S.	N.D.	N.D.	
1	27.9±11.65 N.S.	32.71±6.38 N.S.	N.S.	N.D.	N.D.	
2	32.07±4.23 N.S.	37.16±8.01 N.S.	N.S.	N.D.	N.D.	
3	32.2±6.77 N.S.	33.31±3.48 N.S.	N.S.	N.D.	N.D.	
4	32.11±11.24 N.S.	30.29±5.7 N.S.	N.S.	N.D.	N.D.	
5	33.98±4.79 N.S.	35.61±10.07 N.S.	N.S.	N.D.	N.D.	
6	30.28±5.43 N.S.	50.56±23.21 N.S.	N.S.	?	?	
7	34.75±6.06 N.S.	67.5±8.41 N.S.	(+94.25) ***	N.D.	N.D.	
8	37.81±4.59 N.S.	38.64±7.09 (-42.76)**	N.S.	N.D.	N.D.	
9	54.9±10.05 (+45.2)**	47.15±7.71 N.S.	N.S.	N.D.	N.D.	
10	36.76±5.03 (-33.02)*	28.28±5.88 (-40.02)**	N.S.	?	?	
11	34.34±6.21 N.S.	25.14±4.97 N.S.	N.S.	5.02±1.64	12.74±1.23	(+153.79) ***
12	60.34±6.21 (+93.97)**	51.45±8.7 (+104.65)**	N.S.	9.27±2.86 (+84.66)*	21.99±3.52 (+72.61)***	(+137.22) **
13	66.61±15.6 N.S.	41.84±7.39 N.S.	(-37.19) *	?	?	
14	79.19±18.3 N.S.	76.75±12.3 (+83.44)**	N.S.	24.69±2.61 (+166.34)***	38.6±8.24 (+75.53)**	(+56.34) **
15a	86.8±13.89 N.S.	69.49±10.02 N.S.	N.S.	37.04±4.76 (+50.02)**	69.05±3.35 (+78.89)***	(+86.42) ***
15b	61.76±8.06 (-28.85)*	83.25±8.91 N.S.	(+34.8) **	38.1±10.35 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation.
* $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.D. not detected; N.S. not significant.
G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 28 : Changes in the specific activity (units/mg protein) x 10^2 of phosphate dependent Glutaminase (PDG) during early development of C. carpio and L. rohita at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	C. carpio			L. rohita		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	9.28±1.39	9.88±1.79	N.S.	N.D.	N.D.	
1	14.03±2.13 (+51.19)**	16.52±2.94 (+67.21)*	N.S.	N.D.	N.D.	
2	18.69±3.45 N.S.	19.65±6.13 N.S.	N.S.	N.D.	N.D.	
3	17.85±3.42 N.S.	16.98±1.86 N.S.	N.S.	N.D.	N.D.	
4	17.68±5.71 N.S.	16.2±3.55 N.S.	N.S.	N.D.	N.D.	
5	19.02±3.02 N.S.	19.86±6.75 N.S.	N.S.	N.D.	N.D.	
6	17.17±3.77 N.S.	29.39±3.96 (+47.99)*	(+71.17) **	?	?	
7	20.78±3.37 N.S.	41.77±5.63 (+42.12)*	(+101.01) ***	N.S.	N.D.	
8	22.83±2.61 N.S.	18.92±4.35 (-54.7)***	N.S.	N.D.	N.D.	
9	31.01±4.64 (+35.83)*	24.3±5.19 N.S.	N.S.	N.D.	N.D.	
10	20.54±2.81 (-33.76)*	15.63±2.88 (-35.68)*	(-21.77) *	?	?	
11	18.84±4.41 N.S.	14.08±3.35 N.S.	N.S.	5.53±1.67	16.35±3.23	(+195.66) ***
12	33.91±11.53 N.S.	31.09±5.61 (+120.81)**	N.S.	11.93±2.79 (+115.73)**	36.27±13.88 (+70.44)***	(+204.02) **
13	38.56±11.92 N.S.	26.55±5.45 N.S.	N.S.	?	?	
14	49.27±12.1 N.S.	54.31±6.37 (+104.56)***	N.S.	25.53±4.57 (+114.0)**	49.57±6.92 N.S.	(+94.16) ***
15a	55.13±6.49 N.S.	57.48±9.02 N.S.	N.S.	40.32±11.16 N.S.	112.39±11.66 (+126.73)***	(+178.75) ***
15b	45.54±4.41 (-17.4)*	82.49±10.01 (+43.51)**	(+81.14) ***	40.59±9.91 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.D. not detected; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 29 : Changes in the total activity (units/embryo) $\times 10^3$ of Glutamine synthetase (GS) during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000 m)	G/M
0	27.93±5.68	26.72±18.45	N.S.	35.1±2.68	28.6±5.04	N.S.
1	33.32±6.78 N.S.	40.56±18.45 N.S.	N.S.	82.3±12.63 (+134.47)***	75.08±15.59 (+162.52)**	N.S.
2	31.46±6.41 N.S.	36.07±3.16 N.S.	N.S.	81.12±10.1 N.S.	76.6±7.14 N.S.	N.S.
3	36.06±6.21 N.S.	38.8±12.6 N.S.	N.S.	76.61±6.37 N.S.	75.5±7.25 N.S.	N.S.
4	37.19±6.41 N.S.	32.21±5.64 N.S.	N.S.	76.61±7.77 N.S.	81.13±17.77 N.S.	N.S.
5	37.18±9.48 N.S.	41.4±11.27 N.S.	N.S.	77.74±10.26 N.S.	84.51±14.04 N.S.	N.S.
6	35.75±8.46 N.S.	32.76±7.39 N.S.	N.S.	?	?	
7	44.4±6.18 N.S.	34.32±7.95 N.S.	N.S.	78.9±12.66 N.S.	78.9±7.09 N.S.	N.S.
8	45.77±7.08 N.S.	14.04±7.15 (-59.09)**	(-69.02) ***	85.5±9.7 N.S.	90.1±14.6 N.S.	N.S.
9	52.73±14.94 N.S.	9.54±3.36 N.S.	(-81.91) **	79.6±7.36 N.S.	117.03±5.98 (+29.89)**	(+47.02) ***
10	57.2±10.84 N.S.	44.11±15.58 (+362.37)**	N.S.	?	?	
11	68.7±14.44 N.S.	66.73±11.2 N.S.	N.S.	173.1±11.09 (+117.59)***	126.36±7.44 N.S.	(-27.04) ***
12	69.92±14.65 N.S.	67.6±14.24 N.S.	N.S.	180.2±12.61 N.S.	112.9±14.25 N.S.	(-37.35) ***
13	64.44±13.05 N.S.	63.5±16.1 N.S.	N.S.	?	?	
14	64.85±11.82 N.S.	52.88±9.14 N.S.	N.S.	193.05±12.12 N.S.	138.07±7.04 (+22.29)*	(-28.48) ***
15a	68.64±14.58 N.S.	80.5±11.81 (+52.23)*	N.S.	205.93±12.71 N.S.	153.5±10.19 (+11.18)*	(-25.46) ***
15b	96.72±13.6 (+40.91)*	126.31±13.27 (+56.91)***	(+30.59) **	342.83±21.51 (+66.48)***	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 30 : Changes in the specific activity (units/mg protein) x 10^2 of Glutamine synthetase (GS) during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. changes	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	9.72±1.89	9.61±1.52	N.S.	26.55±4.32	23.46±3.14	N.S.
1	17.74±5.87 (+82.51)*	19.67±3.29 (+104.68)***	N.S.	95.79±13.85 (+260.79)***	90.83±20.64 (+286.32)***	N.S.
2	18.4±4.67 N.S.	18.18±3.73 N.S.	N.S.	95.37±16.85 N.S.	89.8±18.64 N.S.	N.S.
3	19.96±2.76 N.S.	18.6±6.07 N.S.	N.S.	89.37±13.28 N.S.	89.59±15.83 N.S.	N.S.
4	20.65±3.89 N.S.	17.92±3.73 N.S.	N.S.	91.45±13.24 N.S.	96.05±14.24 N.S.	N.S.
5	20.85±5.58 N.S.	22.27±4.18 N.S.	N.S.	110.65±40.04 N.S.	111.98±19.15 N.S.	N.S.
6	20.12±5.06 N.S.	18.89±5.32 N.S.	N.S.	?	?	
7	26.46±2.93 N.S.	21.29±5.25 N.S.	N.S.	119.24±7.96 N.S.	106.39±13.39 N.S.	N.S.
8	27.61±3.95 N.S.	6.73±1.62 (-68.39)***	(-75.62) ***	112.08±26.03 N.S.	102.93±12.7 N.S.	N.S.
9	29.91±9.75 N.S.	4.83±1.48 N.S.	(-83.85) **	88.24±13.66 N.S.	133.05±11.74 N.S.	(+50.78) **
10	33.78±7.79 N.S.	24.09±7.49 (+398.76)***	N.S.	?	?	
11	37.47±6.77 N.S.	37.04±6.16 (+53.76)*	N.S.	191.67±7.96 (+189.62)***	160.12±7.27 (+20.35)**	(-66.46) **
12	34.45±7.79 N.S.	41.42±11.33 N.S.	N.S.	209.84±12.49 N.S.	204.54±7.49 (+27.74)***	N.S.
13	35.69±9.99 N.S.	40.78±14.34 N.S.	N.S.	?	?	
14	40.47±4.96 N.S.	37.39±3.35 N.S.	N.S.	199.99±37.71 N.S.	211.88±14.33 N.S.	N.S.
15a	43.06±5.71 N.S.	66.57±20.01 (+78.04)*	N.S.	221.15±9.29 N.S.	199.08±5.55 N.S.	N.S.
15b	70.66±11.53 (+64.1)*	120.65±24.63 (+81.24)*	(+70.75) **	385.68±28.65 (+74.4)***	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 31 : Changes in the total activity (units/embryos) x 10^2 of arginase during the early development of C. carpio and L. rohita at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Maupun (1000m)	G/M
0	56.3±10.32	56.85±4.61	N.S.	45.53±8.22	48.81±5.99	N.S.
1	71.81±6.23 (+27.55)*	76.03±4.84 (+33.74)***	N.S.	50.39±9.92 N.S.	45.02±5.99 N.S.	N.S.
2	65.08±10.28 N.S.	74.26±5.73 N.S.	N.S.	53.04±13.52 N.S.	73.49±7.98 (+62.57)**	(+38.56) *
3	68.44±15.34 N.S.	68.48±5.37 N.S.	N.S.	65.64±6.57 N.S.	72.49±3.26 N.S.	N.S.
4	67.32±13.46 N.S.	64.87±9.27 N.S.	N.S.	53.7±11.77 N.S.	56.8±6.34 (-21.64)**	N.S.
5	62.83±11.44 N.S.	61.27±8.55 N.S.	N.S.	53.7±11.77 N.S.	53.04±10.23 N.S.	N.S.
6	57.22±6.64 N.S.	53.7±6.54 N.S.	N.S.	?	?	
7	53.86±11.44 N.S.	49.57±7.89 N.S.	N.S.	58.35±3.66 N.S.	57.46±8.43 N.S.	N.S.
8	88.64±8.61 (+64.58)**	82.62±9.09 (+66.67)***	N.S.	57.84±11.68 N.S.	57.46±7.34 N.S.	N.S.
9	63.96±8.62 (-27.84)**	78.54±10.49 N.S.	N.S.	59.9±11.87 N.S.	55.54±5.41 N.S.	N.S.
10	68.44±12.84 N.S.	76.67±5.91 N.S.	N.S.	?	?	
11	78.54±21.17 N.S.	80.41±6.87 N.S.	N.S.	66.1±13.06 N.S.	62.88±5.25 N.S.	N.S.
12	74.05±14.37 N.S.	79.56±3.53 N.S.	N.S.	74.36±11.69 N.S.	60.13±5.25 N.S.	N.S.
13	76.3±16.18 N.S.	89.22±15.39 N.S.	N.S.	?	?	
14	70.69±13.97 N.S.	80.89±8.17 N.S.	N.S.	35.8±6.16 (-51.86)***	36.26±8.14 (-39.7)**	N.S.
15a	86.39±12.29 N.S.	80.51±11.41 N.S.	N.S.	30.29±6.16 N.S.	19.74±4.38 (-45.56)**	(-34.83) *
15b	63.95±11.16 (-29.98)**	44.74±11.13 (-44.43)**	N.S.	20.81±6.36 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 32 : Changes in the specific activity (units/mg protein) x 10 of arginase during the early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	19.8±4.39	19.15±4.66	N.S.	35.34±6.35	34.27±5.54	N.S.
1	36.59±10.54 (+84.8)*	39.22±5.03 (+104.8)***	N.S.	61.08±6.88 (+72.84)**	56.27±7.35 (+64.2)*	N.S.
2	38.69±7.5 N.S.	38.99±4.49 N.S.	N.S.	73.5±12.75 N.S.	90.31±4.04 (+60.49)***	(+22.87) *
3	39.48±8.59 N.S.	35.69±2.45 N.S.	N.S.	80.63±7.24 N.S.	91.51±10.58 N.S.	N.S.
4	40.19±9.23 N.S.	35.85±6.23 N.S.	N.S.	71.93±18.04 N.S.	76.49±14.89 N.S.	N.S.
5	37.27±6.71 N.S.	35.04±5.08 N.S.	N.S.	76.39±17.18 N.S.	76.18±20.07 N.S.	N.S.
6	34.53±5.17 N.S.	32.88±3.71 N.S.	N.S.	?	?	
7	34.32±6.95 N.S.	32.24±5.46 N.S.	N.S.	82.27±4.9 N.S.	86.64±26.5 N.S.	N.S.
8	48.29±6.58 (+40.71)*	42.89±4.31 (+33.03)*	N.S.	73.76±14.86 N.S.	77.61±12.76 N.S.	N.S.
9	38.44±6.95 N.S.	42.8±6.82 N.S.	N.S.	79.64±21.53 N.S.	75.47±7.51 N.S.	N.S.
10	37.0±3.57 N.S.	43.66±4.33 N.S.	N.S.	?	?	
11	46.04±11.82 N.S.	48.12±6.59 N.S.	N.S.	82.55±17.67 N.S.	90.55±8.66 (+19.98)*	N.S.
12	45.64±11.85 N.S.	51.9±8.03 N.S.	N.S.	90.85±15.62 N.S.	108.54±9.11 (+19.87)*	N.S.
13	48.64±11.85 N.S.	62.55±6.06 N.S.	N.S.	?	?	
14	53.1±14.51 N.S.	68.98±12.02 N.S.	N.S.	44.52±8.91 (-50.99)*	66.35±5.45 (-38.87)***	(+49.03) **
15a	63.96±8.69 N.S.	79.41±16.43 N.S.	N.S.	37.94±9.56 N.S.	40.04±5.07 (-39.65)***	N.S.
15b	47.07±7.53 (-26.41)*	57.0±6.86 (-28.22)*	N.S.	28.05±8.91 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 33 : Changes in acetylcholine (ACh) content (mmol/embryo) x 10^3 during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	3.04±0.97	2.59±0.42	N.S.	1.69±0.28	1.57±0.37	N.S.
1	3.48±0.68 N.S.	2.94±0.54 N.S.	N.S.	2.2±0.48 N.S.	2.02±0.48 N.S.	N.S.
2	3.44±0.66 N.S.	3.06±0.71 N.S.	N.S.	1.77±0.26 N.S.	1.65±0.33 N.S.	N.S.
3	3.04±0.73 N.S.	2.64±0.51 N.S.	N.S.	1.77±0.35 N.S.	1.53±0.26 N.S.	N.S.
4	3.59±0.56 N.S.	3.12±0.61 N.S.	N.S.	2.18±0.82 N.S.	1.65±0.37 N.S.	N.S.
5	3.29±0.37 N.S.	2.64±0.61 N.S.	N.S.	1.47±0.31 N.S.	1.88±0.75 N.S.	N.S.
6	2.99±0.76 N.S.	2.93±0.89 N.S.	N.S.	?	?	
7	3.16±0.92 N.S.	2.93±0.73 N.S.	N.S.	1.88±0.37 N.S.	1.88±0.29 N.S.	N.S.
8	3.59±0.89 N.S.	3.42±0.51 N.S.	N.S.	2.2±0.52 N.S.	1.88±0.37 N.S.	N.S.
9	4.06±0.56 N.S.	3.24±0.42 N.S.	N.S.	2.08±0.27 N.S.	2.59±0.53 N.S.	N.S.
10	2.39±0.47 (-41.13)**	2.49±0.39 (-23.15)*	N.S.	?	?	
11	1.79±0.42 N.S.	1.99±0.31 N.S.	N.S.	1.47±0.46 N.S.	3.42±0.32 (+32.05)*	(+132.65) ***
12	1.5±0.47 N.S.	1.25±0.31 N.S.	N.S.	1.35±0.44 N.S.	1.96±0.52 (-42.69)**	N.S.
13	4.48±0.92 (+198.67)***	3.32±0.39 (+165.6)***	N.S.	?	?	
14	4.48±0.76 N.S.	5.25±0.26 (+58.13)***	N.S.	2.2±0.29 (+62.96)*	1.71±0.17 N.S.	(-22.27) *
15a	5.08±0.52 N.S.	5.51±0.35 N.S.	N.S.	3.42±0.46 (+55.45)**	1.84±0.36 N.S.	(-46.2) **
15b	2.82±0.47 (-44.49)***	3.19±0.87 (-42.11)**	N.S.	2.61±0.29 (-23.68)*	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 34 : Changes in the total activity (units/embryo) x 10^3 of acetylcholinesterase (AChE) during early development of <u>C. carpio</u> and <u>L. rohita</u> at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	N.D.	N.D.		N.D.	N.D.	
1	N.D.	N.D.		N.D.	N.D.	
2	N.D.	N.D.		N.D.	N.D.	
3	N.D.	N.D.		N.D.	N.D.	
4	N.D.	N.D.		N.D.	N.D.	
5	N.D.	N.D.		N.D.	N.D.	
6	N.D.	N.D.		?	?	
7	N.D.	N.D.		N.D.	N.D.	
8	N.D.	N.D.		N.D.	N.D.	
9	1.84±0.64	2.0±0.55	N.S.	0.8±0.15	N.D.	
10	8.71±1.64 (+373.37)***	8.0±3.74 (+300.0)*	N.S.	?	?	
11	12.14±2.04 (+39.38)*	14.6±2.83 (+82.5)*	N.S.	5.02±0.6 (+527.5)***	1.62±0.32	(-67.73) ***
12	15.9±2.39 N.S.	17.0±2.76 N.S.	N.S.	6.9±0.81 (+37.45)**	6.3±1.6 (+288.89)**	N.S.
13	19.3±2.26 N.S.	20.5±3.56 N.S.	N.S.	?	?	
14	28.0±2.19 (+45.08)***	30.6±4.95 (+49.27)**	N.S.	10.8±4.7 N.S.	14.1±1.55 (+123.81)***	N.S.
15a	48.0±3.62 (+71.43)***	53.4±6.58 (+74.51)***	N.S.	16.0±1.3 (+48.15)***	18.86±1.82 (+33.76)**	N.S.
15b	66.01±8.09 (+37.5)**	61.9±10.0 N.S.	N.S.	17.7±0.9 (+10.63)*	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.D. not detected; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 35 : Changes in the specific activity (units/mg protein) x 10^3 of acetylcholinesterase (AChE) during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	N.D.	N.D.		N.D.	N.D.	
1	N.D.	N.D.		N.D.	N.D.	
2	N.D.	N.D.		N.D.	N.D.	
3	N.D.	N.D.		N.D.	N.D.	
4	N.D.	N.D.		N.D.	N.D.	
5	N.D.	N.D.		N.D.	N.D.	
6	N.D.	N.D.		N.D.	?	
7	N.D.	N.D.		N.D.	N.D.	
8	N.D.	N.D.		N.D.	N.D.	
9	10.94±3.61	10.39±3.11	N.S.	10.0±3.42	N.D.	
10	51.24±10.88 (+368.37)***	65.37±11.62 (+529.16)***	N.S.	?	?	
11	71.02±13.44 N.S.	87.01±10.01 (+33.1)*	N.S.	62.69±9.19 (+526.9)***	23.5±3.02	(-62.51) ***
12	95.07±13.54 (+33.86)*	111.29±18.13 N.S.	N.S.	83.95±6.17 (+33.91)**	136.27±23.26 (+479.87)*	(+62.32) **
13	122.22±15.33 (+28.56)*	147.46±39.75 N.S.	N.S.	?	?	
14	207.73±27.21 (+69.96)**	266.52±84.86 (+80.74)*	N.S.	131.71±13.4 (+56.89)***	267.15±51.45 (+96.05)*	(+102.83) **
15a	366.92±46.79 (+76.63)**	528.62±10.77 (+98.34)***	(+44.07) ***	206.57±58.13 (+56.84)*	389.83±54.27 (+45.92)**	(+88.72) **
15b	488.11±74.64 (+33.03)*	759.04±28.53 (+43.59)***	(+55.51) ***	237.57±18.05 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * p<0.05; ** p<0.01; *** p<0.001; N.D. not detected; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 36 : Changes in the total activity (units/embryo) x 10^2 of cytoplasmic tyrosine amino transferase (c-TAT) during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	32.93±2.09	33.33±2.23	N.S.	12.06±0.52	11.9±0.43	N.S.
1	19.8±3.32 (-39.87)***	22.16±1.67 (-33.45)***	N.S.	11.17±1.44 N.S.	11.92±0.84 N.S.	N.S.
2	19.58±4.48 N.S.	20.92±1.32 N.S.	N.S.	8.82±1.37 N.S.	9.87±0.69 N.S.	N.S.
3	20.48±4.44 N.S.	18.98±1.18 N.S.	N.S.	7.98±1.01 N.S.	8.4±0.19 N.S.	N.S.
4	19.46±5.73 N.S.	19.7±2.36 N.S.	N.S.	7.36±1.54 N.S.	7.51±0.84 N.S.	N.S.
5	20.48±4.2 N.S.	20.78±1.25 N.S.	N.S.	6.83±0.75 N.S.	6.92±0.76 N.S.	N.S.
6	20.03±2.26 N.S.	21.29±1.59 N.S.	N.S.	?	?	
7	21.49±3.7 N.S.	19.33±1.76 N.S.	N.S.	6.52±1.38 N.S.	6.75±0.76 N.S.	N.S.
8	19.58±3.73 N.S.	22.28±2.13 N.S.	N.S.	5.41±1.19 N.S.	5.99±0.45 N.S.	N.S.
9	20.4±2.95 N.S.	24.57±2.11 N.S.	N.S.	7.18±0.71 N.S.	5.96±1.68 N.S.	N.S.
10	19.85±2.17 N.S.	22.12±4.68 N.S.	N.S.	?	?	
11	22.28±3.85 N.S.	22.12±2.31 N.S.	N.S.	6.56±1.03 N.S.	6.28±0.47 N.S.	N.S.
12	23.29±2.47 N.S.	23.73±5.64 N.S.	N.S.	6.82±1.09 N.S.	5.15±0.83 N.S.	(-24.49) *
13	23.4±4.36 N.S.	24.54±2.12 N.S.	N.S.	?	?	
14	23.4±3.67 N.S.	24.75±3.66 N.S.	N.S.	8.38±0.99 N.S.	6.53±0.81 N.S.	(-22.08) *
15a	20.48±1.87 N.S.	26.18±4.89 N.S.	N.S.	6.92±0.54 N.S.	6.03±0.17 N.S.	(-12.86) *
15b	21.39±1.91 N.S.	27.56±3.12 N.S.	(+28.85) **	8.35±1.33 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 37 : Changes in the specific activity (units/mg protein) x 10^2 of cytoplasmic tyrosine aminotransferase (c-TAT) during the early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	11.55±1.69	11.11±1.91	N.S.	9.36±0.24	9.15±0.47	N.S.
1	10.94±0.91 N.S.	11.25±1.17 N.S.	N.S.	13.66±1.48 (+45.94)**	15.26±1.06 (+66.78)***	N.S.
2	10.98±4.66 N.S.	10.99±1.21 N.S.	N.S.	10.98±3.26 N.S.	12.19±0.67 N.S.	N.S.
3	11.63±4.88 N.S.	9.93±0.94 N.S.	N.S.	9.9±1.85 N.S.	10.56±0.68 N.S.	N.S.
4	11.61±3.36 N.S.	10.79±1.33 N.S.	N.S.	9.4±1.89 N.S.	10.07±0.82 N.S.	N.S.
5	12.17±2.55 N.S.	11.6±5.66 N.S.	N.S.	9.82±1.53 N.S.	9.89±1.72 N.S.	N.S.
6	11.98±0.92 N.S.	13.05±0.99 N.S.	N.S.	?	?	
7	13.69±2.17 N.S.	12.64±1.45 N.S.	N.S.	9.35±2.32 N.S.	10.15±0.86 N.S.	N.S.
8	10.71±2.01 N.S.	11.58±1.13 N.S.	N.S.	7.63±1.63 N.S.	8.21±0.43 (-19.11)**	N.S.
9	12.06±2.54 N.S.	13.22±0.84 N.S.	N.S.	9.63±1.54 N.S.	8.12±0.11 N.S.	N.S.
10	11.65±1.64 N.S.	12.78±0.35 N.S.	N.S.	?	?	
11	13.04±2.03 N.S.	13.11±0.76 N.S.	N.S.	8.13±1.08 N.S.	9.03±0.53 (+11.21)*	N.S.
12	13.94±1.24 N.S.	16.86±1.47 (+28.6)**	(+20.95) *	8.5±1.45 N.S.	8.86±0.97 N.S.	N.S.
13	14.77±2.19 N.S.	17.49±2.18 N.S.	N.S.	?	?	
14	17.26±2.67 N.S.	21.03±4.0 N.S.	N.S.	10.34±0.76 N.S.	12.37±0.63 (+39.62)***	(+19.62) *
15a	15.45±2.05 N.S.	26.02±3.08 N.S.	(+68.41) **	11.85±0.49 N.S.	12.71±0.31 N.S.	(+ 7.26) *
15b	15.66±1.21 N.S.	34.29±1.64 (+31.78)**	(+18.28) ***	11.13±1.96 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 38 : Changes in the total activity (units/embryo) x 10^3 of mitochondrial tyrosine aminotransferase (m-TAT) during early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	N.D.	N.D.		N.D.	N.D.	
1	N.D.	N.D.		N.D.	N.D.	
2	N.D.	N.D.		N.D.	N.D.	
3	N.D.	N.D.		N.D.	N.D.	
4	N.D.	N.D.		N.D.	N.D.	
5	N.D.	N.D.		N.D.	N.D.	
6	N.D.	N.D.		?	?	
7	N.D.	N.D.		N.D.	N.D.	
8	N.D.	N.D.		N.D.	N.D.	
9	N.D.	N.D.		N.D.	N.D.	
10	N.D.	N.D.		?	?	
11	2.82±1.23	2.35±0.71	N.S.	N.D.	N.D.	
12	5.63±1.38 (+99.65)*	4.4±1.07 (+87.23)*	N.S.	1.74±0.35	1.5±0.31	N.S.
13	8.44±1.67 (+49.91)*	10.4±3.35 (+136.36)***	N.S.	?	?	
14	9.0±0.89 N.S.	15.1±0.96 (+45.19)*	(+67.78) ***	2.42±0.19 (+39.08)**	1.5±0.38 N.S.	(-38.02) **
15a	12.11±3.02 N.S.	14.75±3.16 N.S.	N.S.	2.99±0.52 N.S.	2.2±0.55 N.S.	N.S.
15b	14.2±1.18 N.S.	43.0±10.12 (+191.53)***	(+202.82) **	3.57±0.39 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.D. not detected. N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

Table 39 : Changes in the specific activity (units/mg protein) x 10 of mitochondrial tyrosine aminotransferase (m-TAT) during the early development of *C. carpio* and *L. rohita* at different altitudes (values are expressed as Mean ± S.D.).

Dev. stages	*C. carpio*			*L. rohita*		
	Gauhati (100m)	Shillong (1500m)	G/S	Gauhati (100m)	Mawpun (1000m)	G/M
0	N.D.	N.D.		N.D.	N.D.	
1	N.D.	N.D.		N.D.	N.D.	
2	N.D.	N.D.		N.D.	N.D.	
3	N.D.	N.D.		N.D.	N.D.	
4	N.D.	N.D.		N.D.	N.D.	
5	N.D.	N.D.		N.D.	N.D.	
6	N.D.	N.D.		?	?	
7	N.D.	N.D.		N.D.	N.D.	
8	N.D.	N.D.		N.D.	N.D.	
9	N.D.	N.D.		N.D.	N.D.	
10	N.D.	N.D.		?	?	
11	2.44±1.01	1.96±0.67	N.S.	N.D.	N.D.	
12	3.02±2.88 N.S.	3.35±0.35 (+70.92)***	N.S.	1.39±0.16	1.66±0.16	N.S.
13	5.23±0.81 N.S.	5.65±1.14 (+68.66)**	N.S.	?	?	
14	4.53±2.26 N.S.	7.4±0.86 (+7.5)***	N.S.	1.41±0.25 N.S.	1.43±0.11 N.S.	N.S.
15a	5.71±1.28 N.S.	6.17±0.88 N.D.	(+63.36) *	1.51±0.31 N.S.	1.37±0.16 N.S.	N.S.
15b	4.76±0.75 N.S.	16.55±2.88 (+168.23)*	(+247.69) ***	1.63±0.12 N.S.	-	

? stage could not be observed; - embryos were not enough for estimation. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N.D. not detected; N.S. not significant. G/S and G/M columns show the percentage change and level of significance of the data between Gauhati and Shillong and Gauhati and Mawpun respectively for each developmental stage.

FIGURES

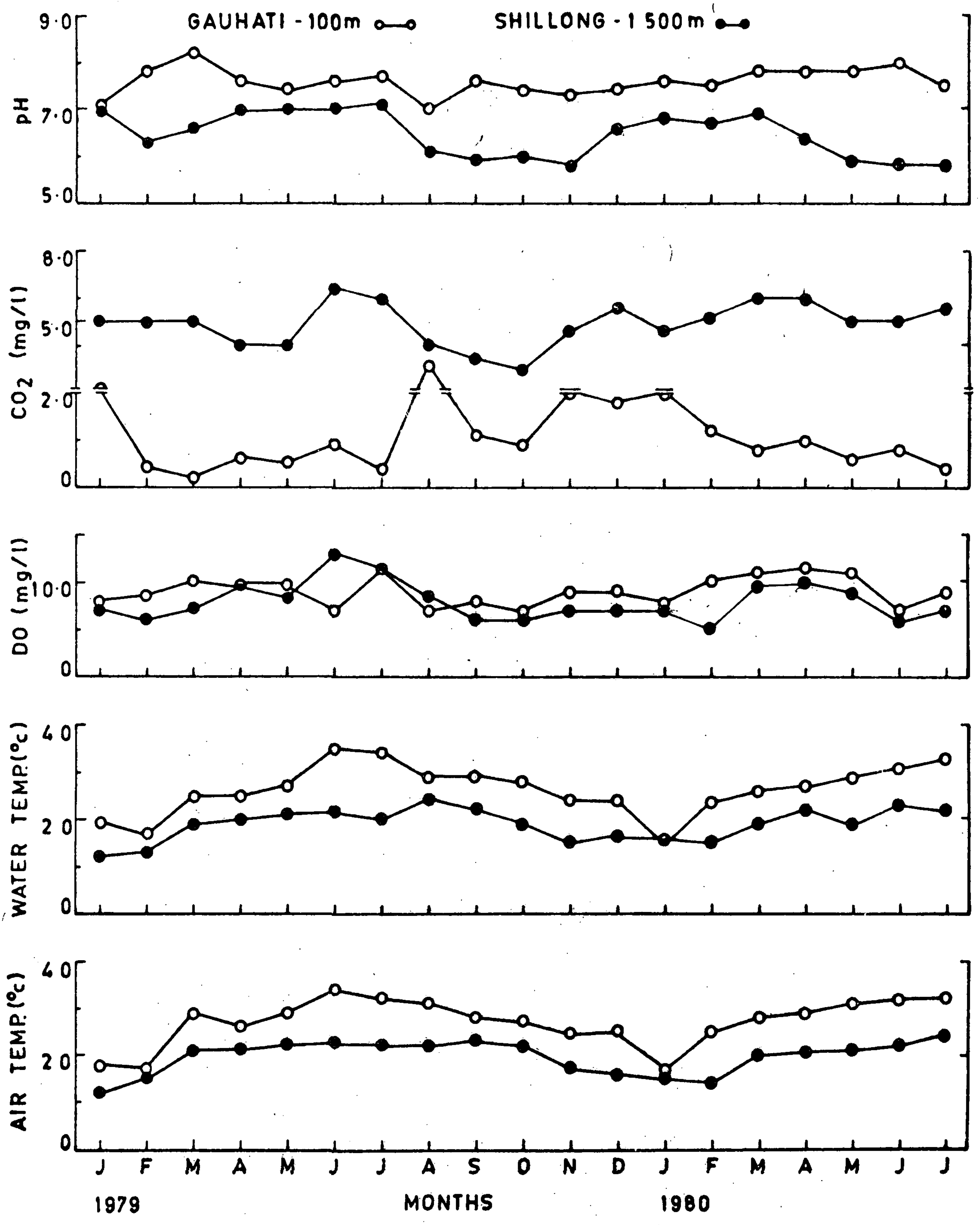
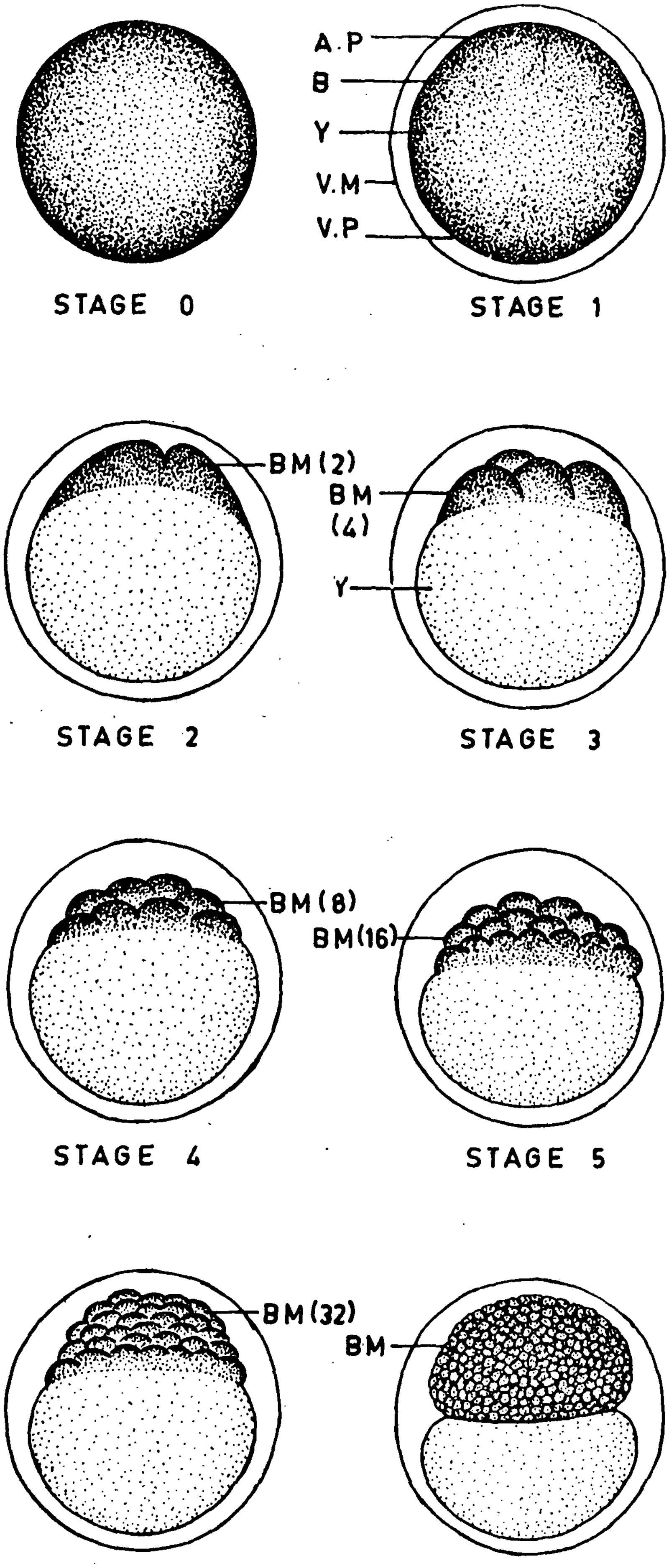


FIG. 1 . SEASONAL VARIATION IN THE PHYSICO-CHEMICAL FACTORS OF THE MANAGED NURSERY PONDS AT TWO DIFFERENT ALTITUDES.



STAGE 0 STAGE 1

STAGE 2 STAGE 3

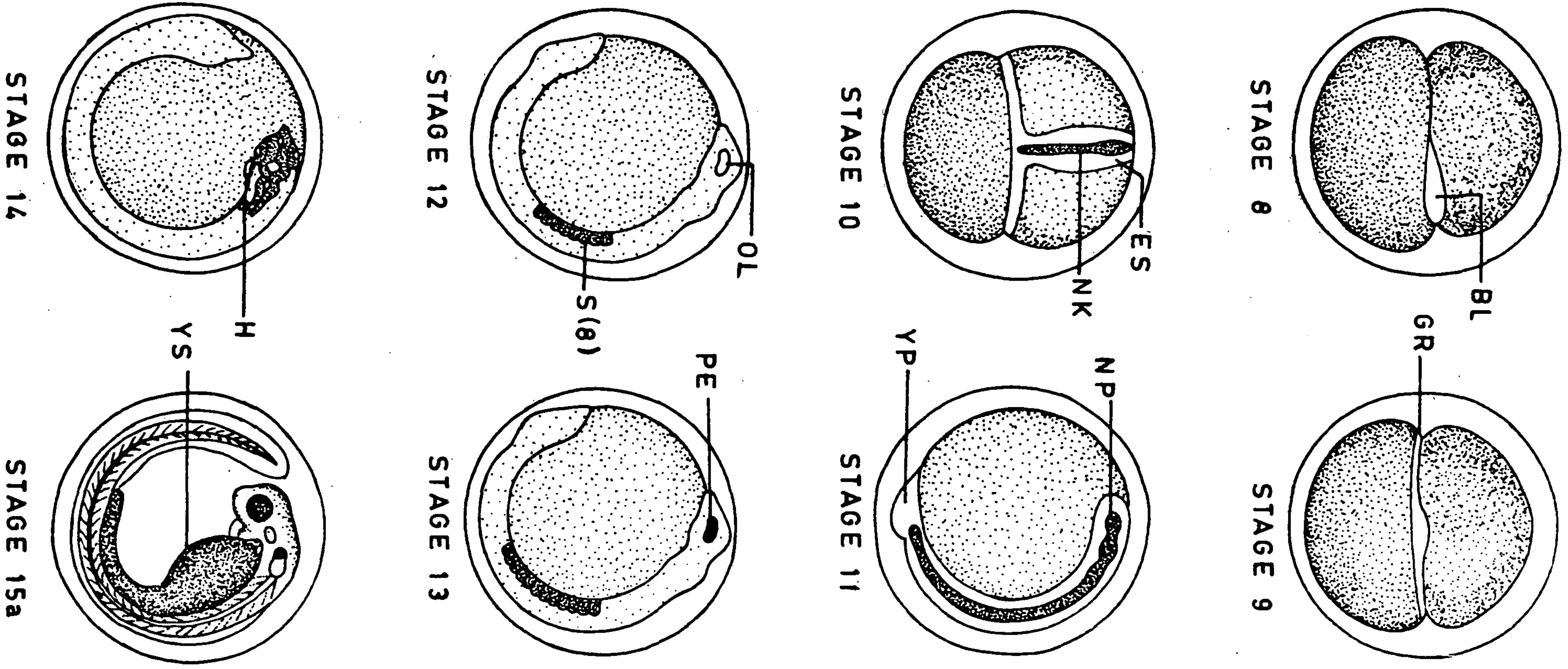
STAGE 4 STAGE 5

STAGE 6 STAGE 7

FIG. 2a. DIAGRAMATIC REPRESENTATION OF DIFFERENT EARLY DEVELOPMENTAL STAGES (0-15b) OBSERVED IN *C. CARPIO*. A.P = ANIMAL POLE; B = BLASTODISC; Y = YOLK; VM = VITELLINE MEMBRANE; V.P = VEGETAL POLE; BM = BLASTOMERE.

Contd.

FIG. 2b. DIAGRAMATIC REPRESENTATION OF DIFFERENT EARLY DEVELOPMENTAL STAGES (0-15b) OBSERVED IN C. CARPIO. BL = BLASTOCOEL; GR = GERM RING; ES = EMBRYONIC SHIELD; NK = NEURAL KEEL; NP = NEURAL PLATE; YP = YOLK PLUG; S = SOMITE; OL = OPTIC LOBE; PE = PIGMENTED EYE; H = HEART; YS = YOLK SAC.



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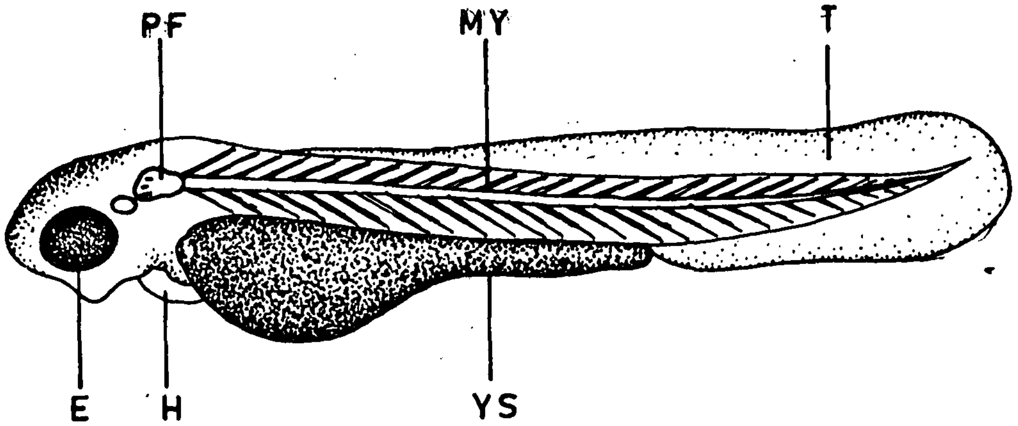


STAGE 15b

FIG. 2c. DIAGRAMATIC REPRESENTATION OF DIFFERENT EARLY DEVELOPMENTAL STAGES (0-15b) OBSERVED IN C.CARPIO. PF= PECTORAL; FIN ; MY= MYOTOMES ; T=TAIL ; E=EYE ; H = HEART ; YS =YOLK SAC.

FIG. 3a. DIAGRAMATIC REPRESENTATION OF DIFFERENT EARLY DEVELOPMENTAL STAGES (0-15b) OBSERVED IN *L. ROHITA*. AP=ANIMAL POLE; B=BLASTODISC; Y=YOLK; VM=VITELLINE MEMBRANE; V.P=VEGETAL POLE; BM=BLASTOMERE; BL=BLASTOCOEL.

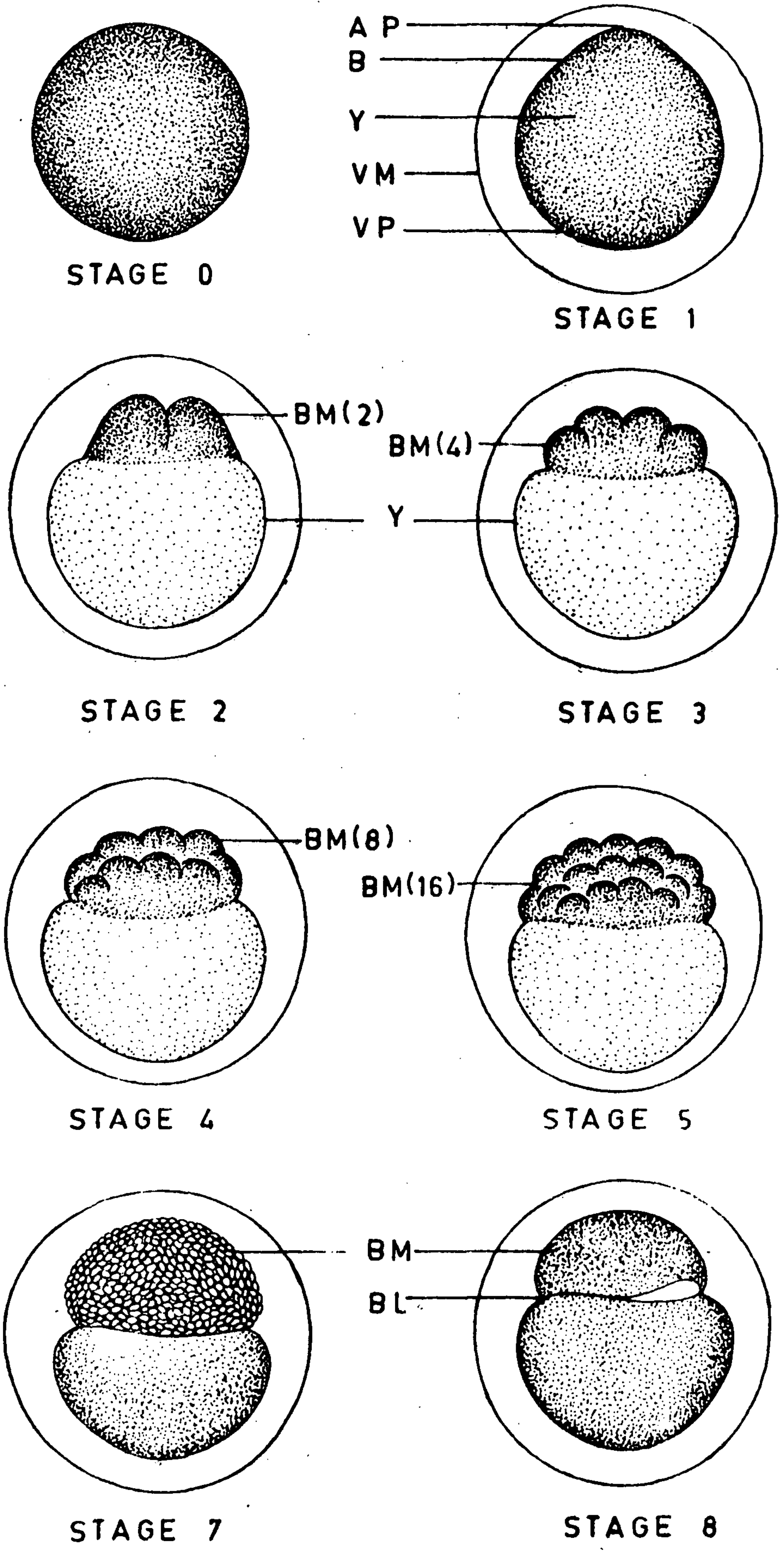
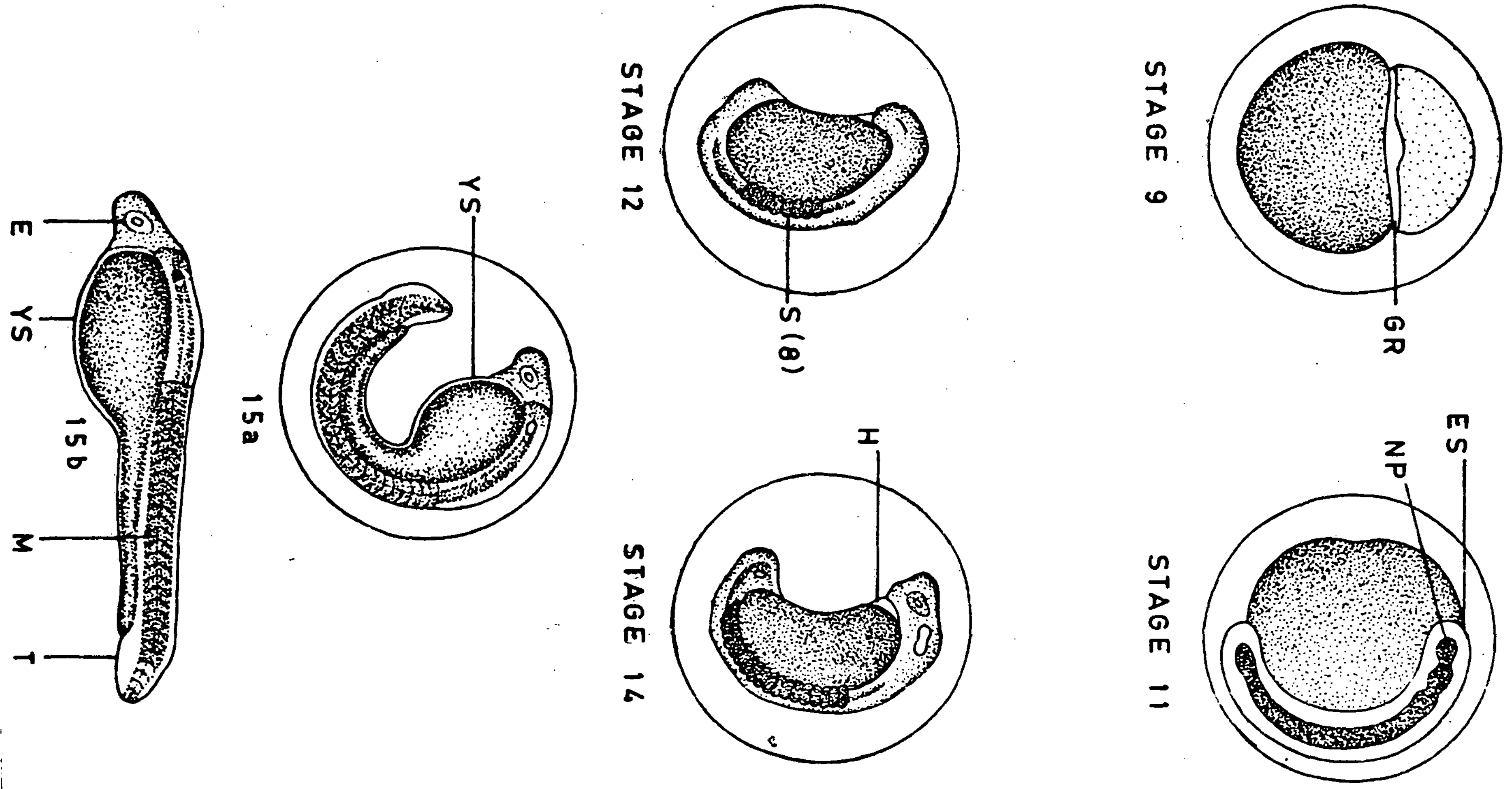


FIG. 3b. DIAGRAMATIC REPRESENTATION OF DIFFERENT EARLY DEVELOPMENTAL STAGES (0-15b) OBSERVED IN *L.ROHITA*. ES=EMBRYONIC SHIELD; GR=GERM BAND; NP=NEURAL PLATE; S=SOMITE; H=HEART; YS=YOLK SAC; E=EYE; M=MYOTOMES; T=TAIL.



STAGE 9

STAGE 11

STAGE 12

STAGE 14

15a

15b

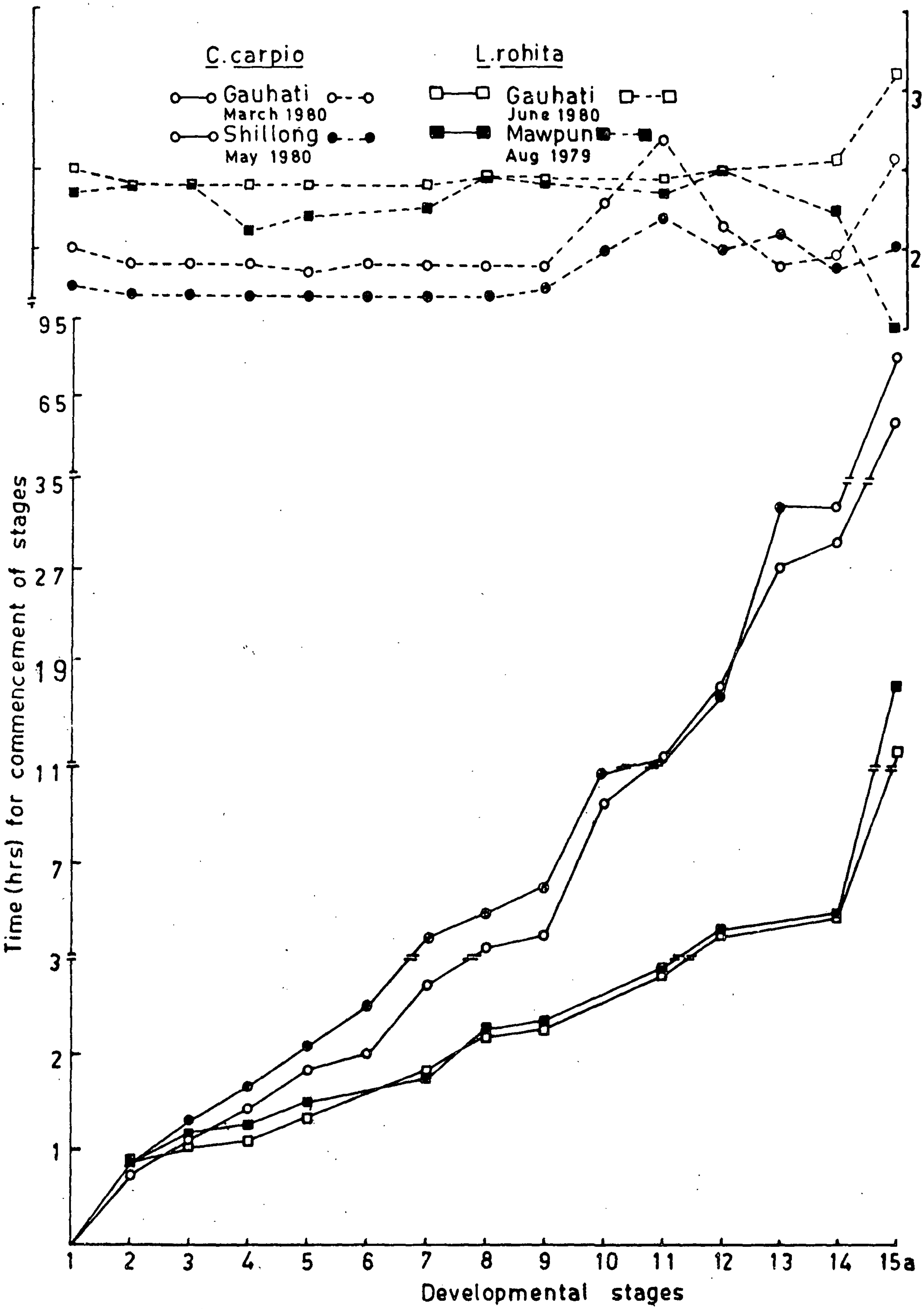


Fig. 4 Time course of development (—) of *C.carpio* and *L.rohita* in relation to water temperature (---)

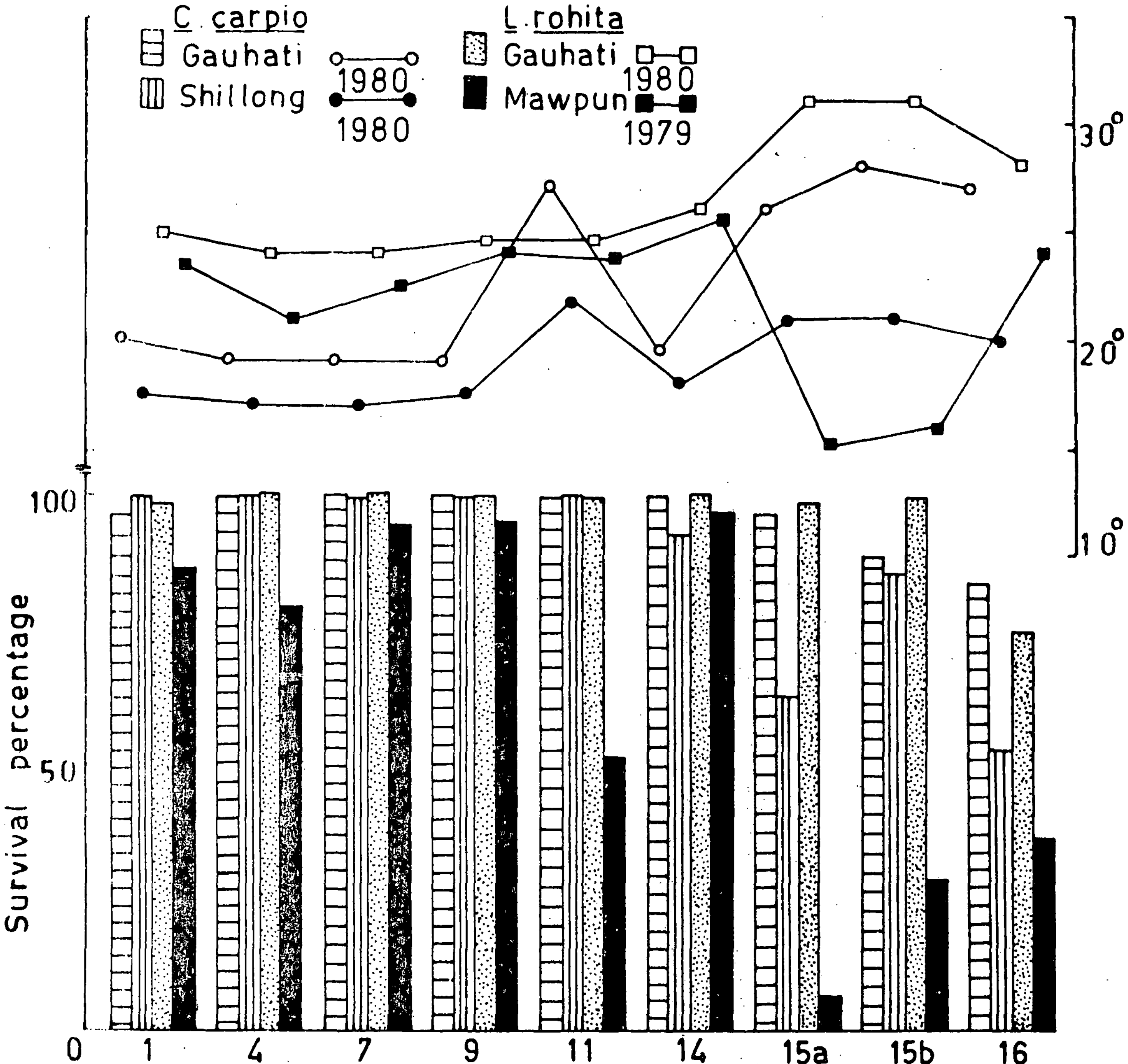


Fig. 5. Survival percentage (□) of some developmental stages of *C. carpio* and *L. rohita* at different altitudes in relation to the variations in water temperature (—)

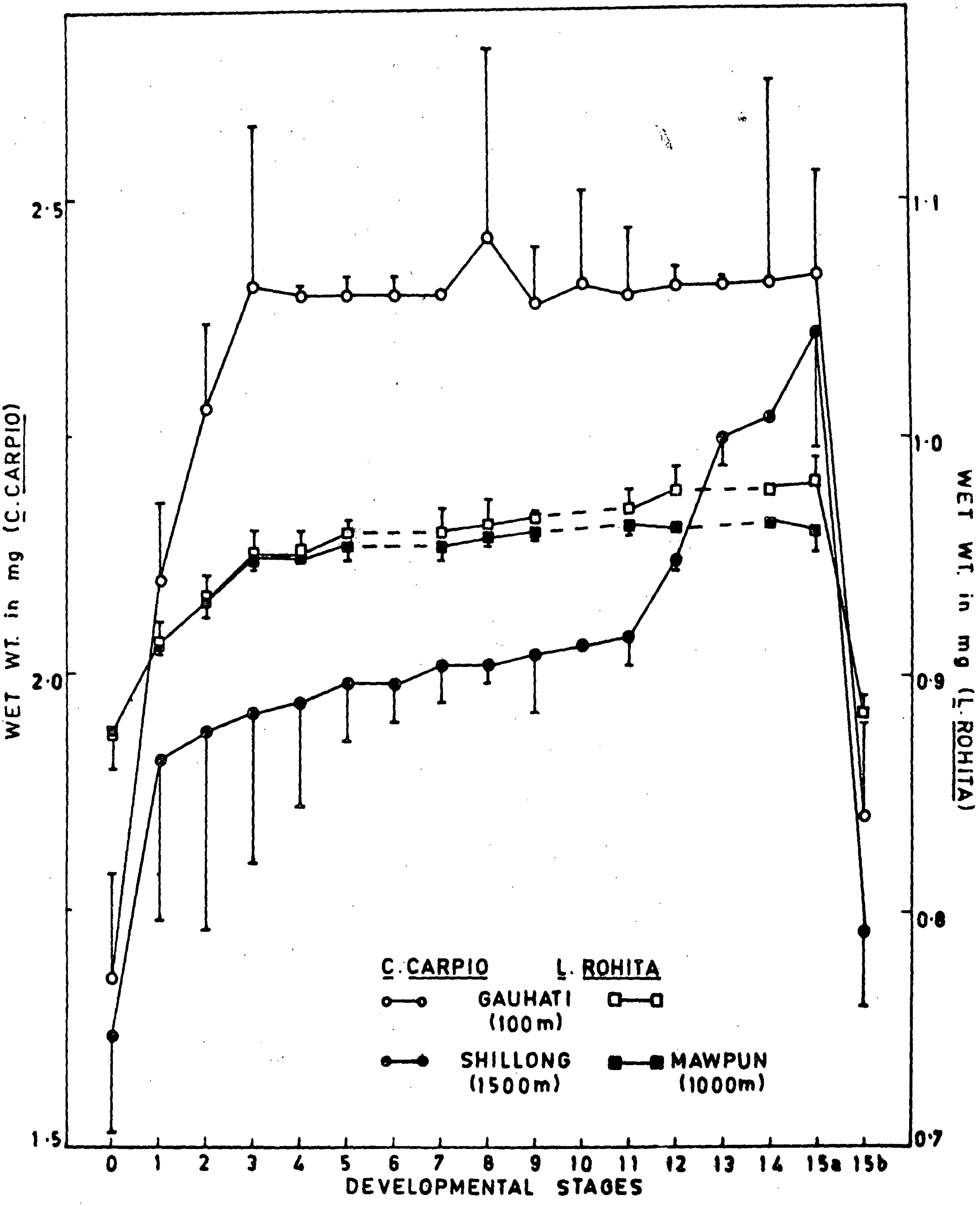


FIG. 6. CHANGES IN THE WET WEIGHT (mg) OF THE EMBRYO DURING EARLY DEVELOPMENT OF C. CARPIO AND L. ROHITA AT DIFFERENT ALTITUDES.

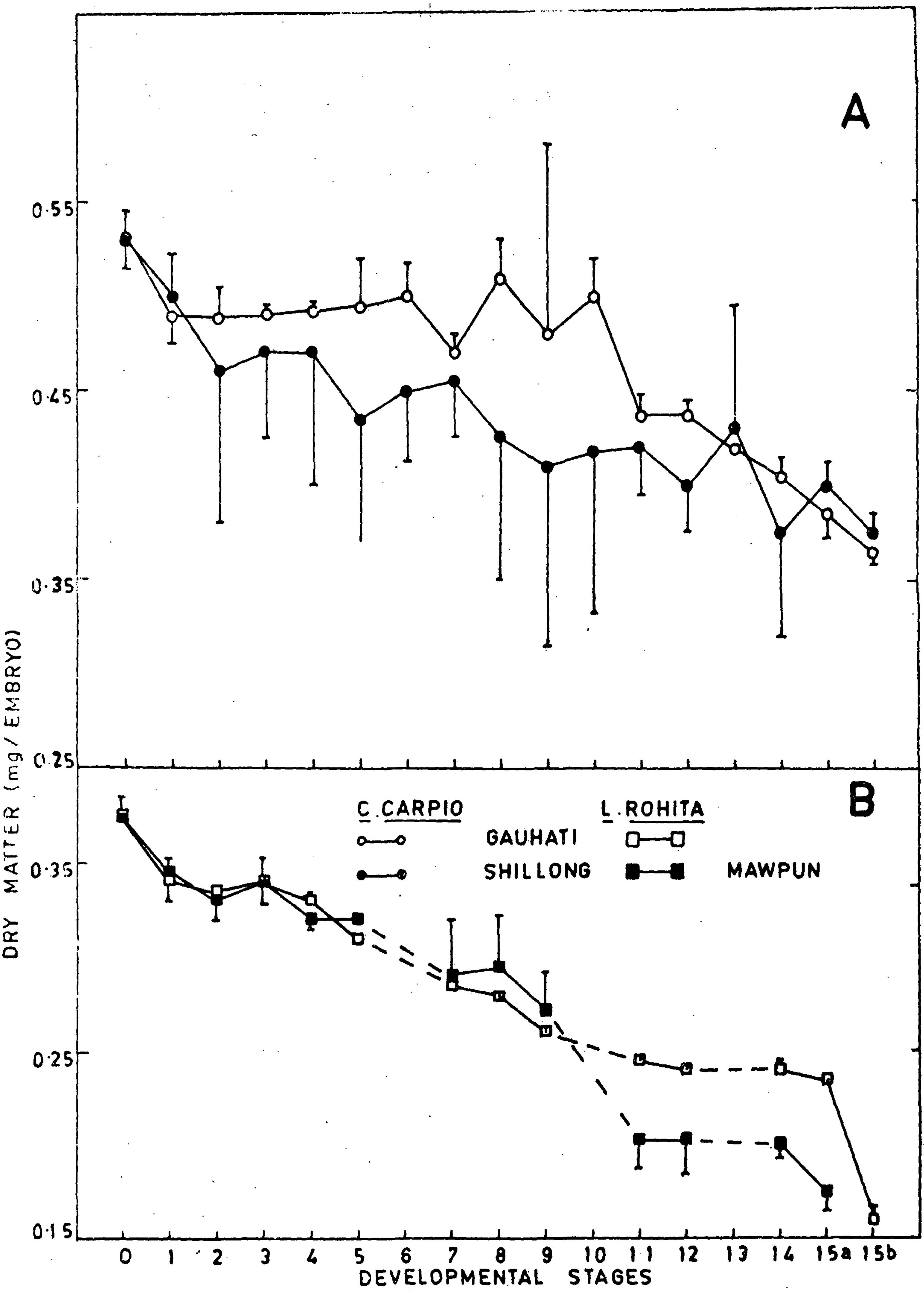


FIG. 7. CHANGES IN THE DRY MATTER CONTENT (mg/ EMBRYO) DURING THE EARLY DEVELOPMENT OF *C. CARPIO* (A) AND *L. ROHITA* (B) AT DIFFERENT ALTITUDES.

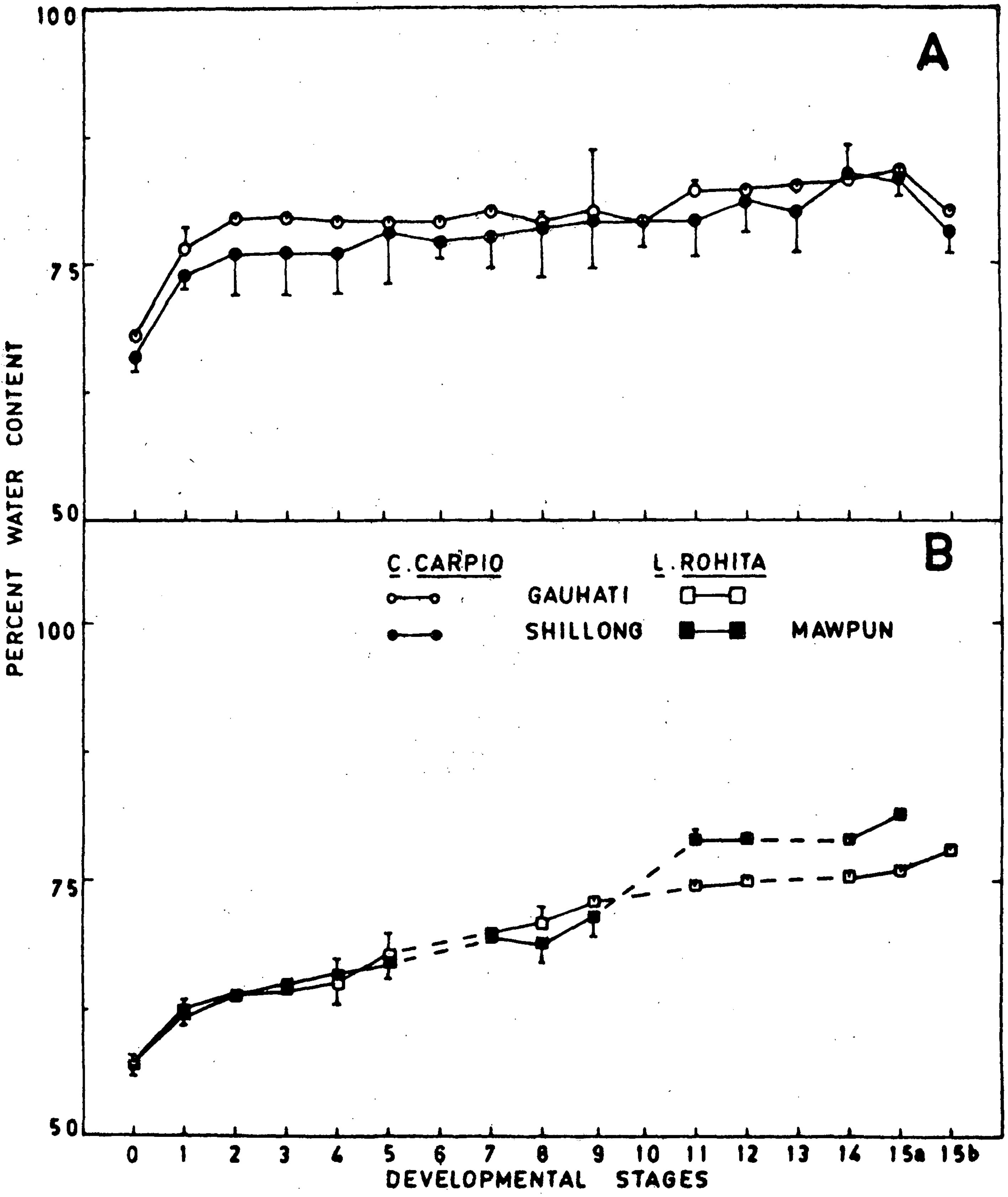


FIG. 8. CHANGES IN PERCENT WATER CONTENT OF THE EMBRYO DURING EARLY DEVELOPMENT OF *C. CARPIO* (A) AND *L. ROHITA* (B)

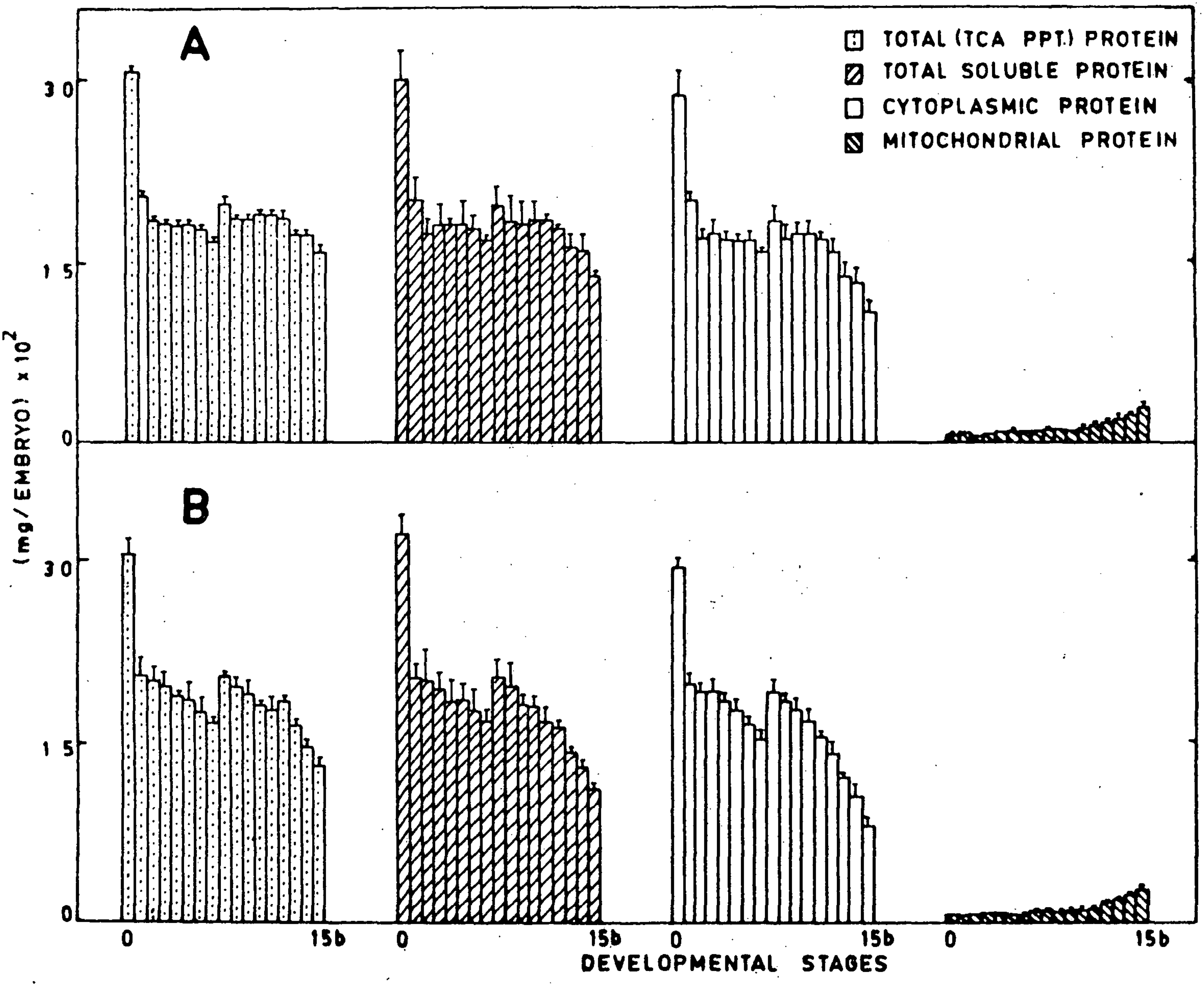


FIG. 9. CHANGES IN TOTAL, SOLUBLE, CYTOPLASMIC AND MITOCHONDRIAL PROTEIN CONTENT (mg/EMBRYO) x 10^2 DURING EARLY DEVELOPMENT OF C. CARPIO AT DIFFERENT ALTITUDES [GAUHATI (A) AND SHILLONG (B)].

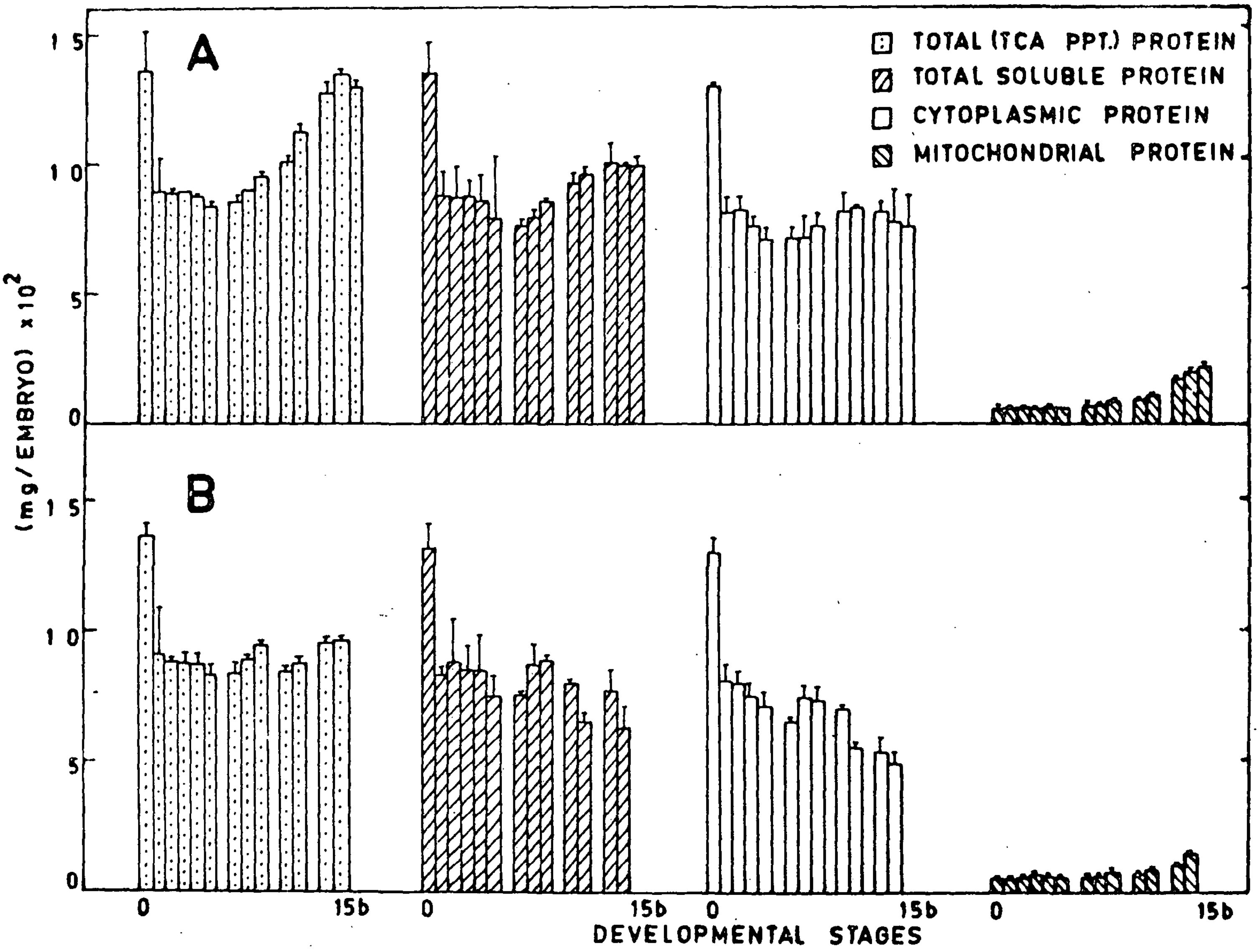


FIG. 10 CHANGES IN TOTAL, SOLUBLE, CYTOPLASMIC AND MITOCHONDRIAL PROTEIN CONTENT (mg/EMBRYO) x 10^2 DURING EARLY DEVELOPMENT OF L. ROHITA AT DIFFERENT ALTITUDES [GAUHATI (A) AND MAWPUN (B)].

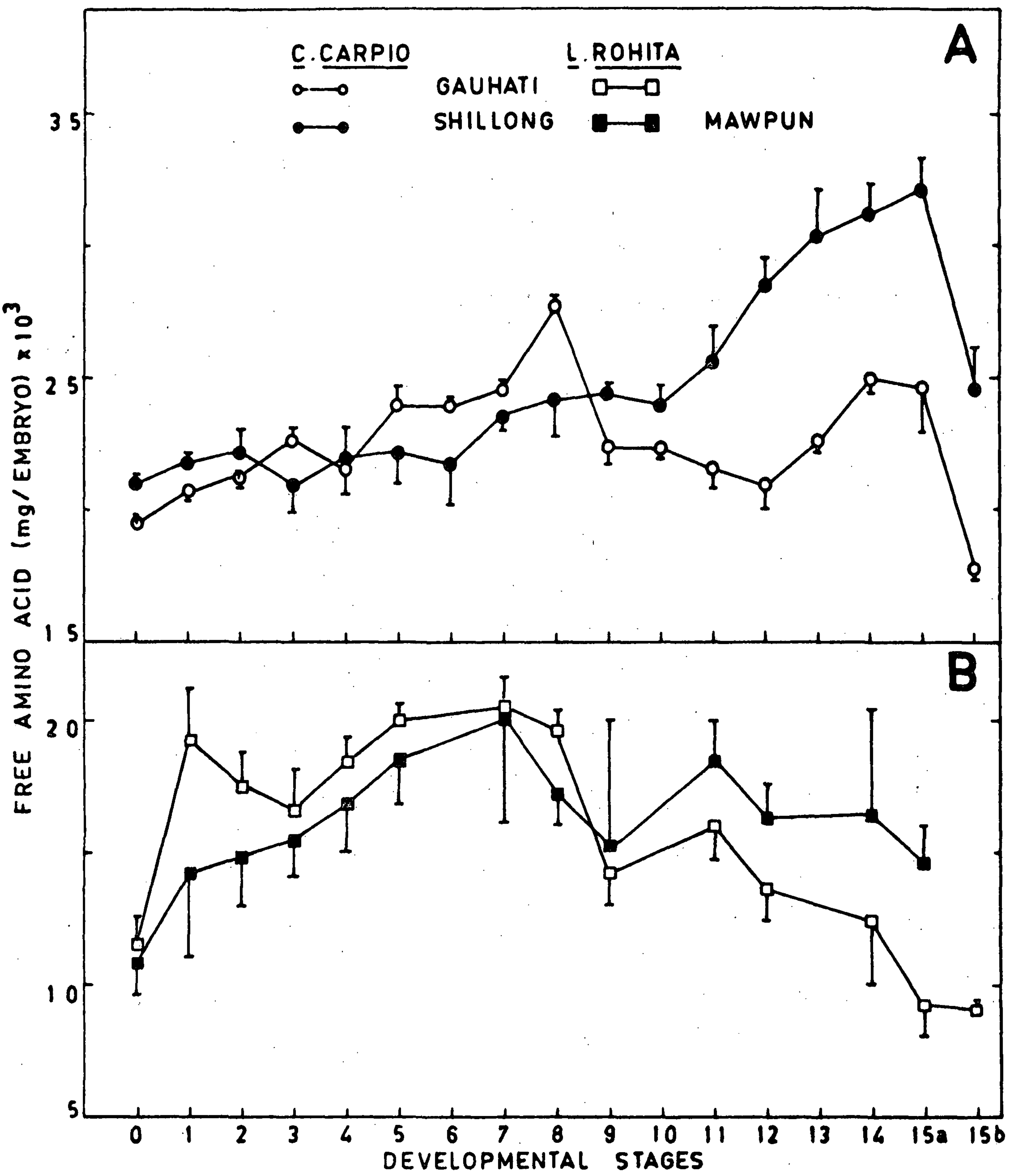


FIG.11. CHANGES IN TOTAL FREE AMINO ACID CONTENT (mg/EMBRYO) x 10^3 DURING EARLY DEVELOPMENT OF C. CARPIO (A) AND L. ROHITA (B) AT DIFFERENT ALTITUDES.

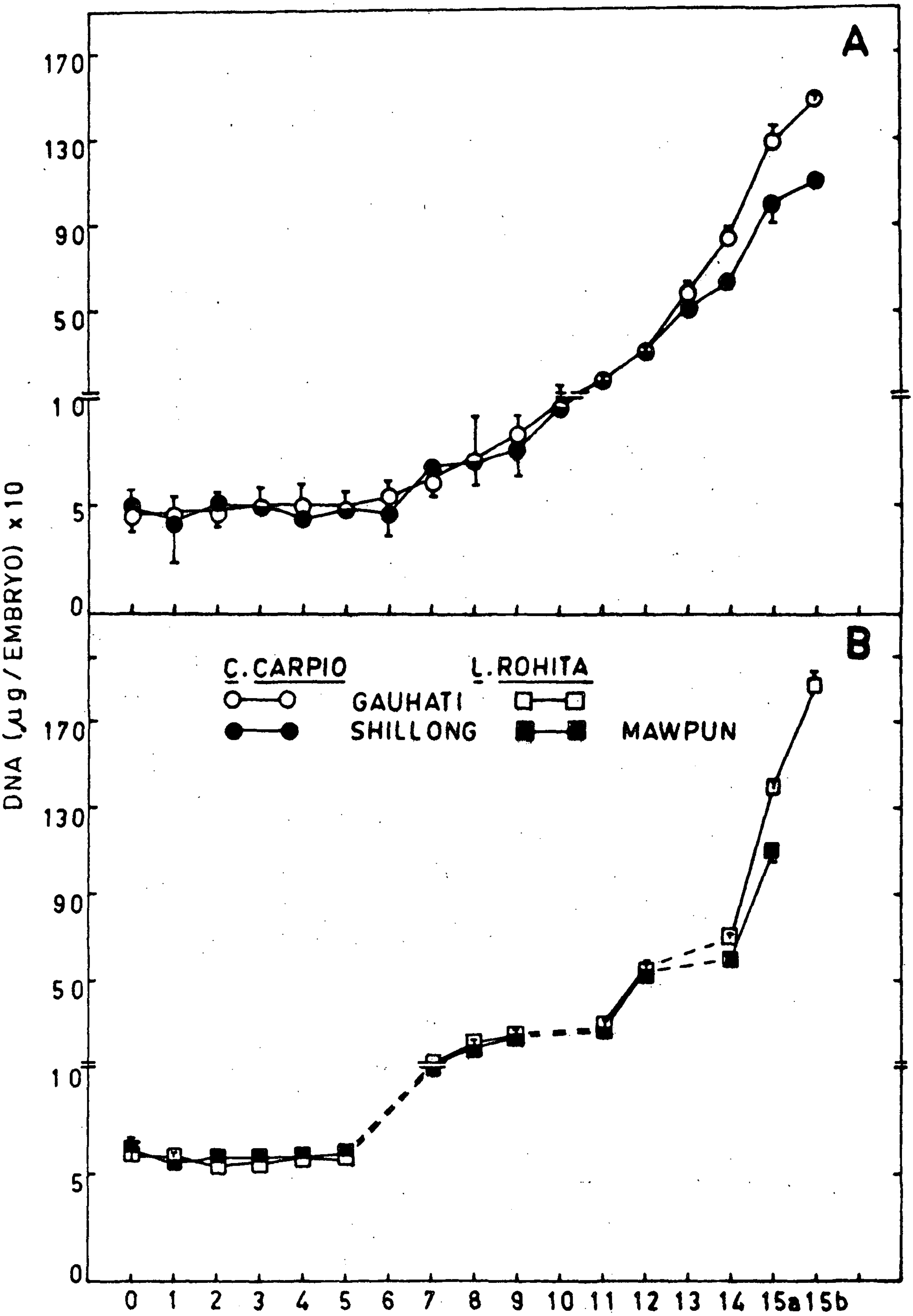


FIG. 12. CHANGES IN DNA CONTENT (µg/EMBRYO) x 10 DURING EARLY DEVELOPMENT OF C. CARPIO (A) AND L. ROHITA (B) AT DIFFERENT ALTITUDES.

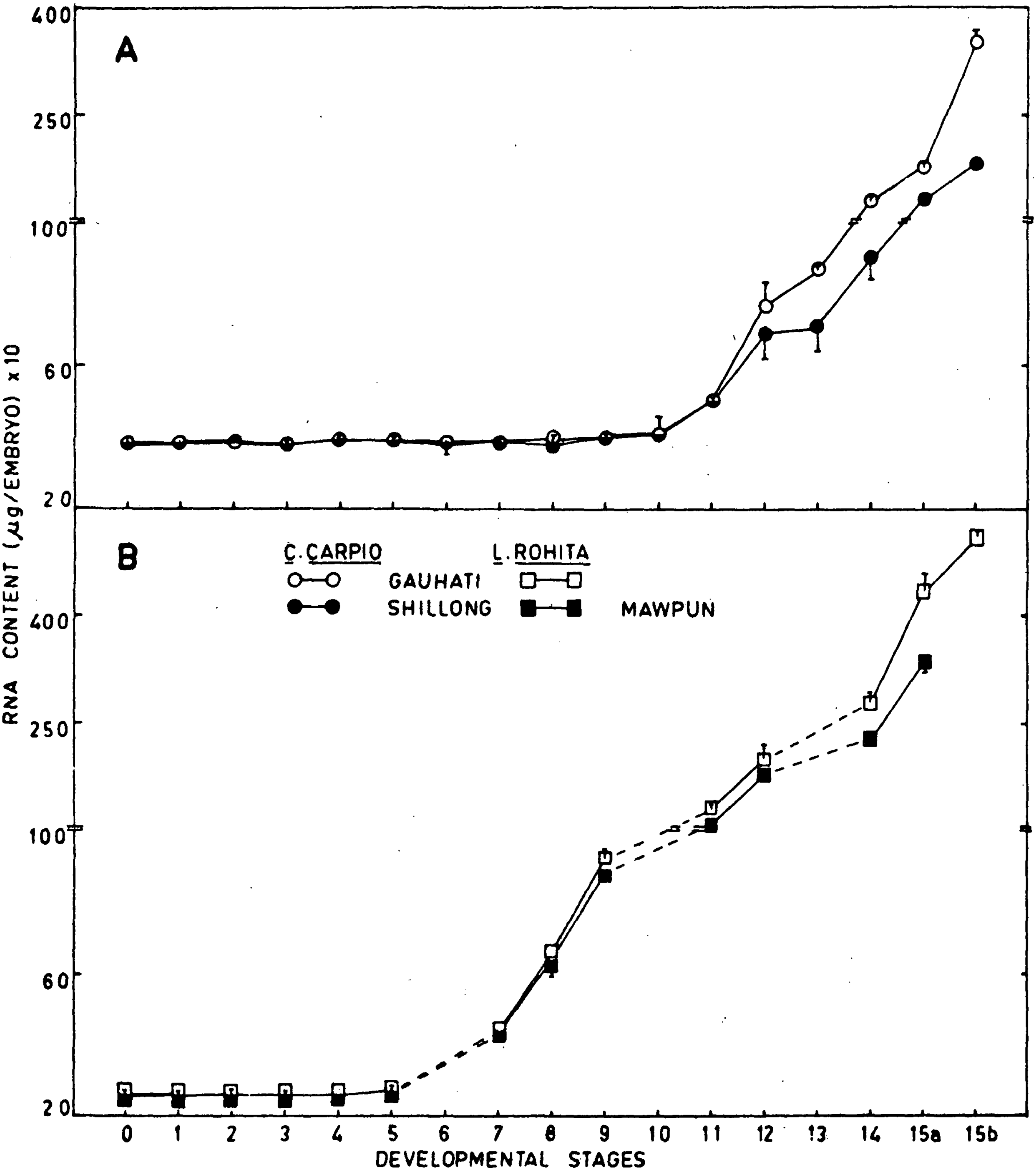


FIG.13. CHANGES IN THE RNA CONTENT (µg/EMBRYO) x10 DURING EARLY DEVELOPMENT OF C. CARPIO (A) L. ROHITA (B) AT DIFFERENT ALTITUDES.

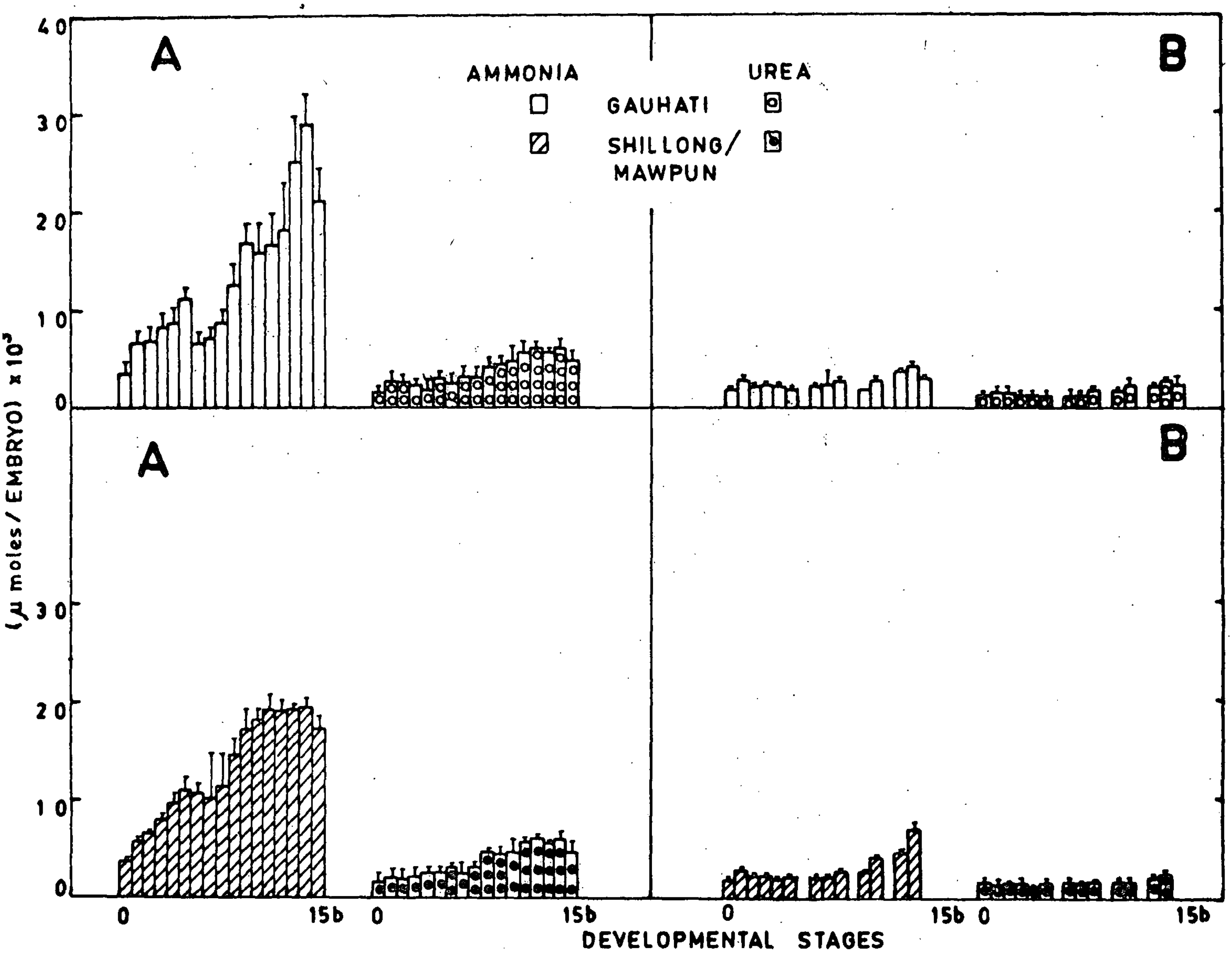


FIG. 14. CHANGES IN AMMONIA AND UREA CONTENT (µmoles/EMBRYO) x 10^3 DURING EARLY DEVELOPMENT OF C. CARPIO (A) AND L. ROHITA (B) AT DIFFERENT ALTITUDES.

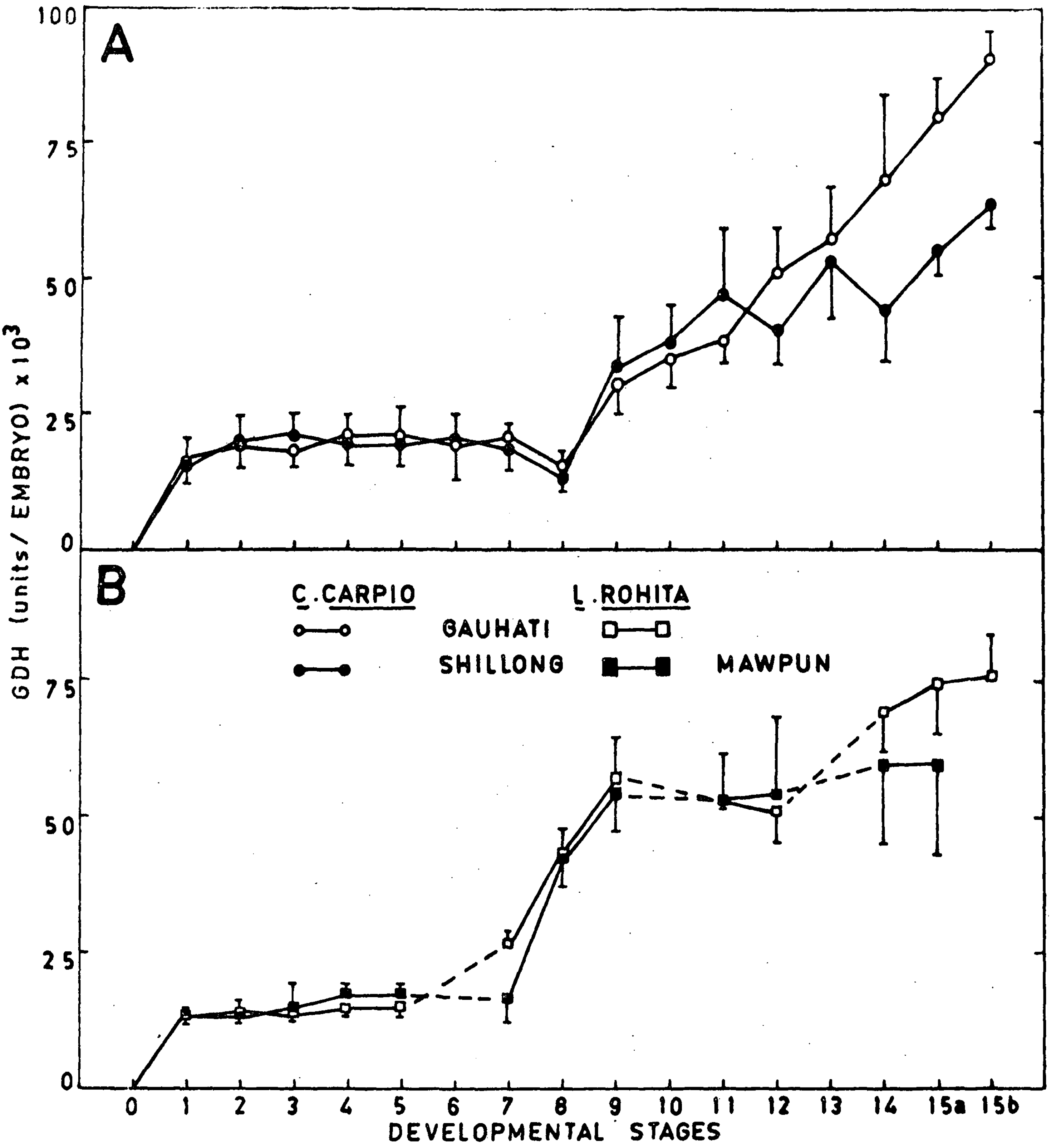


FIG. 15. CHANGES IN THE TOTAL ACTIVITY (units/EMBRYO) x 10^3 OF GLUTAMATE DEHYDROGENASE (GDH) DURING EARLY DEVELOPMENT OF C. CARPIO (A) AND L. ROHITA (B) AT DIFFERENT ALTITUDES.

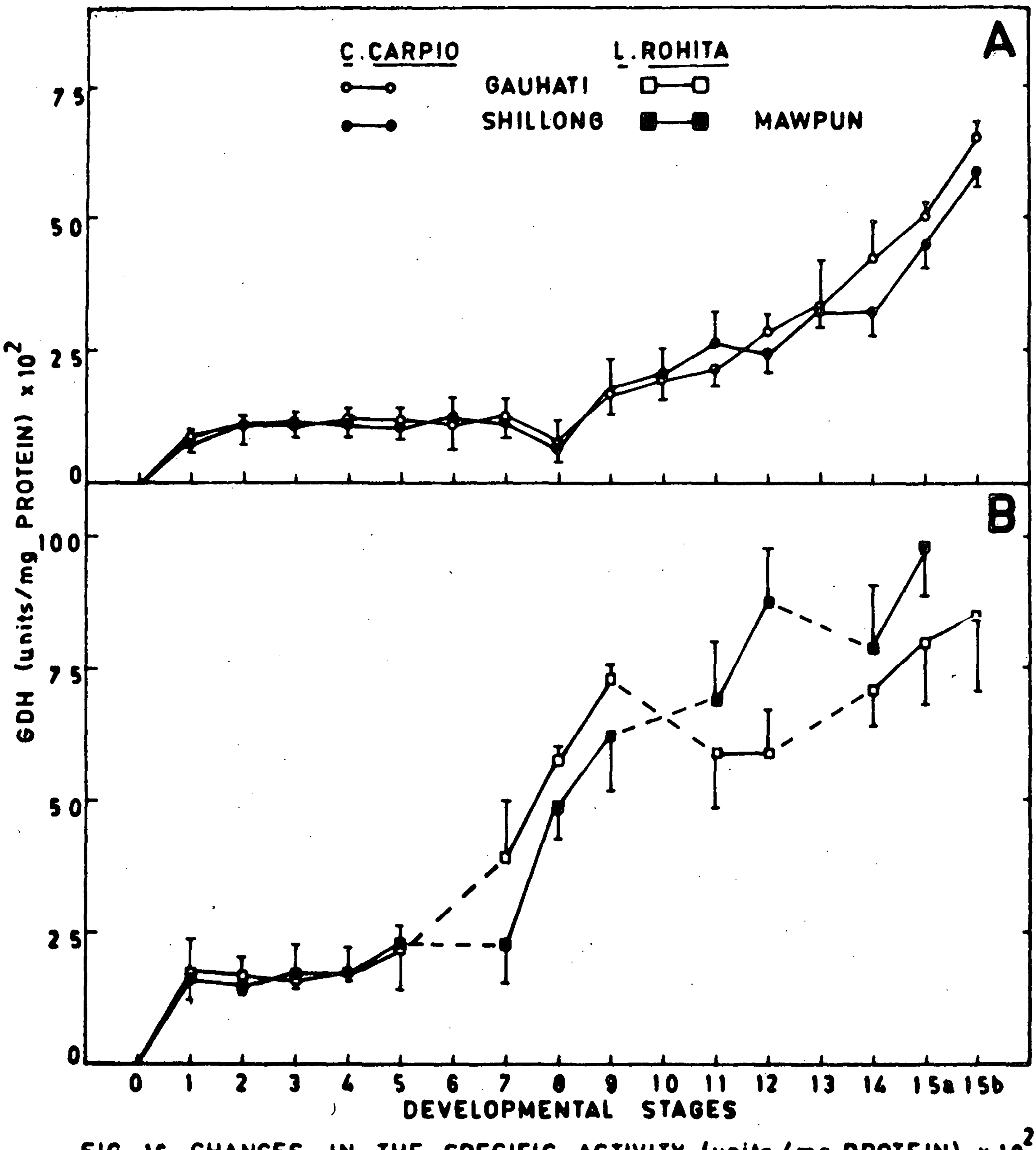


FIG. 16. CHANGES IN THE SPECIFIC ACTIVITY (units/mg PROTEIN) x 10^2 OF GLUTAMATE DEHYDROGENASE (GDH) DURING EARLY DEVELOPMENT OF *C. CARPIO* (A) AND *L. ROHITA* (B) AT DIFFERENT ALTITUDES.

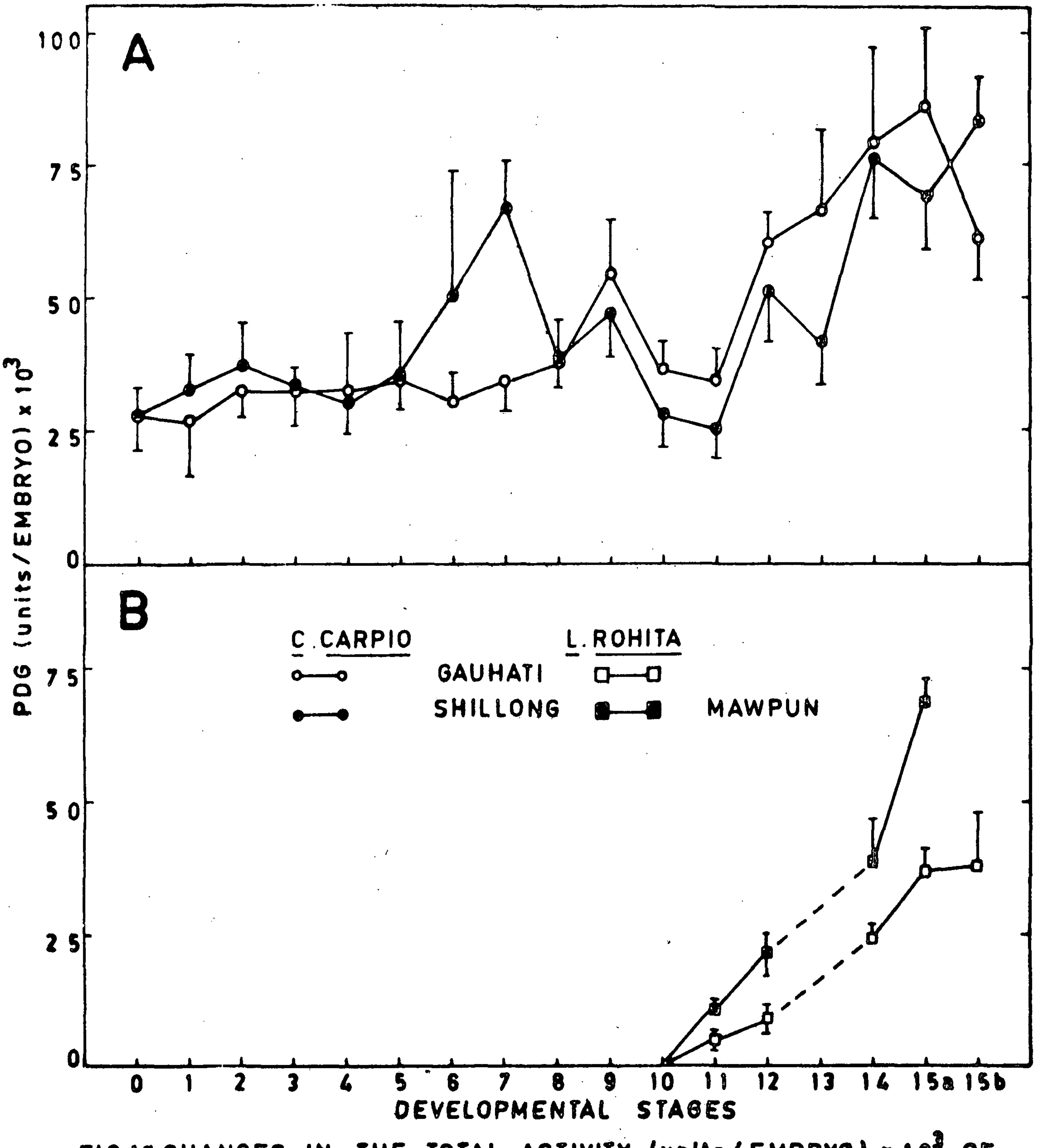


FIG.17. CHANGES IN THE TOTAL ACTIVITY (units/EMBRYO) x 10^3 OF PHOSPHATE DEPENDENT GLUTAMINASE (PDG) DURING EARLY DEVELOPMENT OF C. CARPIO (A) AND L. ROHITA (B) AT DIFFERENT ALTITUDES.

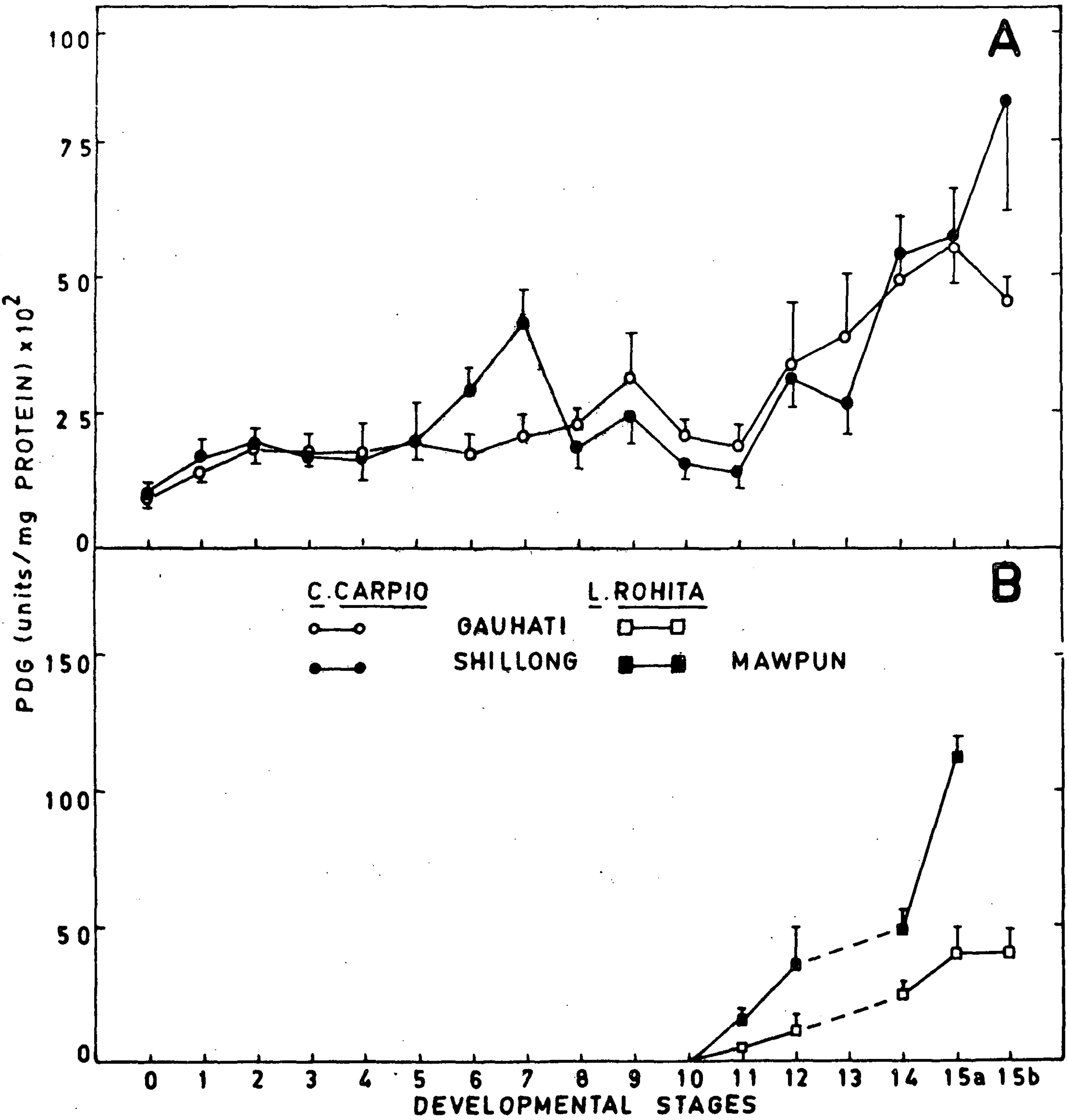


FIG. 18. CHANGES IN THE SPECIFIC ACTIVITY (units/mg PROTEIN) $\times 10^2$ OF PHOSPHATE DEPENDENT GLUTAMINASE (PDG) DURING EARLY DEVELOPMENT OF C. CARPIO (A) AND L. ROHITA (B) AT DIFFERENT ALTITUDES.

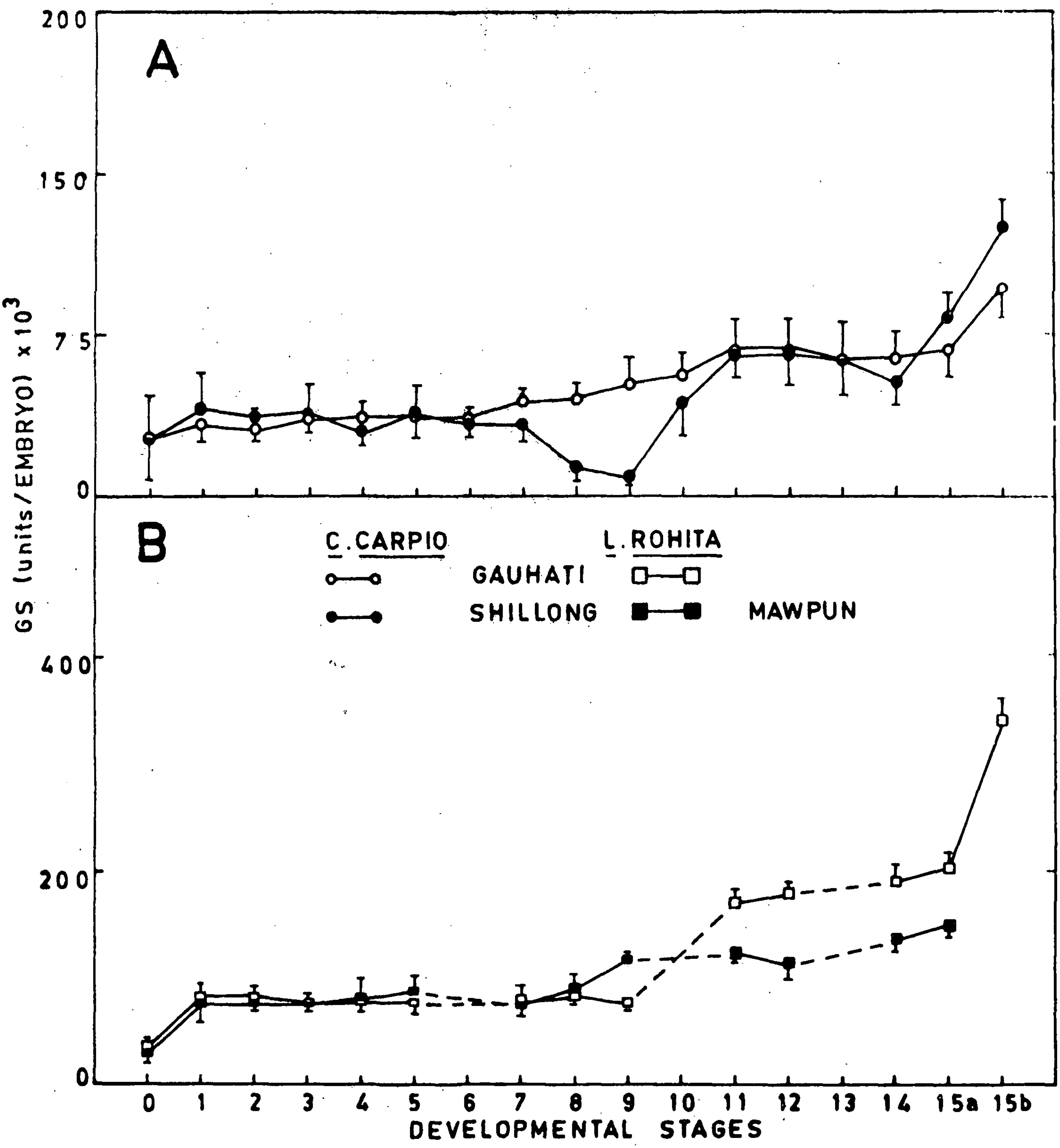


FIG. 19. CHANGES IN THE TOTAL ACTIVITY (units/ EMBRYO) x 10^3 OF GLUTAMINE SYNTHETASE (GS) DURING EARLY DEVELOPMENT OF *C. CARPIO* (A) AND *L. ROHITA* (B) AT DIFFERENT ALTITUDES.

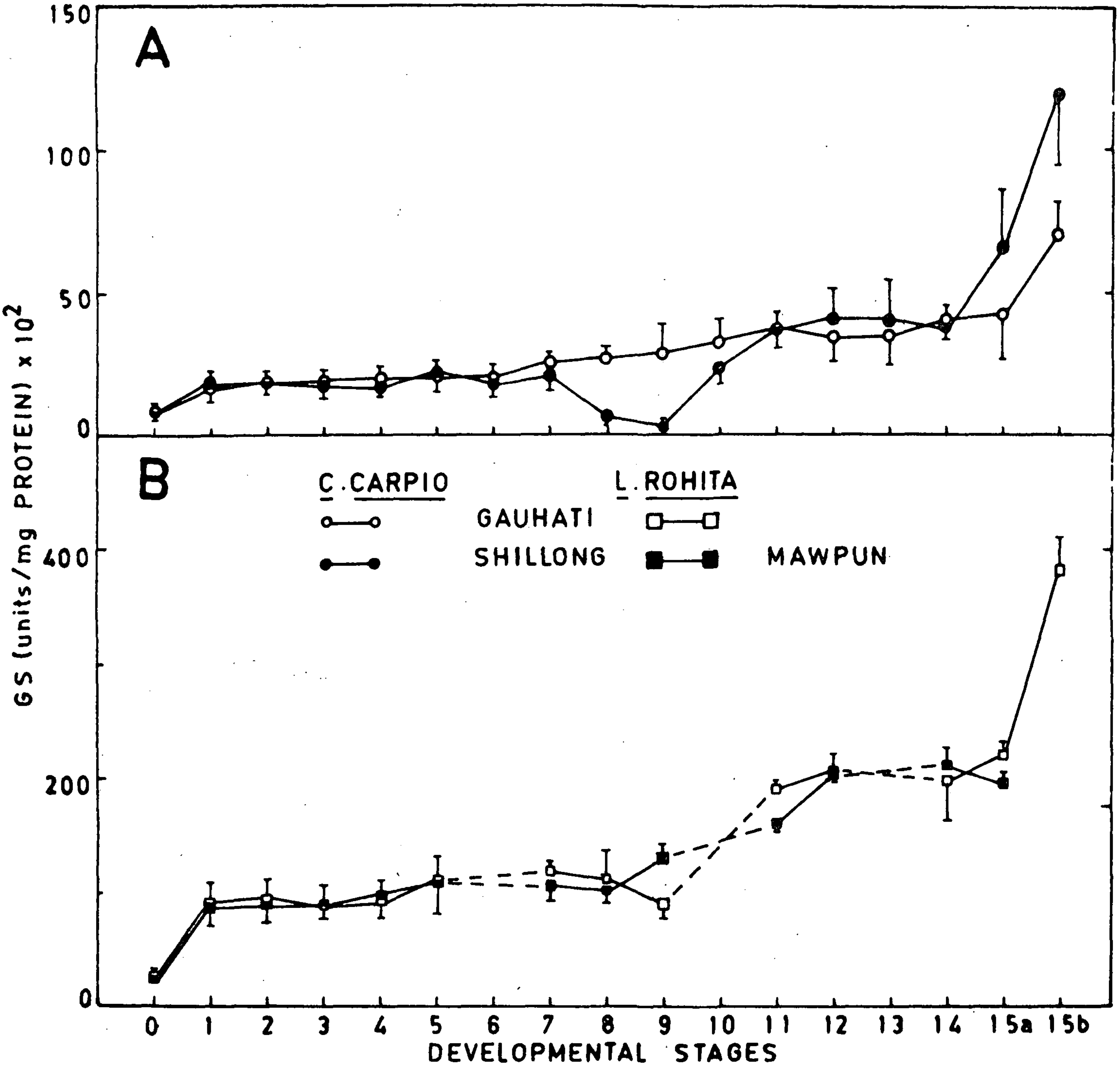


FIG.20. CHANGES IN THE SPECIFIC ACTIVITY (units/mg PROTEIN) x 10^2 OF GLUTAMINE SYNTHETASE (GS) DURING EARLY DEVELOPMENT OF C. CARPIO (A) L. ROHITA (B) AT DIFFERENT ALTITUDES.

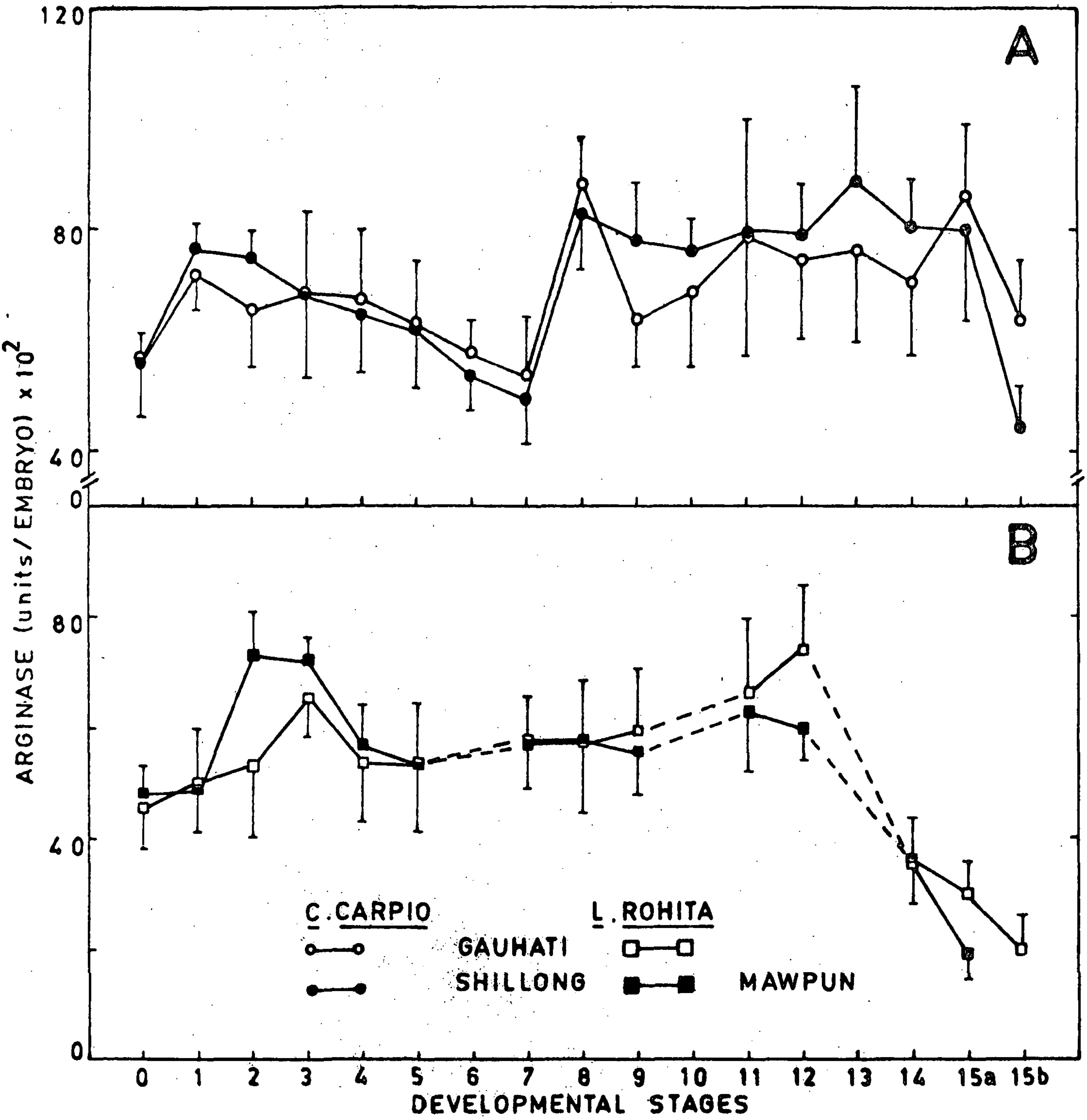


FIG.21. CHANGES IN THE TOTAL ACTIVITY (units/EMBRYO) x 10^2 OF ARGINASE DURING EARLY DEVELOPMENT OF C. CARPIO (A) AND L. ROHITA (B) AT DIFFERENT ALTITUDES.

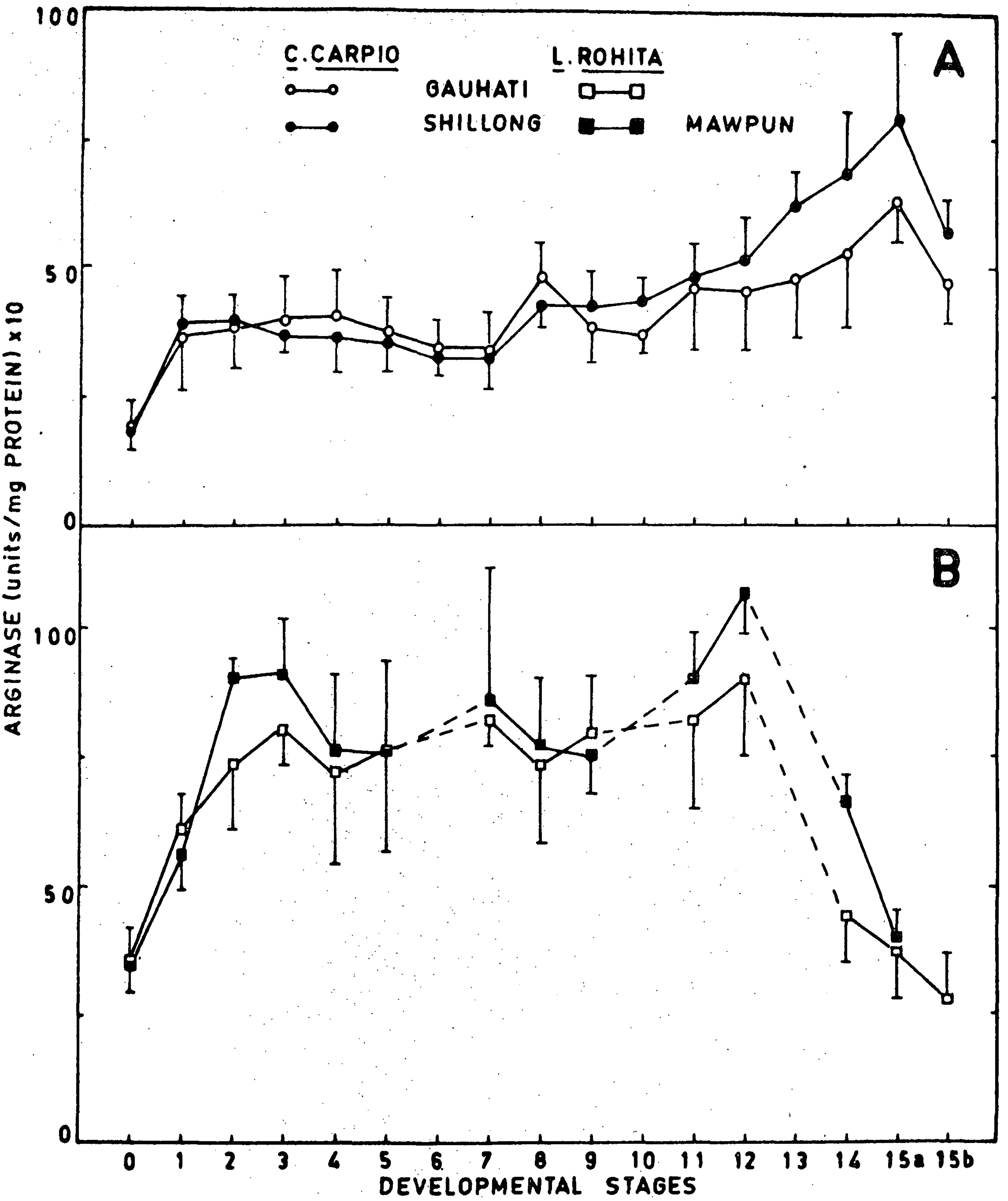


FIG.22. CHANGES IN THE SPECIFIC ACTIVITY (units/ mg PROTEIN) x10 OF ARGINASE DURING EARLY DEVELOPMENT OF *C.CARPIO* (A) AND *L.ROHITA* (B) AT DIFFERENT ALTITUDES.

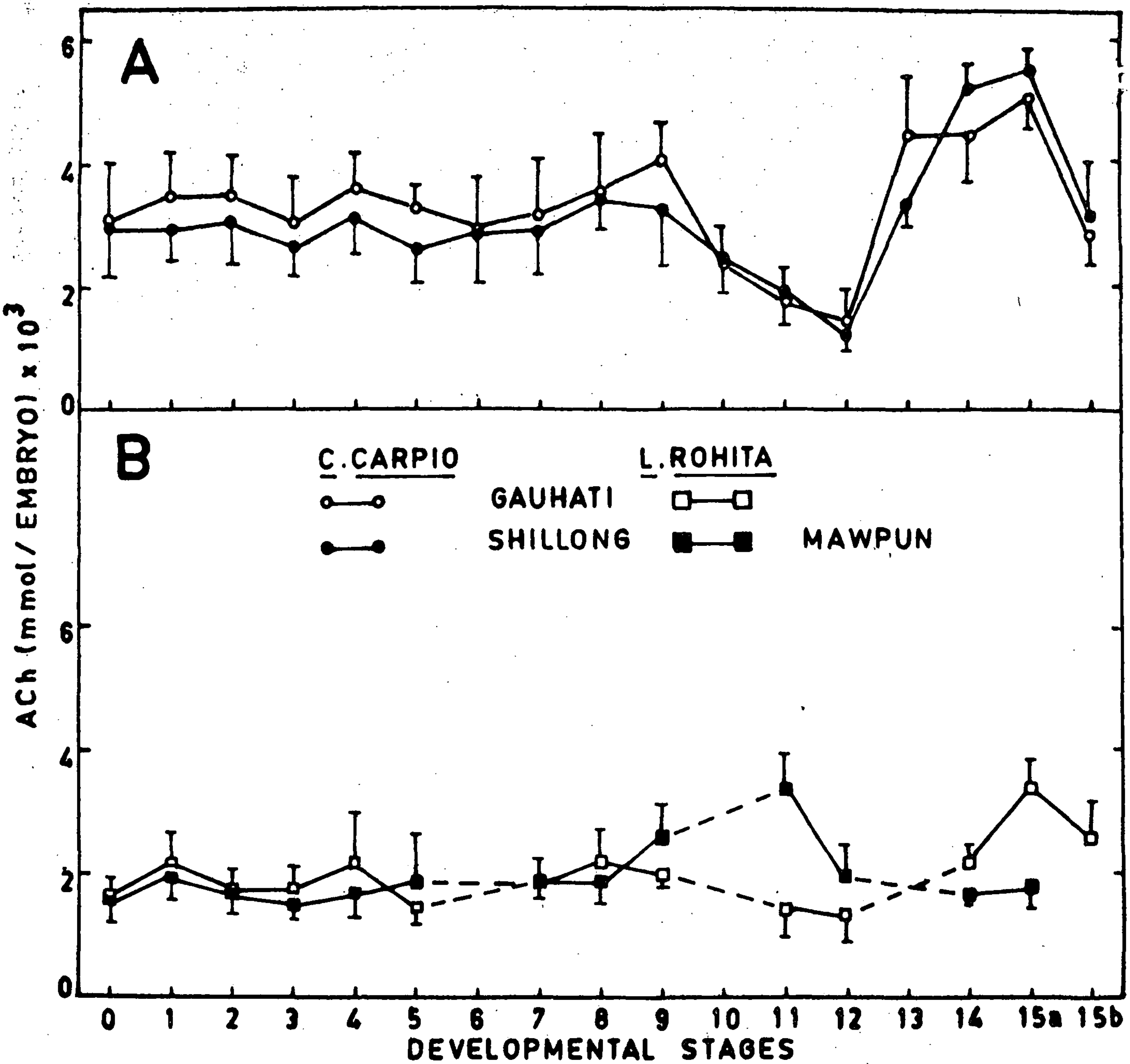


FIG.23. CHANGES IN ACETYLCHOLINE (ACh) CONTENT (mmol/EMBRYO) x 10^3 DURING EARLY DEVELOPMENT OF C. CARPIO (A) AND L. ROHITA (B) AT DIFFERENT ALTITUDES.

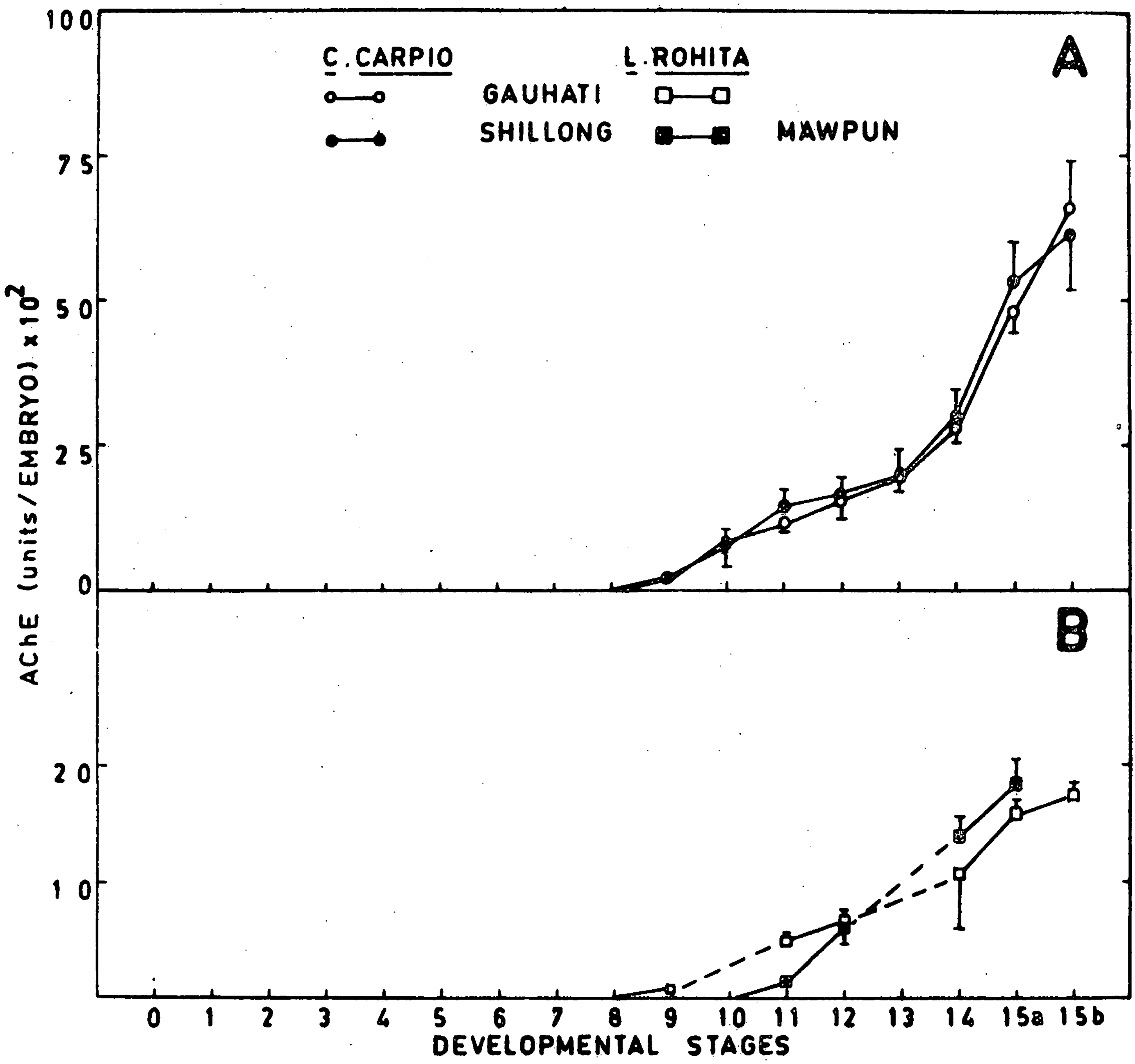


FIG.24. CHANGES IN THE TOTAL ACTIVITY (units/EMBRYO) x 10^3 OF ACETYLCHOLINESTERASE (AChE) DURING EARLY DEVELOPMENT OF C. CARPIO (A) AND L. ROHITA (B) AT DIFFERENT ALTITUDES.

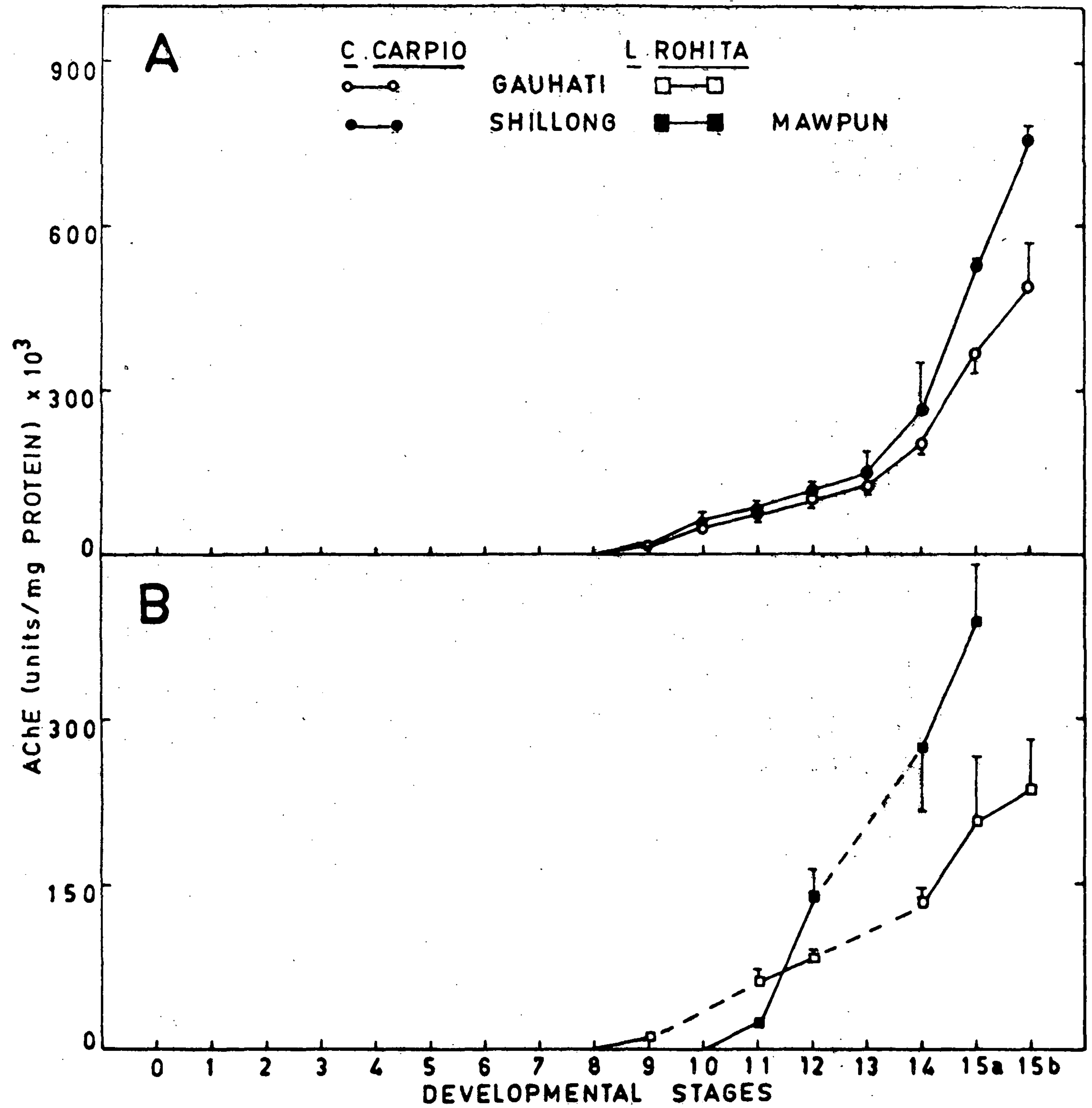


FIG. 25. CHANGES IN THE SPECIFIC ACTIVITY (units/mg PROTEIN) x10^3 OF ACETYLCHOLINESTERASE (AChE) DURING EARLY DEVELOPMENT OF C. CARPIO (A) AND L. ROHITA (B) AT DIFFERENT ALTITUDES.

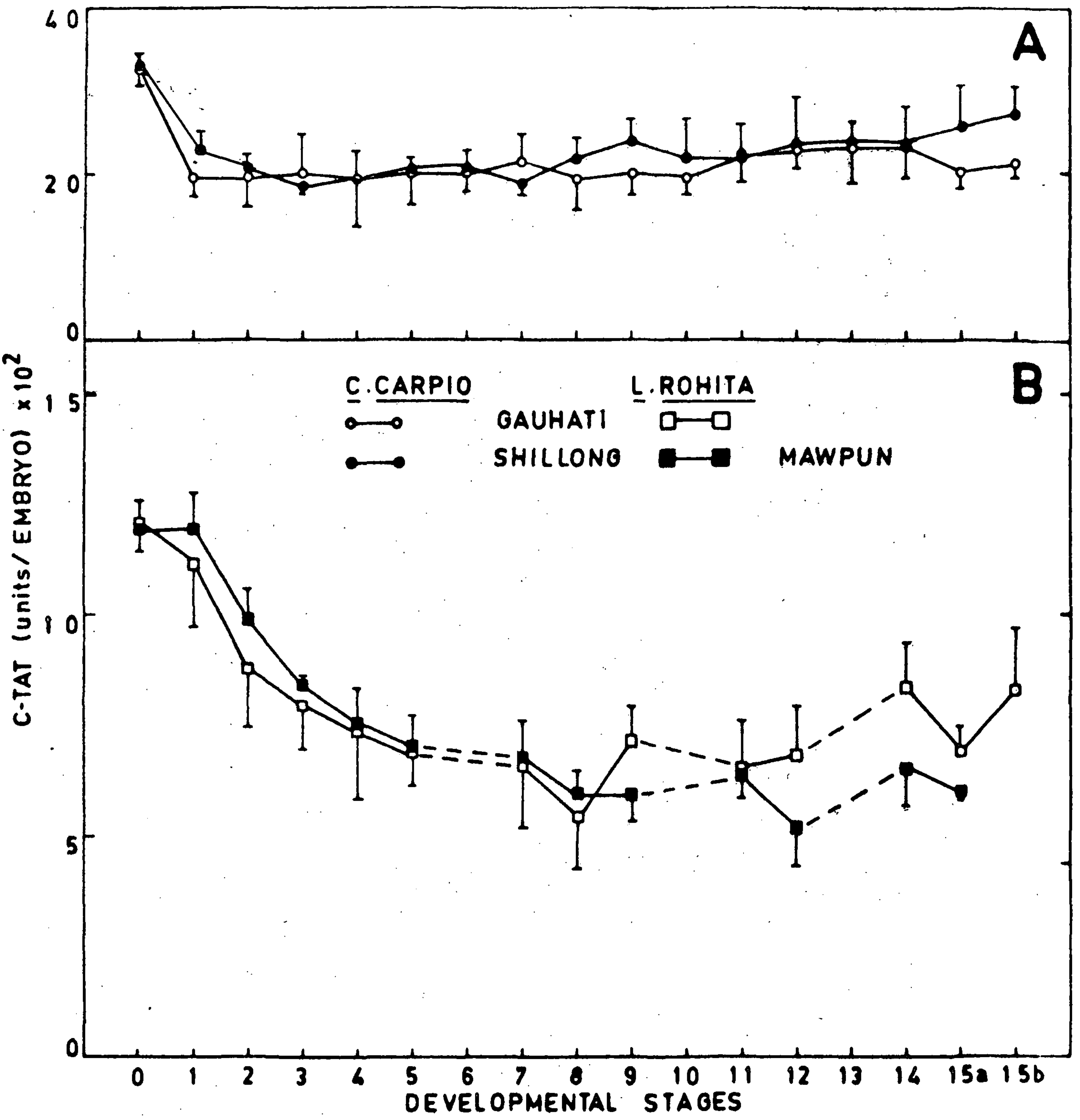


FIG. 26. CHANGES IN THE TOTAL ACTIVITY (units / EMBRYO) x 10^2 OF CYTOPLASMIC TYROSINE AMINOTRANSFERASE (C-TAT) DURING EARLY DEVELOPMENT OF *C. CARPIO* (A) AND *L. ROHITA* (B) AT DIFFERENT ALTITUDES.

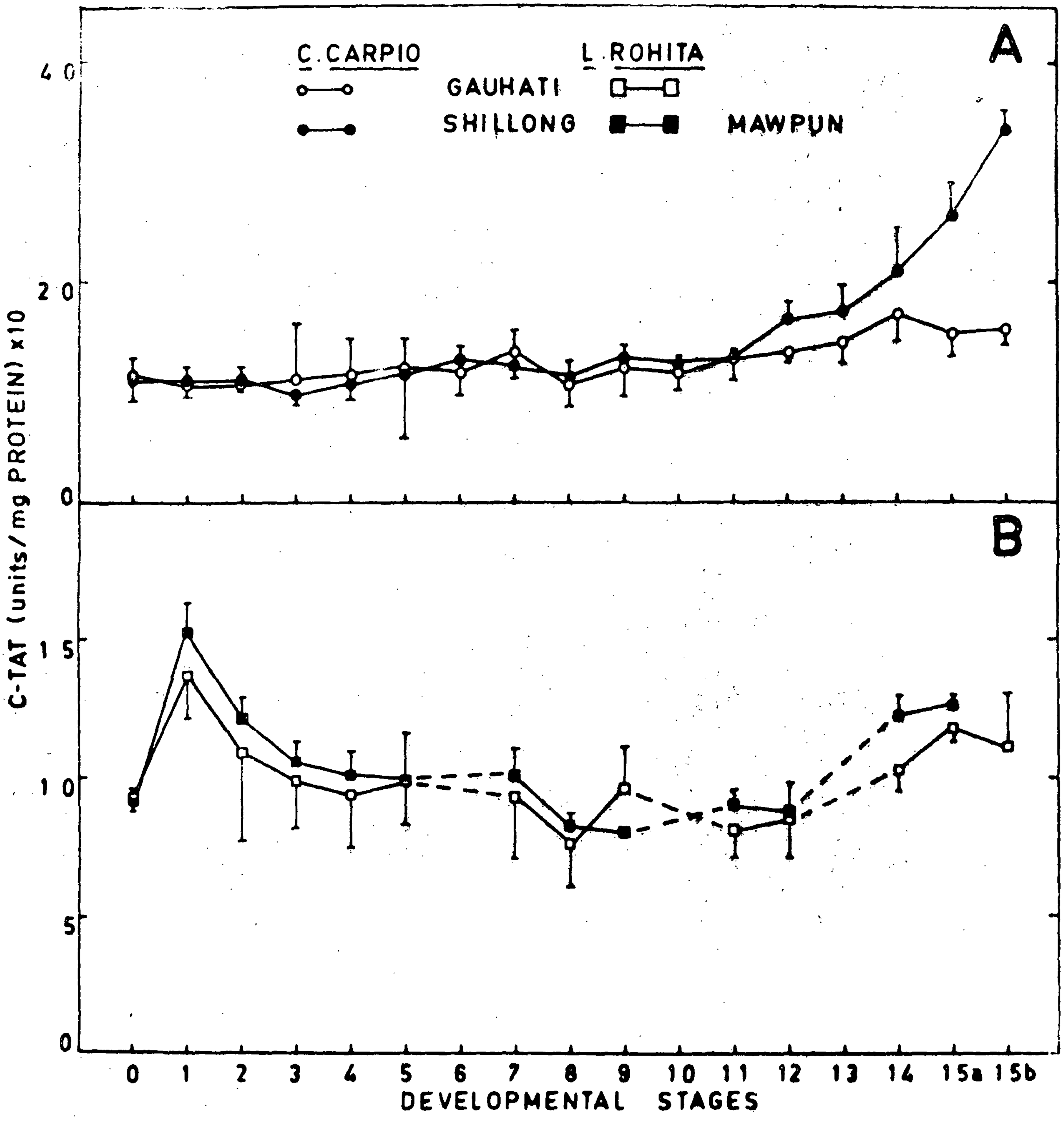


FIG.27.CHANGES IN THE SPECIFIC ACTIVITY (units/mg PROTEIN) x 10 OF CYTOPLASMIC TYROSINE AMINOTRANSFERASE (C-TAT) DURING EARLY DEVELOPMENT OF C.CARPIO (A) AND L.ROHITA (B) AT DIFFERENT ALTITUDES.

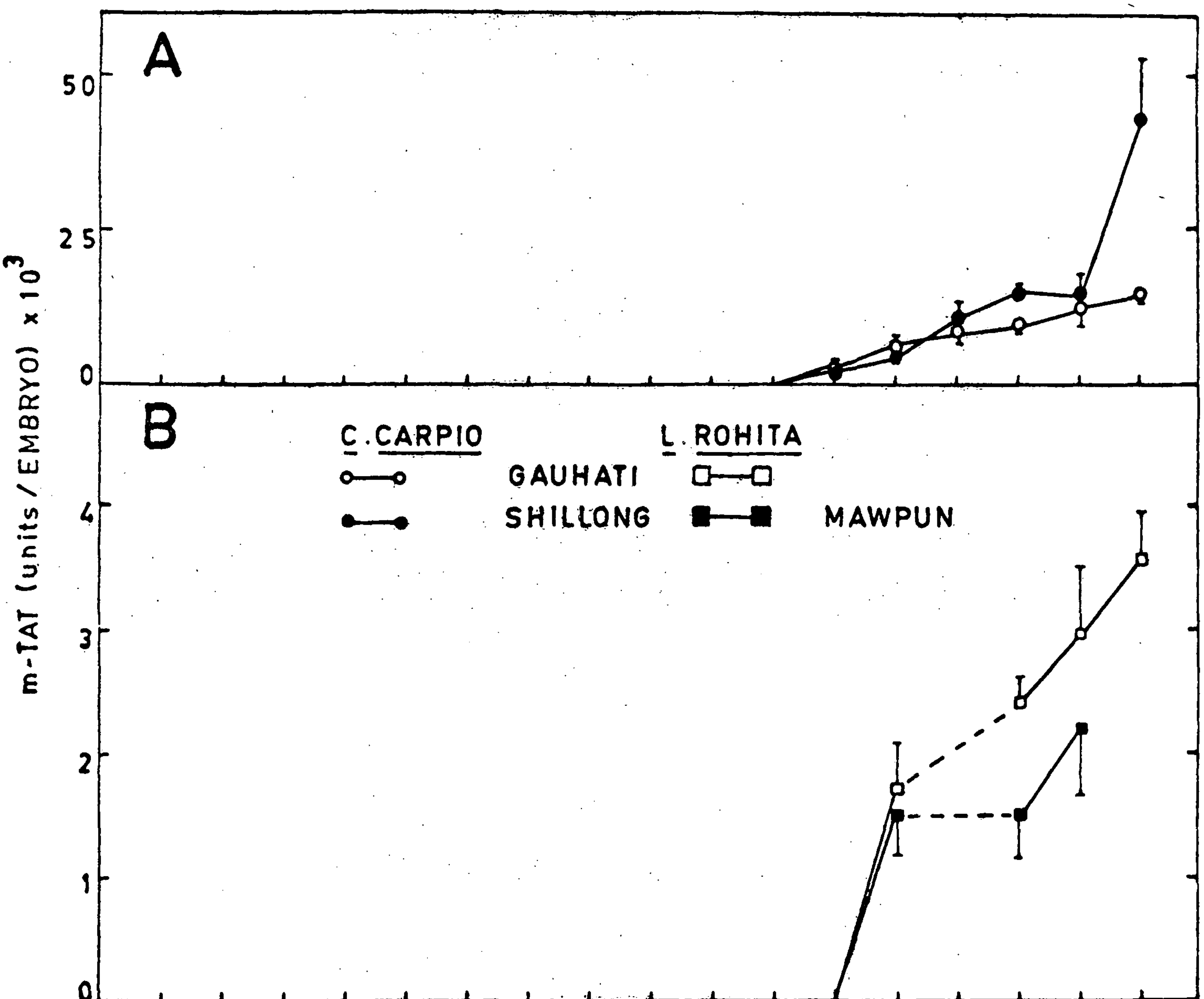


FIG. 28. CHANGES IN THE TOTAL ACTIVITY (units / EMBRYO) x 10^3 OF MITOCHONDRIAL TYROSINE AMINOTRANSFERASE (m-TAT) DURING EARLY DEVELOPMENT OF *C. CARPIO* (A) AND *L. ROHITA* (B) AT DIFFERENT ALTITUDES.

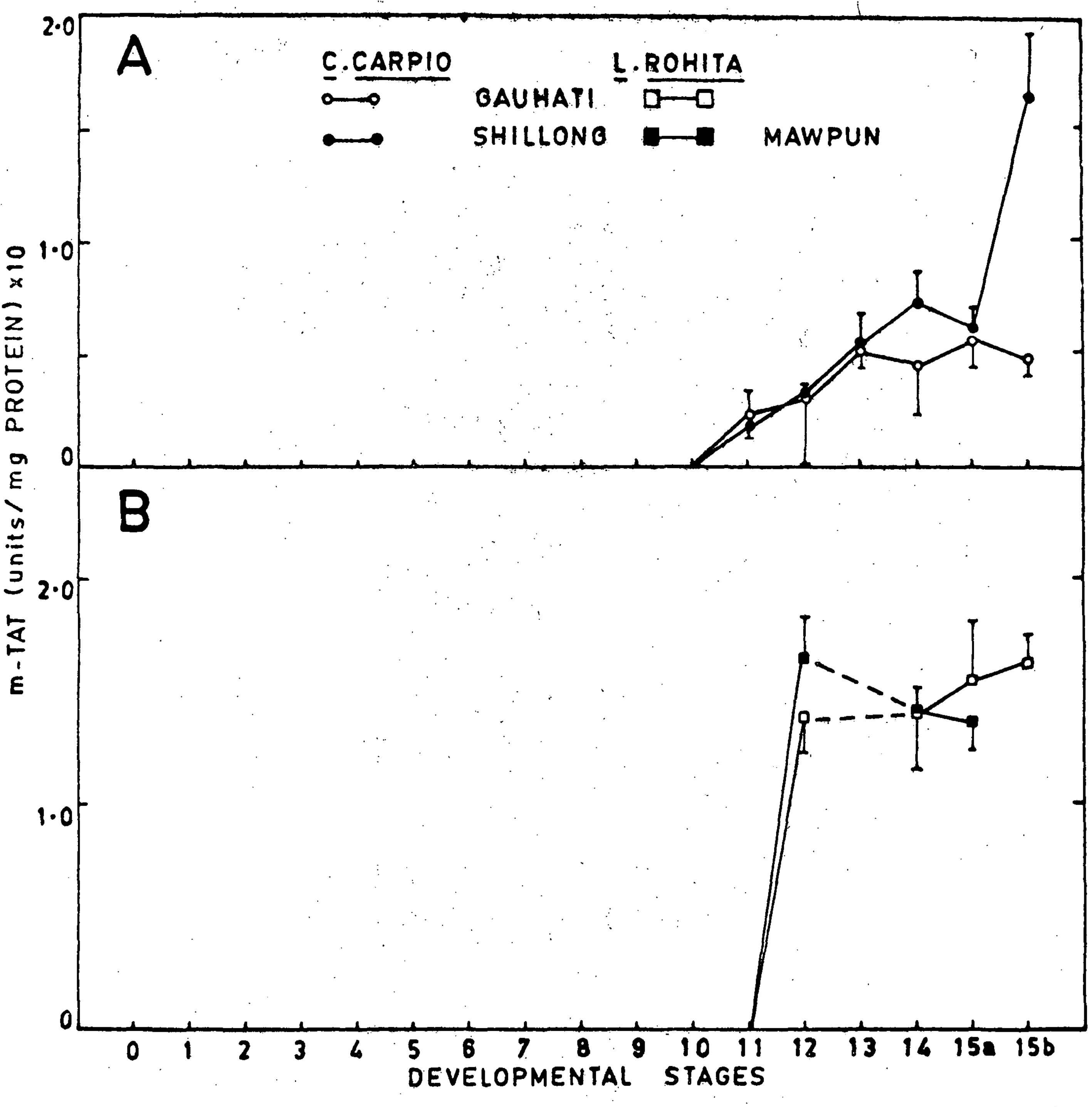


FIG.29. CHANGES IN THE SPECIFIC ACTIVITY (units / mg PROTEIN)x10 OF MITOCHONDRIAL TYROSINE AMINOTRANSFERASE (m-TAT) DURING EARLY DEVELOPMENT OF *C. CARPIO* (A) AND *L. ROHITA* (B) AT DIFFERENT ALTITUDES.

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* Not referred in original.

APPENDIX

APPENDIX-I

BRIEF BIODATA

1. Name : SRI AJIT KUMAR BHAGOWATI

2. Date of birth : January 1st, 1956

3. Permanent Address : At/P.O. Borua Bamun Gaon,
Golaghat, Assam

4. Academic qualifications :

B.Sc. (Hons.)	1975	St. Anthony's College, North-Eastern Hill University, Shillong.	1st Class 2nd Rank
M.Sc. (Zoology)	1977	North-Eastern Hill University, Shillong.	1st Class 1st Rank

5. Academic distinctions :

(a) Awarded 'State Merit Scholarship' of Assam, 1971-72.

(b) Awarded 'Jawaharlal Nehru Book Prize' from the Jawaharlal Nehru Memorial Book Fund, New Delhi, 1975.

(c) Awarded the 'National Scholarship', 1975-77.

(d) Awarded 'Gold Medal' of North-Eastern Hill University, 1977.

(e) Awarded 'Merit Prize' of North-Eastern Hill University, 1977.

(f) Awarded 'Jawaharlal Nehru Book Prize' from the Jawaharlal Nehru Memorial Book Fund, New Delhi, 1977.

(g) Awarded a 'Junior Research Fellowship' from C.S.I.R., New Delhi, 1977-81.

(h) Awarded a 'Senior Research Fellowship' from C.S.I.R., New Delhi, 1981-82.

6. Membership of distinguished societies :

(a) Society of Biological Chemists, India and the Federation of Asian and Oceanian Biochemists.

(b) Indian Society of Cell Biology.

APPENDIX-II

LIST OF PUBLICATIONS

PAPERS IN JOURNALS

1. Inhibition of tyrosine aminotransferase activity during severe hypoxic stress in a fish, *Cyprinus carpio* (with B.K. Ratha).
   Indian J. Biochem. Biophys. (1981) *18*, 445-447.

2. 2. Amino-acid composition of haemolymph of the crab, *Paratelphusa* (*Liotelphusa*) *levis* Wood-Masan during healthy and pathogenic conditions (with A.R. Varman).
   Sci. Cult. (1981), *47*, 225-226.

3. Biochemical composition and nutritional values of three species of hill stream fish belonging to the genus *Garra* from North-Eastern India (with B.K. Ratha).
   Proc. Indian natn. Sci. Acad. (1982) *48B*, 67-72.

ABSTRACTS IN SYMPOSIA/CONFERENCES

1. Acetylcholinesterase rhythm in the brain and muscle of *Cyprinus carpio* and *Heteropneustes fossilis* (with B.K. Ratha & S.N. Ramanujam).
   Conf. Indian Soc. Chronobiol., Varanasi (1979) *Abstract pp. 57.*

2. Induction of hepatic tyrosine aminotransferase by hydrocortisone in two different stages of development of tadpole of *Philautus cherrapunjiae* (with B.K. Ratha & S.N. Ramanujam).
   Cell. Biol. Conf., Calcutta (1980) *Abstract pp. 61.*

3. Regulation of tyrosine aminotransferase activity in liver, brain and muscle of spawning male and female catfish, *Heteropneustes fossilis* (with B.K. Ratha).
   Natn. Symp. Gen. Comp. Endocrinol., Delhi (1980)
   Abstract pp. 13.

4. Some biochemical changes during early development of a fish, *Cyprinus carpio* (with B.K. Ratha).
   Second Cong. F.A.O.B., Bangalore (1980).
   Indian J. Biochem. Biophys. (1981) *18* (suppl.),
   Abstract pp. 203.

5. Effect of photoperiod on the regulation of circadian rhythm of acetylcholinesterase in the brain and muscle, and cytoplasmic tyrosine aminotransferase in the liver of the catfish, *Heteropneustes fossilis* (with B.K. Ratha & S.N. Ramanujam).
   3rd. Symp. Fish Physiol., Bangor, U.K. (1981)
   Abstract pp. 74.

6. Effect of high altitude on the embryonic mortality and changes in acetylcholine and acetylcholinesterase activity level in *Labeo rohita* during early development (with B.K. Ratha).
   Ann. Gen. Meeting Soc. Biol. Chem., India (1981).

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Inhibition of Tyrosine Aminotransferase Acitivity during Severe Hypoxic Stress in a Fish *Cyprinus carpio*

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Effects of hypoxic stress on tyrosine aminotransferase activity in the liver, brain and muscle of a fish, *Cyprinus carpio*, have been studied. Of the three tissues studied, liver exhibited the highest activity followed by brain and muscle. Hypoxic effect resulted in a significant decrease in the total and specific activities of the hepatic enzyme whereas the activities of the other two tissues were not affected. It is suggested that regulation of this enzyme activity in fish is different from that in mammals.

Tyrosine aminotransferase (EC 2.6.1.5) is the rate limiting enzyme catalysing the first step of tyrosine oxidation pathway[1]. This enzyme is known to be an adaptive gluconeogenic enzyme which is quickly induced in mammalian liver and in tissue cultures in response to different hormones and substrates[2]. Physical stresses like starvation[3], exposure to cold[4], laparatomy and partial hepatectomy[5] have also been shown to induce hepatic tyrosine aminotransferase activity significantly. It is reported that various stress factors induce the hepatic enzyme through the adrenal gland by releasing glucocorticoids which, in turn, cause transcription of specific gene[6]. Thus tyrosine aminotransferase has served as an excellent model to study the mechanism of specific gene regulation and metabolic adaptations during stress in higher vertibrates. Information on this enzyme in lower vertibrates is limited and to some extent conflicting. Some earlier findings[7,8] suggest that glucocorticoids fail to induce the hepatic enzyme in animals lower than reptiles, in the phylogenetic series where they serve only as mineralocorticoids and do not show any gluconeogenic activity. However recent reports have shown the induction of hepatic enzyme by starvation and cortisol in fish[9] and hydrocortisone in frog tadpoles[10]. The present study was undertaken to find out the effect of severe hypoxic stress on the tyrosine aminotransferase level in different tissues of a fish, the scale carp (*Cyprinus carpio* var. *Communis* L.).

Ten-month old and sexually mature scale carps (wt, 155-170 g; length, 16.9-17.3 cm) obtained from Assam Govt. Fish Farm, Gauhati, were brought to Shillong in artificially oxygenated water in polythene bags and were maintained in the laboratory. Fishes in one aquarium were aerated to keep the oxygen level optimum and were used as control. In another aquarium, experimental fishes were left without aeration. The fishes in both the aquaria were not given food during experimentation. The oxygen level in the experimental aquarium decreased from 8 to 2 ppm in two days and the fishes started gulping water at the surface due to severe hypoxia. At that stage, 4-5 fishes from each aquarium were killed by decapitation between 14-16 hr of the day. The liver, brain and muscle tissues were immediately removed and deep-frozen at − 15°C. These tissues were thawed and a 10% homogenate for each tissue was prepared with ice-cold 0.25 *M* sucrose with an all-glass homogeniser. The homogenates were centrifuged at 14,000 × *g* for 20 min at 0 ± 2°C. The supernatants were used for the assay of tyrosine aminotransferase activity[11] and protein[12]. The enzyme activity was expressed both as total activity (units/g wet wt tissue) and specific activity (units/mg protein).

The results obtained (Table 1) show that amongst three tissues studied liver exhibited the highest activity followed by brain and muscle. Hypoxic stress resulted in a significant reduction in the total and specific activities of hepatic enzyme. However no significant alteration was observed in the brain and muscle enzyme activities and the cytoplasmic protein content of the three tissues.

The pattern of tissue distribution of the enzyme activities is similar to that of mammals[13], indicating that liver is the major gluconeogenic tissue in fish with higher level of the enzyme activity. It is known that during a physical stress the level of circulating glucocorticoids increases manyfold and they enhance the hepatic enzyme level in mammals[6]. Increase in cortisol level has been reported in fish during stress[14,15]. However the hepatic enzyme could not be induced in the present experiment with the severe hypoxic stress to fish, thus lending support to the earlier suggestion that glucocorticoids do not induce the enzyme, at least in the adult fishes[7,8].

The enzyme activities were not affected both in the

Table 1—Effect of Hypoxia on Tyrosine Aminotransferase Activities and Protein Content in Liver, Brain and Muscle of Control (C) and Hypoxic (H) *Cyprinus carpio*

Total activity (units/g wet wt × 10)		Sp. activity (units/mg protein × 10^3)		Protein (mg/g wet wt)	
C	H	C	H	C	H
		Liver			
552.3±108.7	314.51±83.3 (−43.06) P<0.02	399.5±58.2	239.8±55.8 (−39.98) P<0.01	137.49±10.56	130.43±7.49 NS
		Brain			
16.03±4.95	17.95±7.58 NS	15.9±5.1	17.8±6.2 NS	100.86±7.89	99.99±15.67 NS
		Muscle			
3.63±0.97	3.96±1.3 NS	4.5±0.9	4.9±1.5 NS	80.54±13.41	78.36±14.86 NS

Figures in the parentheses indicate percentage change.
NS, Not significant.

brain and muscle during the hypoxic stress though the hepatic enzyme was significantly suppressed. Such tissue-specific responses are not uncommon in animal systems. Induction of the enzyme in liver by hydrocortisone and glucagon without any effect in brain has been reported in rat[2,4].

The inhibition of hepatic enzyme level in the stressed fishes, in sharp contrast to the reports on mammals, has been quite puzzling because this is perhaps the first report of stress-induced inhibition of this enzyme in an animal. Tyrosine is a non-essential amino acid in fish[16] and is synthesized from an essential amino acid phenylalanine by hydroxylation[17]. All the reactions for synthesis and degradation of tyrosine other than transamination are dependent on the availability of molecular oxygen. Therefore in a hypoxic condition the oxygen-dependent pathways are likely to be affected, resulting in the inhibition of tyrosine synthesis. Thus the decrease in the level of the substrate in liver could be one of the factors responsible for decreasing the hepatic enzyme activity. In a stress condition, hormones like norepinephrine and epinephrine are synthesized at a comparatively higher rate and they are known to concentrate melanin. Thus there is a need for tyrosine to be channelized more in these pathways. Variation in the enzyme activity and hence the amount of tyrosine metabolized *via* transamination may influence availability of tyrosine for these pathways. Therefore the decrease in the enzyme level might be a metabolic adaptation in fish to make the tyrosine available for the excess synthesis of norepinephrine, epinephrine and melanin. The levels of norepinephrine and epinephrine have been shown to increase severalfold during stress in fish[18]. It has also been shown in mammals that norepinephrine suppresses hepatic tyrosine aminotransferase activity[19]. This decrease has been attributed to the formation of a complex due to competitive binding of norepinephrine with pyridoxal phosphate cofactor and thus decreasing the rate of apoenzyme synthesis[20]. During hypoxic stress the liver glycogen is catabolized resulting in elevated blood glucose level[21]. Decrease in the enzyme activity with enhanced blood glucose level has been shown in mammals[22] and frogs[23].

Grossman *et al.*[24] have proposed that tyrosine aminotransferase being a gluconeogenic enzyme, the ratio of tyrosine and glucose would be of importance in regulating the enzyme activity. Therefore it will be premature to assign any specific reason for the inhibition of this hepatic enzyme during hypoxic stress in fish. However it is clear that the mechanisms of regulation of enzyme activities in fish are different from those of mammals.

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Amino-acid composition of haemolymph of the crab, *Paratelphusa* (*Liotelphusa*) *levis* Wood-Mason during healthy and pathogenic conditions

It is well established that the composition of the haemolymph of insects and other groups of arthropods varies markedly in relation to different environmental conditions[1] as well as during various pathogenic conditions[2,3], such as starvation[4], fungal infection[5], ultra violet rediation and others In comparison to other groups of arthropods very little work has been done on the haemolymph of crustaceans especially in relation to pathogenic conditions. Recently the authors investigated the free amino-acid constituents of the haemolymph of the fresh water crab *Paratelphusa* (*Liotelphusa*) *levis* Wood-Mason during normal life and during starvation as well infection by the aquatic fungus, *Blastocladiales* sp. and the results are presented here.

The fresh water crabs, *Paratelphusa* (*L.*) *levis* were collected from the local streams and cultured in aquaria in the laboratory. The crabs, infected with *Blastocladialis* sp. were also collected in nature and utilized for our study.

Alive carbs (intermoult stage) were anaesthetised by keeping them at 4.5°C in a refrigerator. Haemolymph was collected by gently puncturing through the body cuticle and subsequent aspirating in small glass capillaries. The collected haemolymph samples were poured in clean centrifuge tubes and centrifuged. Free clean serum was collected and submitted to descending paper chromatography. Whatman No. 1 chromatogram paper was used with the solvent system n-butanol : acetic acid; water (4 : 1 : 5 vol./vol.). Chromatograms were run for about 4-5 hrs. at room temperature (28°C). Standard amino-acids (BDH) were also run for comparison. Detection of amino-acids was done by spraying with ninhydrin dissolved in n-butanol and subsequent heating in a hot oven at 100°Cc for 3-5 minutes. Identification of the amino-acids was carried out by comparing the Rf values of the amino-acids of the haemolymph with those of known amino-acids.

The haemolymph of *Paratelphusa* (L.) *levis* is straw coloured and viscous. The clear serum was directly spotted as the sample on the Whatman No. 1 paper and chromatography was carried out. The results presented in Table 1, show that five amino-acids, namely methionine, lysine, cysteine, cystine and arginine were presented in the normal healthy haemolymph.

TABLE I : Free amino-acid composition of the serum of *Paratelphusa* (L) *levis* during normal condition, starvation and fungal infection

Name of the amino-acids	Normal condition	Starvation	Fungal infection
Methionine	++	+++	+++*
Lysine	++	+	+
Cysteine	++	++	+
Cystine	+++	+++	—
Arginine	+++	+	++

* Key :
+++ : very intensively present
++ : intensively present
+ : present
— : negative

Perusal of Table 1 indicates that the amino-acid composition of the haemolymph of *Paratelphusa* (L.) *levis* is very unique in having only five amino-acids, namely methionine, lysine, cysteine, cystine and arginine. During starvation lysine and arginine seem to be immediately metabolically utilised by the crabs. Similarly during infection with *Blastocladialis*, cystine seems to be totally utilised by these animls.

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Biochemical Composition and Nutritional Value of Three Species of Hillstream Fish belonging to the Genus *Garra* from North-Eastern India

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The biochemical constituents like total protein, free amino acids, DNA, RNA and phosphates were estimated in muscle, liver, kidney, brain and gill of three species of hillstream fish belonging to the genus *Garra* (*G. gotyla gotyla; G. annandalei; G. lissorhynchus*). The fishes were collected from different localities and showed tissue-specific variations in their constituents. Nutritionally, these fishes are as good as other important fishes. These wild fishes have served the people of this hilly region as a good source of protein nutrition and may be exploited for commercial purposes.

Key Words: Biochemical constituents; *Garra* species; Adaptational and nutritional value

Introduction

Fishes are one of the major sources of protein nutrition for human beings. Therefore, efforts are being made all over the world to exploit both the marine and freshwater bodies for fish production. Besides culturable fishes, the wild fishes also provide a major bulk of fish protein. Hence, studies on different sources and nutritive values of wild fishes are necessary. North-Eastern part of India is particularly rich in hill streams whose major fish fauna consists of the *Garra* species. There are about eight species of genus *Garra* reported till now in these hill streams (Menon 1974 and Majhi 1980). Some of them occur in large numbers and grow to a moderate size. They have been serving as a staple food for the people of this region. Some reports on the biology and ecology have appeared on a few species only (Hora & Mukerji 1936, Hora 1937, Fraser 1937, Jones 1941, Agrawal & Tyagi 1969, Somvanshi 1976, 1980 and Somvanshi & Bapat 1979). However, no information is available on the biochemical composition of these fishes to assess their nutritional values.

The present report deals with some preliminary investigations on some biochemical constituents like the total protein, free amino-acids, DNA, RNA and phosphates in different tissues of the three species of *Garra* (*G. gotyla gotyla*, *G. annandalei* and *G. lissorhynchus)* from North-Eastern India.

Materials and Methods

Fish

G. gotyla gotyla (length 11–14 cm; wt. 16–19 g) and *G. annandalei* (length 9–10.5 cm; wt. 13–16 g) were collected during April 1979 from Pagladia river, a torrential stream at Uttarkuchi (26°51'N and 91°25'E), Assam, at an altitude of about 265 m. *G. lissorhynchus* (length 6–7.5 cm; wt. 8–10 g) were collected during May, 1979 from Umkhrah stream at Shillong (25°34'N and 91°56'E), Meghalaya, at an altitude of about 1400 m. The fishes were collected between 7–10 A.M. when the water temperature was 17–18°C in both the places. The sexes could not be distinguished in these fishes because the gonads were not developed and there was no other morphological difference. The tissues (muscle, liver, kidney, brain and gill) were immediately removed after collection on the spot and were transported to the laboratory in ice. They were kept at—15°C till the analyses were made. All the estimations were done within a week of collection. Some tissues like brain and kidney were very small in size and therefore, tissues from 2–3 fishes were pooled for each set. Six–seven sets of estimations were done from 12–15 fishes.

Analytical procedures

A 10% homogenate was made for each sample with ice-cold 0.012M Tris-HCl buffer (*p*H 7.4). The tissue fractionation was done following Schneider (1957) for estimations of different biochemical constituents. The cold acid-soluble fraction was used for free amino acid and phosphate estimation and the final residue after nucleic acid extraction was dissolved in 1N KOH for protein estimation. The DNA and RNA were estimated in the hot TCA soluble nucleic acid extract.

Total free amino acid was estimated colorimetrically following the ninhydrin method of Spies (1957), using glycine as standard. Inorganic, labile, bound and total phosphates were estimated by the method of Fiske and Subbarow (1925) with KH_2PO_4 as standard. DNA and RNA were estimated by diphenylamine and orcinol reagents respectively following Schneider (1957), using calf thymus DNA and yeast RNA as standards. Protein was estimated by Folin-Ciocalteau reagent (Lowry et al. 1951) with bovine serum albumin as standard.

All chemicals and biochemicals were of analytical grade and were obtained from either Sigma Chemical Co., USA or Glaxo Laboratories, India. The data were expressed as mg/g wet wt of tissue.

Results

The concentrations of various chemical components studied have been expressed in mg/g wet wt of muscle, liver, kidney, brain and gill of *G. gotyla gotyla*, *G. annandalei* and *G. lissorhynchus*. The concentrations of total protein and free amino acids have been shown in table 1, DNA and RNA in table 2 and the phosphates in table 3. There have been variations in the concentrations of different components in different tissues. However, in general, there were no significant interspecific differences among the three species studied for a specific component in a particular tissue except for DNA.

Table 1 *Tissue protein (P) and free amino acid (FAA) contents* (mg/g wet wt) in muscle, liver, kidney, brain and gill of* G. gotyla gotyla, G. annandalei *and* G. lissorhynchus

	Muscle		Liver		Kidney		Brain		Gill	
	P	FAA	P	FAA	P	FAA	P	FAA	P	FAA
G. gotyla gotyla	168.8 ±14.19	0.975 ±0.019	150.33 ±24.26	1.785 ±0.034	109.45 ±8.02	1.37 ±0.04	62.28 ±6.34	0.677 ±0.802	38.37 ±4.04	0.49 ±0.025
G. annandalei	166.3 ±14.56	0.93 ±0.13	151.73 ±14.01	1.6 ±0.018	106.56 ±7.56	1.12 ±0.02	61.41 ±5.59	0.625 ±0.02	37.39 ±5.06	0.32 ±0.01
G. lissorhynchus	164.89 ±15.38	0.908 ±0.044	149.45 ±13.96	1.48 ±0.24	107.5 ±7.05	1.03 ±0.19	51.73 ±5.89	0.542 ±0.015	37.16 ±5.11	0.265 ±0.036

*Values are mean ± standard deviation.

Table 2 *DNA and RNA contents (mg/g wet wt.) in muscle, liver, kidney, brain and gill of* G. gotyla gotyla, G. annandalei *and* G. lissorhynchus

	Muscle		Liver		Kidney		Brain		Gill	
	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
G. gotyla gotyla	0.983 ±0.05	1.81 ±0.13	0.718 ±0.013	2.36 ±0.07	0.851 ±0.01	1.86 ±0.14	1.07 ±0.02	1.64 ±0.025	0.789 ±0.014	1.399 ±0.127
G. annandalei	1.017 ±0.01	1.79 ±0.12	0.729 ±0.03	2.37 ±0.025	0.867 ±0.05	1.85 ±0.07	1.112 ±0.011	1.72 ±0.019	0.808 ±0.03	1.59 ±0.16
G. lissorhynchus	1.04 ±0.014	1.81 ±0.14	0.795 ±0.016	2.48 ±0.035	0.91 ±0.01	1.87 ±0.04	1.16 ±0.019	1.72 ±0.03	0.823 ±0.019	1.55 ±0.19

Table 3 *Inorganic (Pi), labile (Pl), bound (Pb) and total phosphate (Pt) contents* (mg/g wet wt.) in muscle, liver, kidney, brain and gill of* G. gotyla gotyla, G. annandalei *and* G. lissorhynchus

	Muscle	Liver	Kidney	Brain	Gill
G. gotyla gotyla					
Pi	30.0 ±4.0	45.0 ±5.0	37.33 ±7.64	25.66 ±4.1	63.0 ±14.36
Pl	4.0 ±0.5	7.33 ±1.18	4.0 ±0.5	3.33 ±0.95	3.66 ±1.07
Pb	27.33 ±4.5	24.66 ±12.9	18.0 ±8.5	16.0 ±5.6	20.67 ±7.25
Pt	61.33 ±13.06	77.0 ±13.42	59.33 ±6.42	44.99 ±11.0	87.33 ±21.52
G. annandalei					
Pi	29.66 ±4.22	43.0 ±12.65	36.66 ±4.73	22.33 ±2.13	66.0 ±14.0
Pl	2.83 ±0.6	7.5 ±0.5	3.83 ±1.27	5.17 ±2.24	2.16 ±0.79
Pb	29.18 ±5.4	20.5 ±6.0	17.83 ±6.9	16.5 ±2.2	15.5 ±5.54
Pt	61.66 ±12.0	73.0 ±8.0	58.33 ±9.53	44.0 ±4.36	83.66 ±18.03
G. lissorhynchus					
Pi	33.6 ±4.73	47.0 ±8.54	39.0 ±14.35	25.0 ±4.0	70.0 ±10.0
Pl	3.5 ±1.0	6.0 ±0.59	3.83 ±0.61	3.83 ±0.79	1.66 ±0.79
Pb	23.5 ±5.5	17.0 ±8.0	11.83 ±6.5	19.83 ±6.9	7.67 ±2.26
Pt	60.23 ±13.24	70.0 ±14.35	54.66 ±9.56	48.66 ± 5.6	79.33 ±9.65

*Values are mean ± standard deviation

Among the tissues, protein concentration was highest in muscle followed by liver, kidney, brain and gill. Free amino-acids were maximum in liver followed by kidney, muscle, brain and gill. DNA level, in general, was maximum in *G. lissorhynchus* and minimum in *G. gotyla gotyla*. However, these differences between the species were not significant. The tissue distribution of DNA was in the order of brain > muscle > kidney > gill > liver. The concentration of RNA was higher in liver and there were not much variations among the other four tissues studied. The tissue distribution of different phosphates was as follows: total and inorganic phosphates: gill > liver > kidney > muscle > brain; labile phosphate; liver > kidney > muscle > brain>gill; and bound phosphate showed highest level in muscle and no definite order in other tissues.

Discussion

The wild fishes constitute one of the major sources of cheap nutrition for the rural population. The nutritional value of different fishes depends on their biochemical compositions like protein, amino-acids, vitamins, mineral contents, etc. The protein concentrations in different tissues of the three species of *Garra* studied by us are well comparable with those of many commercially important fishes (Zaitsev et al. 1969 and Ogino & Takeda 1978). Thus, in spite of their relatively smaller sizes, these wild *Garra* species have served the hill people of this region as a good source of protein nutrition and could be exploited commercially.

The type of tissue distribution of proteins and free amino acids observed in this study, might be due to the structural and physiological differences among the tissues. Muscle contains a large amount of structural proteins with low turnover rate whereas liver and kidney are highly active metabolic tissues rich in functional proteins with higher turnover rates. Hence, these tissues had higher concentrations of protein. The total free amino-acid level in these species seems to be lower than those reported in carp muscles (Yudaev 1950 and Siddiqui et al. 1973). This indicates that the amino-acid pool in these fishes might have been depleted for either a higher rate of protein synthesis or used for energy production due to poor feeding.

DNA content of a tissue might indicate the cell number (Goss 1966), as the DNA content per cell is a constant factor for a given species. It has been earlier reported that these three species of *Garra* have same number of chromosomes (2N=50) (Majhi 1980). The DNA concentrations per unit wet weight of tissues are different due to their variable cell sizes. Such tissue-specific variations have been reported earlier in other fishes (Love 1970, Jafri & Mustafa 1976 and Mustafa 1977).

The level of RNA reflects the rate of metabolic activity of a tissue (Leslie 1955, Bulow 1970). The RNA content as studied by us shows a higher level than that of carp muscle RNA concentration previously reported by Mustafa (1977). This may be an indication of higher rate of metabolism in these fishes. The metabolically active tissues like liver, kidney and muscle have also more RNA than other tissues.

The total phosphate content in tissues was similar in the three *Garra* species. This may be due to their similar feeding pattern. Ogino and Takeda (1978) have shown a direct correlation between the tissue phosphorous content and dietary phosphorous level in rainbow trout. Most of the phosphates in these tissues are present in free or bound form. The labile phosphates contribute to a lesser amount. The free phosphate level indicate the metabolic state of the tissue. But a very high level of free phosphate observed in the gill may be due to the accumulation of phosphate from surrounding water. Such accumulation of phosphorous has been shown in developing embryos of carp by Moroz and Luzhin (1976). The highest level of labile phosphate with a fairly higher amount of free phosphate in the liver of the three species studied, indicate the higher metabolic status of this tissue.

Taking into consideration the observed higher levels of protein, RNA and phosphates with a depleted free amino acid pool in general, it may be suggested that the *Garra* species have a higher metabolic rate as an adaptation for life in the fast-flowing streams, and they could be developed as a source of protein nutrition for the people of this region.

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