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Seasonal Variations in Protein, RNA and DNA Concentrations in Major Carps, *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*

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Abstract Seasonal variations in the concentrations of protein, RNA and DNA, and the RNA/DNA ratio were studied in the liver of major carps, *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* during their prematurity phase. These biochemical parameters maintained strikingly similar patterns of change in the three species. Alterations in nucleic acids were reciprocal to that of protein. Magnitude of such interrelation, however, varied with the season. Protein level was generally low during winter and high in the summer months, attaining peak value in March and June. Higher concentrations of RNA and DNA were registered in December and June and a sharp decline was evident in January as well as July. During the remaining months, protein, RNA and DNA varied intermittently, but not profoundly.

Study of seasonal variations in biochemical makeup of fishes forms an important and interesting aspect of biochemical studies. Attempts have been made to correlate the changes of chemical constituents with the factors of internal and external environments of these poikilothermal animals. Of all the extrinsic factors, temperature and food supply are known to influence the body composition rather profoundly. Amongst the intrinsic factors (the life processes) the most effective ones include the growth pulses, appetite and sexual maturation. Despite considerable data on seasonal biochemical cycles of fishes little information is available on the dynamics of changes in nucleic acids. The present study was designed to analyse the behaviour of protein, RNA and DNA in fish tissue associated with the natural "biological clock" of the three economically most important species of carp, *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*. These constitute capture and culture fisheries of great magnitude in the plains of the Indian sub-continent. The interaction of exogenous and endogenous factors manifesting ultimately on the overall living standard of the fishes and on the macromolecules studied have been elaborated. Liver was selected for these investigations since earlier work has unambiguously proved the sensitivity of this internal organ (Bulow, 1971; Satomi and Ishida, 1976; Satomi and Tanaka, 1978).

Materials and methods

Specimens of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* belonging to the first year class were captured from a culture pond at Aligarh (27°34′30″N, 78°04′26″E). Live specimens were transported to the laboratory in plastic tanks. Sampling was carried out at monthly intervals from October, 1981 to July, 1982 and at fixed hours in the forenoon. Out of each catch 100 specimens of *Catla catla* and 50 each of *Labeo rohita* and *Cirrhina mrigala* were randomly set aside for investigations. Disparity in the number of observations was due to the composition of catch in which *Catla catla* predominated. Immediately on arrival at the laboratory the fishes were measured for body length and weight, and were then decapitated. Without loss of time the liver of each individual was dissected out for assays. Ponderal index (condition coefficient, K) was determined as:

$$K=\frac{W}{L^3}\times 100$$

where,

W=body weight (g)

L=total length (cm)

Examination of gonads revealed that fishes were in immature phase, according to the ICES scheme detailed by Qasim (1973) and successfully adopted by Mustafa (1977a).

Known weights of liver sample were processed

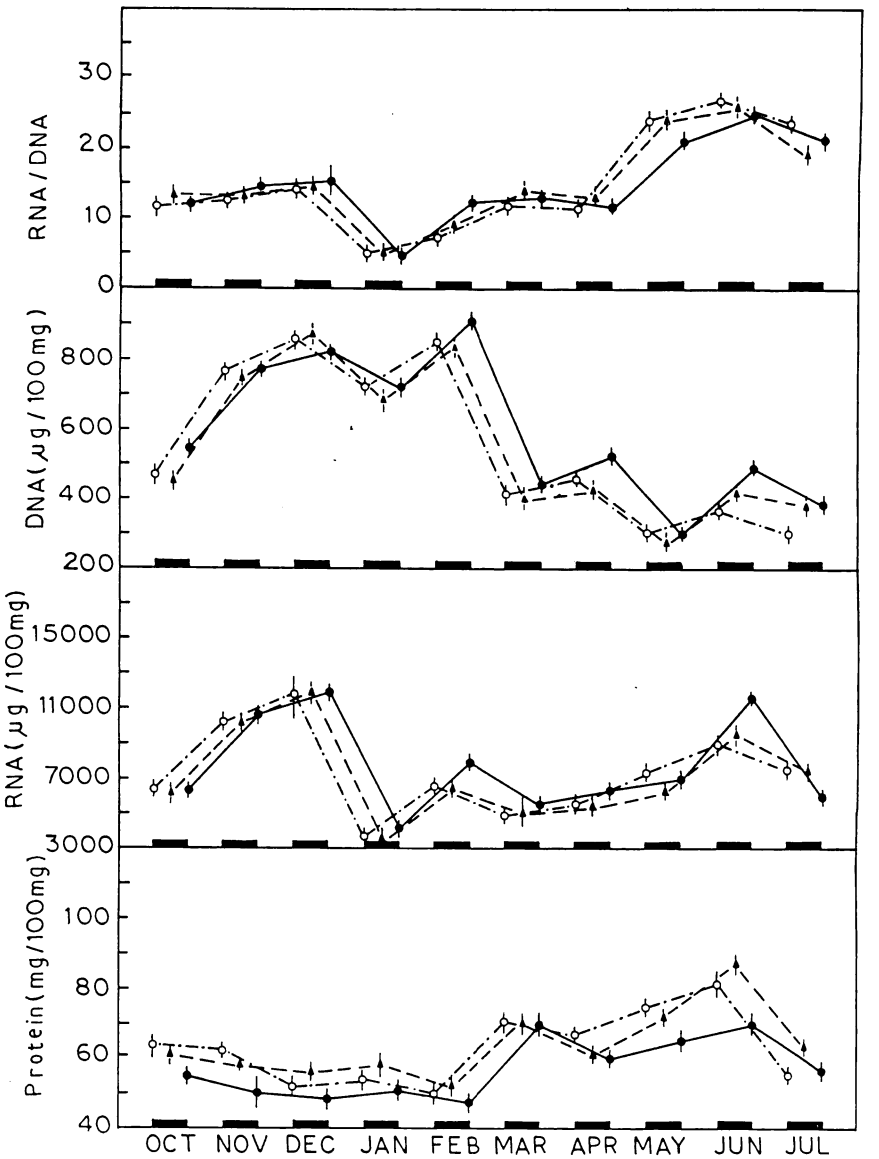

Fig. 1. Seasonal changes in protein, RNA, DNA, RNA/DNA in *Catla catla* (●——●), *Labeo rohita* (▲– –▲) and *Cirrhina mrigala* (○–·–○).

for obtaining dry, fat-free tissue powder (Mustafa and Zofair, 1983) which was used for quantitative determination of protein, RNA and DNA. Protein was assayed by the procedure of Lowry *et al.* (1951). Bovine serum albumin was used as the standard. RNA was isolated and estimated following the technique of Schneider (1957). A calibration curve was prepared using purified yeast RNA. DNA was extracted employing the procedure of Webb and Levy (1955) and quantitated by the methodology of Ashwell (1957). Highly polymerised calf-thymus DNA was used for preparing the standard curve. Protein was expressed as mg/100 mg, and RNA and DNA as µg/100 mg dry, fat-free tissue.

Results and discussion

Seasonal changes in the concentrations of protein and nucleic acids in hepatic tissue of teleosts, *Catla catla*, *Cirrhina mrigala* and *Labeo rohita* have been graphically represented (Fig. 1). The striking similarity in the pattern of seasonal fluctuation in these parameters points to the identity in biochemical adaptations of the three species of carp investigated. However, relatively faster growth rate, higher weight per unit of body length as evidenced by greater ponderal index (*Catla catla*, 1.366±0.014 SE; *Labeo rohita*, 1.198±0.020 SE; *Cirrhina mrigala*, 1.035±0.011 SE) and more profound turnover of RNA in *C. catla* signify better growth potentiality of this species under identical ecological conditions. The changes in RNA and DNA concentrations maintained the same trend which was reciprocal to that of protein for the major part of study period. On the face of it, a negative progression between RNA and protein seems paradoxical in the light of earlier findings of Mustafa and Jafri (1977), Mustafa (1979) and Mustafa and Mittal (1982), emphasizing the role of RNA as the organizer of protein biosynthesis. However, when the quantities of these macromolecules per unit weight of dry, fat-free tissue are considered, the present data are no surprise. After the extensive removal of acid-soluble substances, defattening and dehydration involved in processing of samples for chemical assays, the protein, RNA, DNA are the ones that remain intact and are the main substances left in cells whose biochemical constituents have been so extensively drained out. Any change in protein mass will be gravimetrically adjusted by the nucleic acids. The quantitatively inverse relation between protein and nucleic acids must be viewed in this light. If a tissue sample already deprived of substantial amounts of its constituents is to further loose the protein, it is certain that a larger number of underweight cells with a preponderance of RNA and DNA will be required to make an equivalent weight of the sample. This is a compensatory adjustment and may also imply variation in the pathways of the synthesis of nucleic acids in the living cells. Precentage of protein declined from October to December but concentrations of RNA and DNA increased in these months. Lowering of environmental temperature (onset of winter) may have affected feeding activity or caused a decrease in the protein assimilation efficiency of the body to a level unmatched by its mobilization. In such a case, increase in RNA/DNA ratio can not imply enhanced protein synthesis. Rather, this study strikes a note of caution against an extreme use of the ratio technique in assessing protein level and general robustness of fish. It is necessary to understand the internal environment of the fish before applying the RNA/DNA ratio method for its conventional purpose. Subsequent to December to February protein remained fairly stable, but RNA, DNA, RNA/DNA ratio declined in January and recovered to some extent in February. January being the peak winter month, it is likely that marked reduction in food intake may be linked with the biochemical picture obtained. Bouche (1975) has reported decrease in the levels of messenger, transfer and ribosomal RNAs in carp liver during winter. Evidently, the little quantity of nutrients entering the body failed to meet the maintenance requirements, needless to say to provide for storage in the liver. Protein stability in this period strengthens the view that the fish prefers accumulation of nutrients other than protein. Under the stressful condition of winter the RNA meets the same fate as other cytoplasmic inclusions and hence its concentration declined. While these changes are taking place little increase in protein "dilutes" the DNA but this can be attributed exclusively to the gravimetric adjustment of constitutents in unit weight of tissue, as explained earlier in this discussion. Possibility of an actual breakdown of DNA can be ruled out. Fall in RNA/DNA ratio provides support to this hypothesis, since it can occur when RNA loss exceeds the decrease in DNA concentration. Changes in the external environment in February when winter tends to wane out, are accompanied by reversal of same pathways in the internal environment of the fish. Food intake must increase and perhaps the efficiency of protein synthesis from the raw materials in the diet enhances to sustain the growth process. Notable increase in protein concentration in March explicits that besides its diversion to growth, the nutrient also piles up in the liver. This manifests in decline in the concentration of RNA and DNA. In April decrease of protein is evident, but not accompanied by any notable sequential variation in nucleic acids. Worth elaborating are the changes occurring in May and June, since during this period the liver

replenishes the lost reserves, possibly through some hitherto unexplained endogenous "feed back mechanism", which turns out a larger quantity of protein in the cells. Inasmuch as the specimens investigated were virgins and in pre-maturity phase, the biochemical changes can not be linked with the build-up of gonads in this breeding season of mature fishes. In the absence of this powerful factor of internal environment the fishes maintain the positive trend of feeding intensity, food assimilation and growth in the warm water environment. A larger quantity of RNA is synthesized. This activity is obviously the result of food intake. The published information available to date leaves no doubt on the close relation between the amount of food consumed and RNA level of tissues (Brachet, 1955; Leslie, 1955; Bulow, 1970, 1971; Mustafa, 1977b; Buckley, 1979, 1980; Mustafa and Mittal, 1982). That the RNA is instrumental in epigenetic synthesis of protein has also been explained by these authors. If the views of Love (1958) and Mustafa (1977a, 1978) are given credence, epigenetic DNA synthesis must be taken for granted to control the volume of cell cytoplasm growing due to accumulation of larger quantities of protein, RNA, etc. Increase in the RNA/DNA ratio in recovering fishes can be considered as indicator of protein synthesis and growth.

Outbreak of the monsoon which in this part of the world is characterized by torrential rains and flooding of the environment, seemed to bring about a decline in concentrations of protein and nucleic acids. With an abrupt rise in water level, sharp decrease in amount of food/unit volume of water is obvious. This may account for marked decrease in food intake. Earlier publications emphasize that deprivation of fish of adequate amounts of food causes decrease in protein level (Love, 1980; Mustafa, 1983), loss of RNA (Bulow *et al.*, 1978; Mustafa and Mittal, 1982), and decline in RNA/DNA ratio (Bulow, 1970). Presumably, the stress condition identified adversely affects the cytoplasmic DNA rather than the one housed in the nucleus. The response of DNA, however, needs more investigations.

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Catla catla, *Labeo rohita* および *Cirrhina mrigala* の蛋白質，RNA と DNA 濃度の季節的変動

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Calta calta, *Labeo rohita* および *Cirrhina mrigla* の蛋白質，RNA と DNA 濃度，および RNA と DNA の比率の季節的変動を成熟前の個体を用いて調べた．これらの値は 3 種において よく似た変化の パターンを示した．核酸の変化は蛋白質と逆のパターンを示したが，両者の関係の度合いは季節によって異なっていた．蛋白質の値は一般に冬期に低く，夏期で高く，3 月と 6 月に最高値に達した．RNA と DNA 濃度は 12 月と 6 月に高く，1 月と 7 月に急激に低下した．他の期間では RNA と DNA は断続的に変化したが，顕著な変化ではなかった．