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SEASONAL DYNAMICS OF THE TISSUE LEVELS OF TOTAL PROTEIN, FREE AMINO ACID AND RIBONUCLEIC ACID IN AN INDIAN AIR-BREATHING TELEOST, CHANNA PUNCTATUS

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The tropical freshwater air-breathing teleost, Channa punctatus, reveals a seasonal metabolic reorganization with reference to its biochemical tissue compositions. The summer-adapted and winter-adapted C. punctatus seem to be two biochemically distinct populations. Besides, the different reproductive phases viz. prespawning, spawning, post-spawning, preparatory-I and preparatory-II are also biochemically distinct.

Keywords: Seasonal — protein — free amino acid — RNA

Introduction

While most of investigations have been conducted on the effects of seasonal variation on the levels of biochemical tissue composition in marine and freshwater fishes of temperate and of polar regions, as summarized by Shulman /16/ and Love /5/, little is known about the tropical freshwater fishes, more particularly about the air-breathing fishes. The Indian air-breathing fishes undergo for summer estivation during the summer period when the ambient temperature enhanced to 38 ^{o}C or more. Whatever reports are available, with regards to biochemical tissue compositions, of Indian air-breathing teleosts /2, 9, 11, 12/, the data recorded have been expressed per unit of tissue wet weight. In the present investigation an attempt has been taken to explore the dynamics of tissue protein, free amino acid and ribonucleic acid i.e. RNA (expressed as per unit of DNA) of the male murrel, Channa punctatus during an annual cycle.

The present investigation has been aimed at answering the following questions:

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(i) Is there a rhythmic annual cycle of alteration of biochemical tissue compositions (expressed per unit of DNA) of C. punctatus?

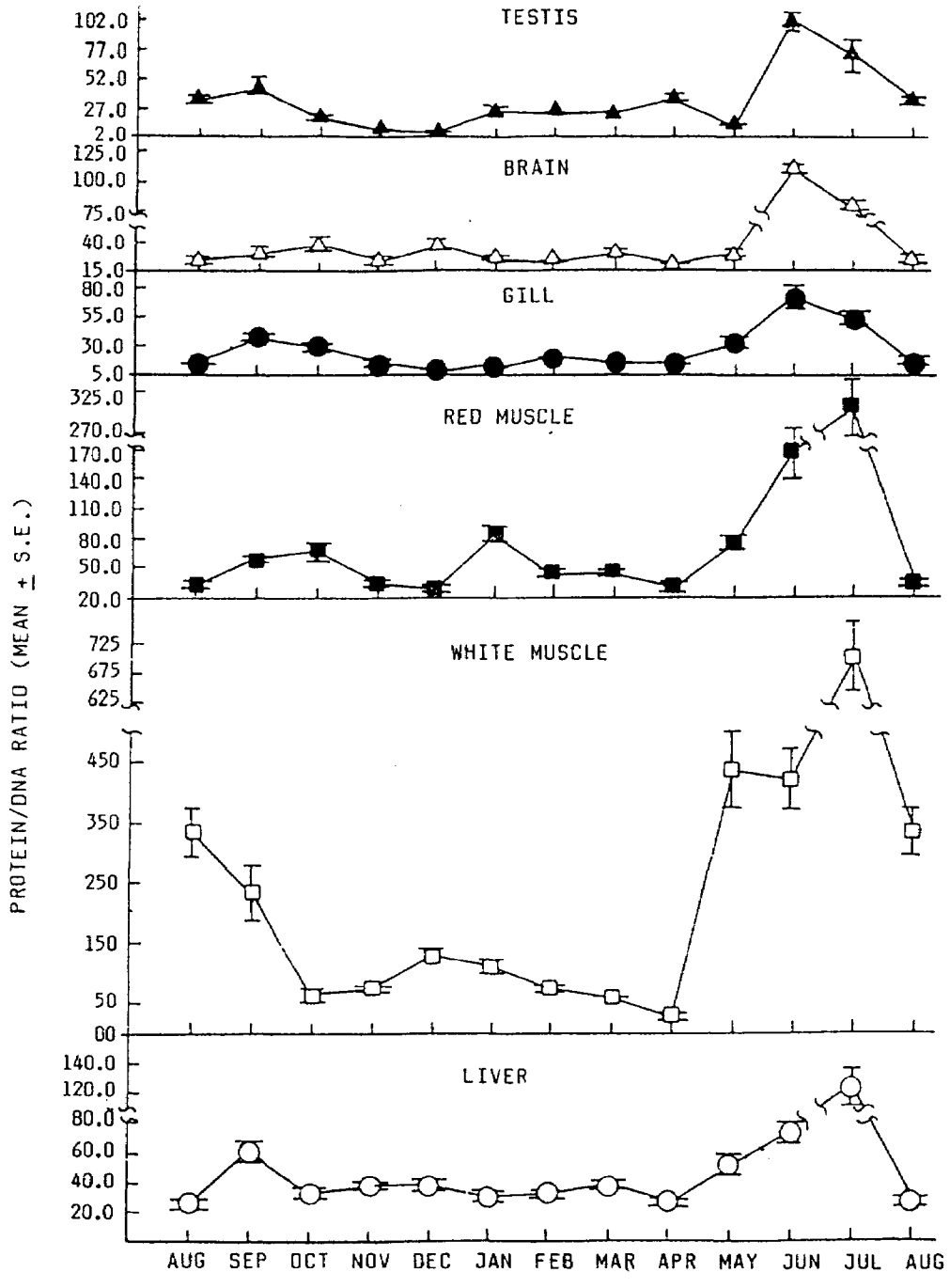
(ii) Can such an annual cycle be broken down into specific periods, which are quantitatively distinct stable states through which populations pass in the course of a year /16/?

Materials and Methods

Male Channa punctatus, of 30.0-40.0 g weight groups were collected during the second week of each month in an annual cycle from the near by villages of Santiniketan, W. Bengal, India. The total protein, free amino acid, RNA and DNA were extracted from the different tissues viz. liver, white and red muscles, gill, brain and testis. The extraction was started with homogenization in 5% ice cold perchloric acid and then subsequent extraction of free amino acids, lipids (not reported here), RNA, DNA and protein as per the experience of Roy et al. /15/. In brief, the free amino acid was collected from the first supernatant after centrifuging at 800 x g for 20 min. at room temperature. Before hydrolyzing the RNA in 0.3 N sodium hydroxide at 37 ^{o}C, all the lipid fractions were washed out with proper organic solvents. DNA was collected by hydrolyzing the residue in 10% perchloric acid at 90 ^{o}C. Finally, the protein was collected and dissolved in 1 N sodium hydroxide. The quantification of total protein was done with folin ciocalteau reagent /6/, of free amino acid with ninhydrin reaction /10/, of RNA with orcinol /8/ and of DNA with diphenylamine reagent /4/ with slight modification as per the experience of Das and Prosser /3/. All the results were statistically analysed (analysis of variance, linear and multiple correlation, "t"-test etc.) with the help of FORTRAN IV computer language.

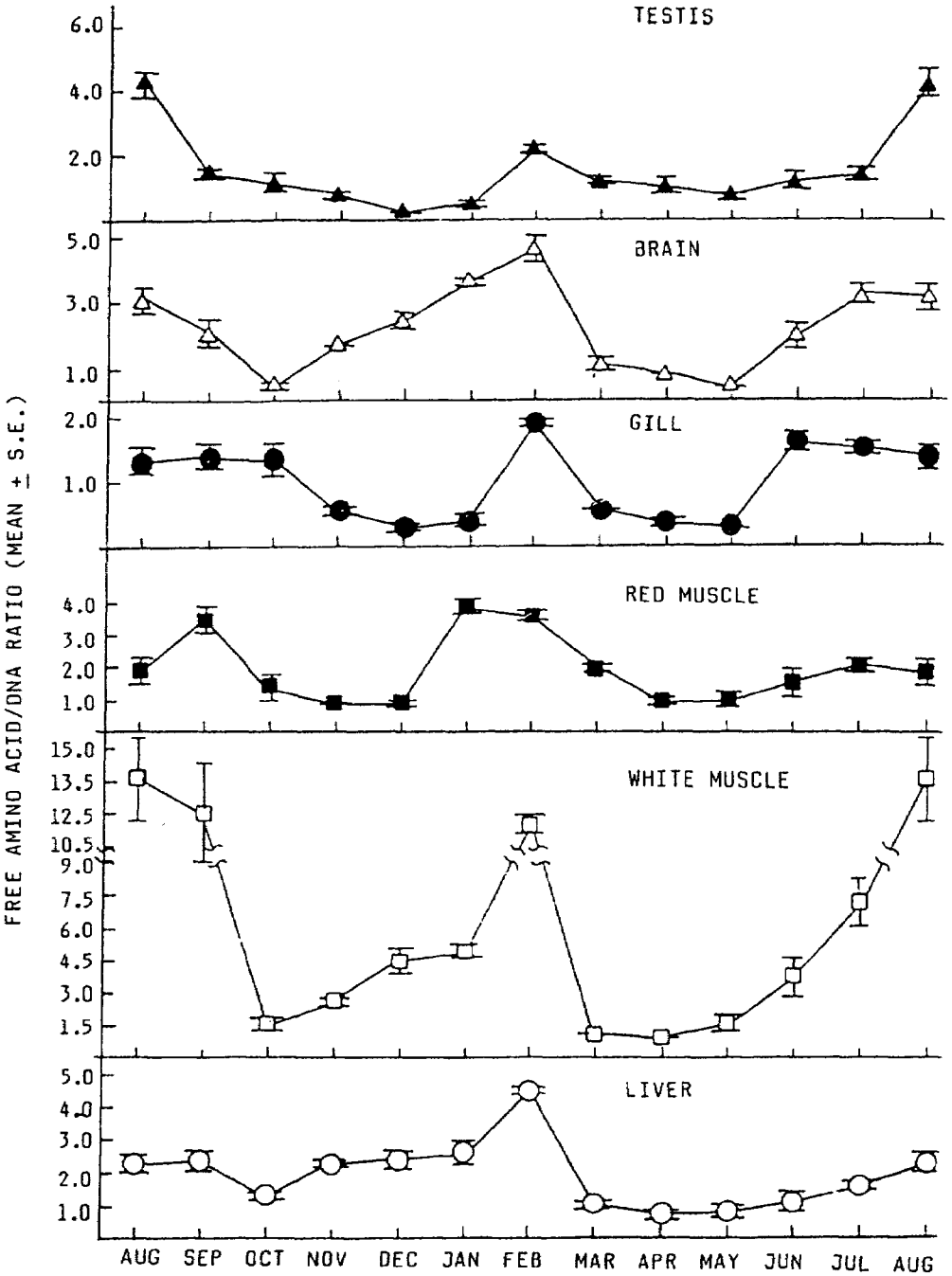
Results

There is a significant alteration of the water content in each tissue of C. punctatus during seasonal variation /2/ but the tissue level of DNA content has been found to be more or less constant (except in the gonadal tissue) during the an annual cycle of this fish (data not shown here). Hence it is misleading to express any biochemical tissue composition with respect to another variable like wet weight of tissue. The data is expressed here with terms of per unit DNA which is almost stable through the annual cycle. Figures 1 to 3 represent the annual (monthly) variation of the value of total protein, free amino acid, RNA (expressed per unit DNA) were statistically significant ($p < 0.001$). There is a positive linear correlation between the total protein content (per unit DNA) and free amino acid concentration (per unit DNA) in white muscle, gill and testis and between total protein concentration (per unit DNA) and RNA content (per unit DNA) in red muscle and testis.



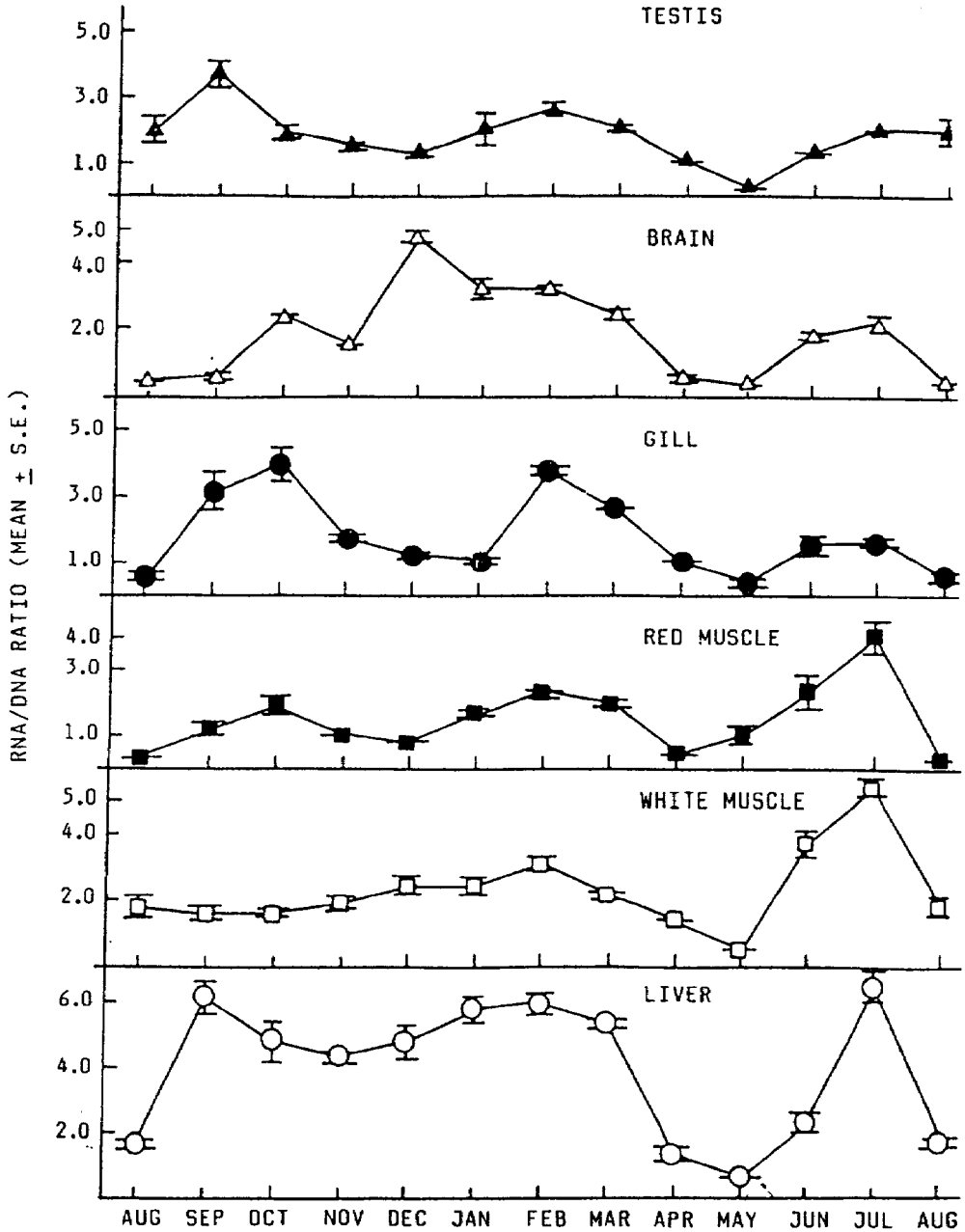
	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG
Aver.Max.Temp.(°C)	32.0	32.2	31.0	28.5	23.0	20.0	24.5	35.4	38.6	40.9	34.2	32.2	32.0
Aver.Min.Temp.(°C)	24.4	26.3	22.2	15.0	11.2	10.9	11.5	18.7	23.8	30.6	25.6	25.4	24.4

Fig. 1. Seasonal variation of the protein concentration (expressed as per unit DNA) in different tissues of Channa punctatus (Mean ± S.E. of six fishes)



	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG
Aver.Max.Temp. (°C)	32.0	32.2	31.0	28.5	23.0	20.0	24.5	35.4	38.6	40.9	34.2	32.2	32.0
Aver.Min.Temp. (°C)	24.4	26.3	22.2	15.0	11.2	10.9	11.5	18.7	23.8	30.6	25.6	25.4	24.4

Fig. 2. Seasonal variation of the free amino acid concentration (expressed per unit DNA) in different tissues of Channa punctatus (Mean ± S.E. of six fishes)



	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG
Aver.Max.Temp. (°C)	32.0	32.2	31.0	28.5	23.0	28.0	24.5	35.4	38.6	40.9	34.2	32.2	32.0
Aver.Min.Temp. (°C)	24.4	26.3	22.2	15.0	11.2	10.9	11.5	18.7	23.8	30.6	25.6	25.4	24.4

Fig. 3. Seasonal variation of ribonucleic acid (RNA) concentration (expressed per unit DNA) in different tissues of Channa punctatus (Mean ± S.E. of six fishes)

Discussion

A scrutiny of the Figs 1 to 3 reveals that most of the changes are in the form of oscillation from a theoretical basal line value /16/, which may be drawn round the year. This theoretical basal line value is tabulated in Table 1. The tissue specificity of the level of protein, free amino acid and RNA is quite clear from the data presented.

Table 1

Basal line value of biochemical tissue compositions in Channa punctatus

Parameter	Liver	White muscle	Red muscle	Gill	Brain	Testis
DNA (mg%)	0.236	0.036	0.097	0.209	0.122	0.245
Protein/DNA	47.941	223.488	81.262	27.099	39.431	31.882
Free amino acid/DNA	1.908	5.448	2.202	0.971	2.120	1.317
RNA/DNA	4.117	2.327	1.573	1.863	1.930	1.791

Patterns of rhythmic oscillation in biochemical tissue composition

It is quite evident from the Figs 1 to 3 that the total protein, free amino acid and RNA contents (expressed as per unit DNA) of the tissues of male Channa punctatus undergo distinct seasonal rhythmic fluctuations in an annual cycle. While a mathematical analysis describing the harmonic (periodic) function of the seasonal alteration of whole body protein and fat content of the black sea fishes (Pickerel, Surmullet, Anchevy, Spart etc.) have been conducted by a group of Russian workers /16/, such biometric analysis for the Indian fishes is utterly lacking. This is the first time an attempt has taken to treat the observed data with such harmonic equation. It is noticed that due to such biometric analysis the major "peak" (primary peak) is appearing repeatedly same time (month) in an annual cycle. Thus it is giving strong support about the circa-annual rhythmicity in the biochemical tissue composition of Channa.

Protein

The major noteworthy alteration of the tissue levels of total protein is build-up, characterizing the pre-spawning period viz., June—July (Fig. 1). In the State of West Bengal this fish spawns in late monsoon i.e. August—September. This characteristic rise in the protein level during pre-spawning period has been focused particularly in the graphic representation of harmonic function for the dynamics of protein concentration in different tissues (liver, muscles, gill, brain and testis) of C. punctatus (data not shown here). Augmentation of the protein content (percent yield value) in muscle of another air-breathing teleost, Clarias batrachus /17/, and in Chela cluppeides /18/ or of gonad in Mystus vittatus /1/ during the annual maturation of these fishes have been recorded. This augmentation in the protein content in different tissues may be a preparatory process for meeting the nitrogen demands and energy needs for spawning in this fish. A small spawning elevation in the level of some tissue protein (testis, liver and gill) is noticeable during August—September and this is followed by post-spawning depletion in October—November. This has been attributed to the massive mobilization of protein towards energy requirement for spawning as the level of nitrogen excretion and ammonia quotient are maximum in the month of September /13, 14/. Besides, a significantly high level of tissue protein is maintained during the winter months (December to February), particularly in white muscle, red muscle and testis and to some extent in liver and brain over that of summer months (April—May). The role of environmental temperature causing such a season specific change of tissue level protein, in the same fish is already described by Roy et al. /15/.

Free amino acid

The seasonal variation of cellular total free amino acid concentrations is characterized by a late 'winter peak' in all the tissues of Channa punctatus (Fig. 2). This season specific alteration is amplified by the treatment of the date in the plot of harmonic function particularly in liver, red muscle, and brain (data not shown). It is interesting to notice that in these tissues an inverse correlation depicted between the total protein concentration and free amino acid content during the winter period, suggesting an accumulation of the tissue level of free amino acid apparently at the cost of total protein in these tissues. This has been further confirmed

by the data on the absolute rate of protein synthesis and the rate of protein degradation in liver and red muscle /7/ of Channa punctatus. The characteristic summer depletion in the tissue levels of free amino acid in all the tissues is quite evident from the data. This corroborates the findings regarding augmented level of ammonia nitrogen excretion /14/ as well as of the ammonia quotient /13/ of this fish during the summer months, suggesting enhanced protein mobilization and utilization of free amino acid as the source of energy in summer populations. Besides, a gradual build up in pre-spawning period and a spent up phase during post-spawning period in the dynamics of total free amino acid and total protein in white muscle, gill and testis also reveal a direct relationship between the accumulation of these biomolecules with the breeding activity of this fish Channa.

RNA

A high level of RNA seems to be maintained throughout the winter months (December—February) in almost all the tissues of C. punctatus (Fig. 3). This accumulation is particularly focused when the data were processed with the above-mentioned harmonic equation particularly in liver, gill and brain (data not shown here). A spawning peak in the testis and liver may be correlated with the increase of biosynthesis of nucleic acid and thus the maximal value of gonado somatic and hepato somatic indexes during this time is noticed in this fish (data not shown here) and as well as in Mystus vittatus /1/.

Periods in the annual cycle

It has been possible in many cases to describe a summer adapted and winter adapted population of a particular species /14/, besides the usual reproductive forms. The annual cycle of rhythmic oscillation in the steady-state levels of total protein, free amino acid and RNA in the different tissues of the tropical murrel, Channa punctatus reveal two distinct periods or phases, one in winter (January—February) and another in summer (April—May). While the winter period is characterized by the accumulation of these biomolecules, the summer period is marked by a significant depletion of these biomolecules. Besides, like other seasonal breeders, the five different reproductive phases are also observed in the seasonal dynamics of biochemical tissue compositions in this fish. The reproductive phases viz.

pre-spawning (June--July), spawning (August—September), post-spawning (October--November), preparatory-I (December to February) and preparatory-II (March to May) are distinguishable which have been found to be characterized by the specific reorganization of the cellular biomolecular composition in particular period. The preparatory-I and preparatory-II are coinciding with winter and summer population, respectively, in regards to seasonal dynamics of biochemical tissue composition.

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