

## ORIGINAL ARTICLE

# Tissue Specific Variations in protein and RNA: DNA ratio of Freshwater fish *Channa Punctatus*

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### ABSTRACT

Analysis of RNA, DNA and protein of laboratory reared fishes was carried out considering these as bio-indicator for nutritional condition of an organism. In the present study freshwater fish *Channa punctatus* was used as experimental model to assess the RNA: DNA ratio in fresh, fed and starved condition, which showed slightly, changed RNA: DNA ratio whereas protein's concentration was remarkably, changed in starved condition. Tissue specific changes in concentration of protein along with RNA: DNA ratio was seen in liver, intestine, muscle, gills, kidney and brain. Brain tissue was the most undisturbed tissue in all three conditions making it vital organ for mental process stability in adverse condition. Present study suggests usefulness of RNA: DNA ratio as a bio-indicator.

**Keywords:** Protein, RNA, DNA, Liver, Muscle, Gill, Intestine, Kidney, Brain.

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## INTRODUCTION

The nucleic acid derived indices, especially RNA: DNA ratio, have been widely used as a bio-indicator of the physiological as well as nutritional status of aquatic organisms in natural environment. It has been a popularly explored as the quickest method for assessing important issues in aquatic ecology without the laborious work of counting measuring and identification process [1]. Marine habitat attracted special attention of nucleic acid derived indices. Significant studies have been done in marine ecology than freshwater ecology. High mortality rate in the larval stages of the, fishes has been seen due to the poor feeding conditions and seasonal variations in biotic as well as abiotic factors [2,3]. Nucleic acid derived indices RNA: DNA ratio has been applied in the determination of potential survival of fishes in aquatic environment. Thus RNA: DNA ratio can be used successfully to increase the fish production in the aquaculture by giving valuable information on nutritional status of the fishes especially availability of the food. Variability of RNA: DNA ratio by changes in feeding levels has been demonstrated. [4- 6]. In the present study *Channa punctatus* was used as an experimental model to assess the effect of starvation, feeding, and immediate conditions in different tissues of this fish on protein, and RNA: DNA ratio.

## MATERIALS AND METHODS

Live specimens of the freshwater fish *Channa Punctatus* with average weight of 45 to 60 gm were captured from the Wadali lake of Amravati City, Maharashtra State. Live specimens were brought to the laboratory and were divided into three equal groups (n=5) of five fishes in each group. The first group was immediately sacrificed for the estimation total protein, RNA, and DNA from different tissues (Liver, Intestine, Muscle, Gills, Kidney, and Brain). Other two groups (control and fed) were kept in laboratory condition for acclimatization for one week. They were regularly fed during acclimatization period of one week. After acclimatization one group (n=5) was kept for starvation and other group (n=5) was continued with the feeding. Feeding was done on everyday considering the carnivorous feeding habits of *Channa Punctatus*. The diet provided was in the form of dried prawns. Aquarium water was removed and refilled

daily till the experimental period. After two weeks all fishes from both groups were sacrificed and tissues like Liver, Intestine, Muscle, Gills, Kidney, and Brain were removed immediately for the estimation of Total protein, RNA and DNA. 10mg of tissue of different organs was used for the estimation of protein and 100mg of tissue was used for the estimation of RNA and DNA.

The total protein estimation was done by the procedure of Lowery et al., [7]. Extraction of RNA and DNA was done by the procedure of Halliburton et al., [8]. Estimation of RNA was done by orcinol method of Ashwell [9] and estimation of DNA was done by diphenylamine method of Burton [10]. Data were compared within the three groups (fresh, fed and fasting), expressed as a feeding conditions in further discussion. Values are expressed in mg/100mg for protein and  $\mu\text{g}/100\text{mg}$  for RNA and DNA.

## RESULTS AND DISCUSSION

The RNA: DNA ratio is considered as a bio-indicator for nutritional status of fishes as DNA amount is stable under changing environmental conditions [11-14], whereas the amount of RNA which is primarily involved in protein synthesis varies with the changing environmental conditions along with physiological conditions [11].

In aquaculture today feed accounts major part of project cost. Assessment of RNA: DNA ratio can be useful for the cost effective business of freshwater fish culture. As RNA: DNA ratio can correlate with food availability of water, accurate output of RNA: DNA ratio can results in proper feeding of fishes in aquaculture thus by reducing the project cost. Starvation studies done by [11, 15] shows immediate decrease in RNA and protein while DNA remain roughly constant. Clemmesen [13] explained RNA: DNA ratio an index of the cell's metabolic intensity and its use as measurement for recent growth in fishes. [16] reported usefulness of Protein: RNA ratio as an indicator of starvation studies with phosphorous.

Availability of food in respect to seasonal changes to the *Channa punctatus* can be a cause depletion of its production. The status of RNA: DNA ratio shall be helpful to know about requirement of food at proper time without loss in production growth of fishes.

Protein				RNA: DNA Ratio			
Tissue	Fresh Fish	Fed Fish	Starved Fish	Tissue	Fresh Fish	Fed Fish	Starved Fish
Liver	25.11	15.40	13.8	Liver	2.05	1.97	1.59
Intestine	33.31	17.57	11.70	Intestine	1.62	1.73	1.38
Muscle	37.85	24.91	15.90	Muscle	3.77	2.79	2.88
Gills	21.32	20.07	12.11	Gills	2.61	2.50	2.24
Kidney	23.86	21.09	16.84	Kidney	1.67	1.50	1.45
Brain	19.74	17.31	17.03	Brain	2.00	2.07	2.04

Table No. 1-Protein content as per feeding conditions Table No. 2- RNA/DNA ratio in as per feeding conditions.

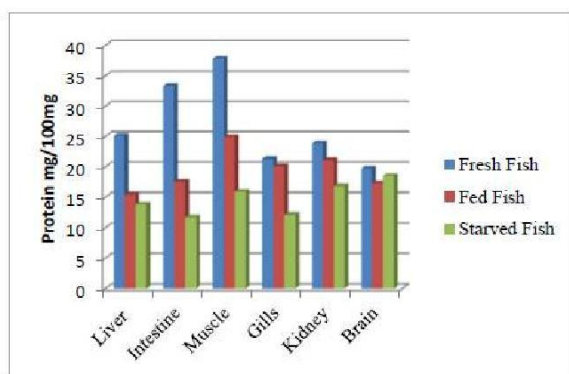


Figure No.1

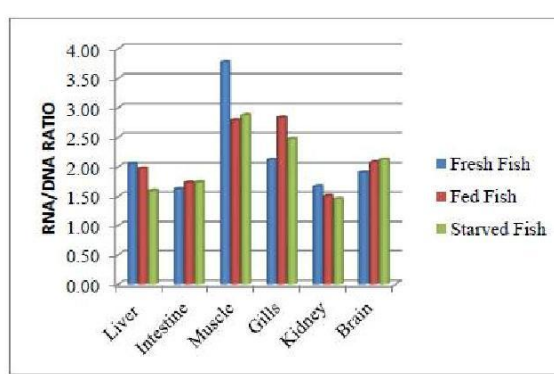


Figure No.2

Fig 1 and 2: Graphical representation of protein content and RNA/DNA ratio in different organ as per feeding conditions.

Higher RNA: DNA ratio indicates good growth condition of the organism whereas the lower RNA: DNA ratio indicates poor growth condition of the organism, which could be due to the environmental changes like adverse variations in water productivity and also inadequate availability of the food. Assessment of usefulness of RNA/DNA ratio is mostly done one larval and early juvenile fish. Limited work has been done to assess the effect of fasting and feeding conditions on nucleic acid ratio and protein content on the

different tissues of freshwater fishes. Response of individual tissue of fish to nucleic acid may be different in varied physiological conditions [17]. Growth pattern of different organ may vary and interpretation of RNA/DNA ratio in whole fish could lead to difficulty in assessing as an index condition [18].

In the present study effect of feeding conditions on protein in relation to nucleic acid was studied in different organ's tissue of *Channa Punctatus*. It was observed that protein content was high in liver (Fig. no. 1) of fresh fish considering it as a freshly captured fish, whereas fish fed for two weeks only on prawn feed was less protein content (Fig. no.1) than fish deprived of food for two weeks. RNA/DNA ratio showed similar trend as that of protein. It was high in liver of fresh fishes, where as it was decreased in fed state further it was lowered in starved fishes. The usual interpretation is that RNA/DNA ratio is sensitive to changes in nutritional status of fish. Amount of DNA per cell is stable under changing nutritional condition, whereas cellular RNA involved in protein synthesis fluctuates with nutritional condition of fish [19]. Thus the RNA/DNA ratio indicates metabolic intensity which is deeply influenced by nutritional status of the diet [12]. Similar observation reported in liver of catfish *Clarias Batrachus* by [20]. [5,4, 21] also reported the rise in protein and RNA content in response to feeding condition. Segnini and Chung, [22] observed higher RNA/DNA ratio in fed fish compared to starved fish.

It is observed in our study that, protein content of the muscle tissue in fresh fish was highest as compared to liver, intestine, gills, brain and kidney being it, as an important functional organ of fish structure. It is stated that muscle is the source of amino acid and energy store for other tissue during starvation [23, 24, 25]. Higher content of protein in the muscle tissue is due to its importance for mobilization activity for survival from the predators [25]. The trend of declination of protein as like liver was also seen in muscle protein of fed and starved condition, however RNA/DNA ratio in starved was slightly higher compared to fed condition. Though, the protein concentration was decreased in starved fishes as compared to fed fish muscle (Fig. No. 2). Musfata and Zofair, [26], in their work on seasonal variation of nucleic acid in major carps found gravimetric adjustment by nucleic acid in response to change in protein mass, and stated that if tissue loose the protein in great extent large number of underweight cell with preponderance of RNA DNA will require to make equivalent weight of the sample. Okumura et al., [27], reported declined in the indices during starvation over fed condition.

Intestine, gills, and kidney showed high protein content in fresh tissue compared to fed and starved, and it was decreased in starved than that of fed state. RNA/DNA ratio showed similar pattern in response to the protein content except in intestine of fed fish where it was slightly higher than fresh fish. Protein content was second in highest in intestine as compared to the other tissue. This could be due to the presence of traces of liver and pancreas in sample homogenate, as also reported by Oliver et al., [25], that content of protein in intestine was higher due to presence of liver and pancreas traces in homogenate.

No appreciable difference was noted in the protein content and RNA/DNA ratio in the brain of starved and fed fishes. The biochemical, constituents of brain usually does not get changed in response to moderate adverse conditions rather than extreme conditions. In the present study fishes were starved for only two weeks which did not exert pressure on this important organ to get catabolised. This stability of brain is to keep the mental process unimpaired [20]. Except brain, all the tissues were observed with appreciable changes in protein and RNA/DNA ratio in response to different feeding conditions of two weeks.

## CONCLUSION

In the present study response of the protein and RNA/DNA ratio to the feeding conditions was significantly observed. Study results support the established hypothesis of use of RNA/DNA ratio as a bio-indicator to assess the nutritional status and starving condition of an organism. Thus study emphasize towards the more assessment of RNA/DNA ratio as a useful bio-indicator in response to different tissues.

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