

# Seasonal variations of protein content in the muscle of Freshwater Fishes from Amravati

G.D. Hande

Assistant Professor, Department of Zoology, Shri Shivaji Science College, Amravati, MS India

**ABSTRACT:** The present investigations deal with to evaluate seasonal changes in protein content of muscle tissue of healthy, naturally infected and artificially Fungal Infected *Channa punctatus* and *Clarus batrachus* during August 2011- March 2013. Seasonal variation in protein content of muscle tissues of fishes has been analyzed during the study period. The highest muscle protein content was recorded in summer and the lowest in winter season. The muscle protein content was high from March to July and low during September to February. It is further observed that as fungal infection increased protein content decreased. The results indicate that the protein content of the fish depends on season but also to a great extent in reaction to food and reproductive cycle. The present study is the first to describe the seasonal variation in the protein content of muscle of freshwater fishes of Wadali Lake, from Amravati.

**Keywords:** Freshwater fishes, muscle protein content, seasonal variations.

## I. INTRODUCTION

Fish is a vital source of food for people. It is man's most important single source of high-quality protein, providing 16 % of the animal protein consumed by the world's population, according to the Food and Agriculture Organization [1]. In many Asian countries over 50% of the protein intakes comes from fish while in Africa the proportion is 17.50% [2]. From the view point of Inland fish production, India ranks third in the world [3]. Potentially the vast and varied inland fishery resources of India are one of the richest in the world. But so far, the availability is concerned; the average animal protein available per inhabitant per day in India is 6 gm. Good quality of fish for consumption can only be produced in an environment free from pathogens. Fish, having great economic importance, are affected immensely by variety of pathogenic agents in various ways. Several reports indicate high mortality of juvenile fish and reduced breeding potentiality of adults after long term microbial infections[4,5,6]. Large scale mortality of the fresh water fishes is often experienced due to environmental stresses followed by pathogenic attacks and parasitic infections causing a tremendous loss to the world economy.

Thus, the concentration of various nitrogen and protein fractions of *C. batrachus* show marked cyclic changes from season to season. The higher levels of various protein and nitrogen fractions, observed in April, May or June, seem to be partially associated with the high feeding activity of the fish. Similarly, the decline in the values of these constituents during the monsoon and winter months may be the result of less active feeding. Similar observations were obtained with *Heteropneustes fossilis* and the lower values of blood protein during January and July were correlated to lower consumption of food. Similar type of correlation was also indicated between various tissues. The quality of food also affects considerable, the concentration of various protein fractions in fish tissue. Higher values of protein in the blood of *H. fossilis* were recorded only in those months when fish consumed mainly proteinous food. Such type of correlation was also pointed out in the muscle protein of trout and certain other fishes. Besides feeding, the seasonal cycle of protein and nitrogen fraction of *C. batrachus* also seemed to be influenced by maturation cycle and depletion of gonad. The maximum level of total protein was recorded during the period when the fish was gravid and almost ready to spawn. Similar observations were made in the murrel, *Ophicephalus punctatus*, which confined maximum protein in gravid condition. The declining values of the above fractions during the spawning months (July-August) presumably point towards the utilization of these reserves. Incidentally, the fish during the spawning months has been found to be active and agile and this also results in the utilization of some of the muscle reserves for energy. During the post-spawning month and especially with the commencement of recovery period, normal life is resumed and this is marked with an increase in the protein content. Depletion in protein during spawning was also reported in cod muscle.

## II. MATERIALS AND METHODS

### Study area:

Wadali Lake is located at 20°93"N and 77°75"E and at an elevation of 343m in Amravati, Maharashtra (India). It is in the vicinity of Sant Gadge Baba Amravati University campus towards South – East direction of the university in the Pohara range of hills. The lake was constructed to meet the severe water scarcity problem erupted in 1899. The lake is surrounded by open hills towards east which drain water during monsoon. The lake also receives waste water from the Wadali zoo and forest quarters from South and drainage from SRP (State Reserve Police) Camp from North. It is also

watered by a percolation channel from upper Wadali Lake. When it fills to its maximum capacity the excess water overflows through an outlet, located on west shore of the lake to form Amba Nalla.

The water from the lake is being used for the drinking purpose and fishery activities, where the fishing of *Channa punctatus* (Phool-dhok), *Catla catla* (Katla), *Labeo rohita* (Rohu), *Clarias* species (Mangri), *Wallago attu* (Shivada), *Mystus seenghala* (Singala) is carried out on commercial scale.

### Estimation of Protein:

Estimation of Protein contents in the muscle tissue of control naturally infected and artificially infected *C. punctatus* and *Clarius batrachus* carried out by Folin Phenol Method [7]. The phenolic group in tyrosine and tryptophan residues (amino acid) in a protein produces a blue purple color complex (with maximum absorption in the region of 660 nm. wavelength) with folin-ciocalteu reagent which consists of sodium tungstate molybdate and phosphate. The intensity of color is directly proportional to the amount of the protein present in the sample.

### Preparation of tissue sample:

Muscles were isolated from the healthy, naturally infected and artificially infected fishes, and 100 mg of muscle tissue was weighed and homogenized separately with 2.5 ml of 0.25 M sucrose solution in ice cold condition. The homogenates were centrifuged for 10 minutes at 6000 rpm and the clear supernatant fluids were taken for protein estimation.

$$= \frac{\text{O.D. of unknown}}{\text{O.D. of standard}} \times \frac{\text{Conc. of standard}}{\text{Tissue taken}} \times 1000$$

(Unit: mg of protein/gm of tissue)

Statistical analysis was carried out, Student 't' test was used,  $p < 0.05$  was regarded as moderately significant and  $p < 0.001$  as significant [8].

## III. RESULTS AND DISCUSSION

Fish proteins contain all the essential amino-acids, those which are present in milk, eggs and mammalian meat proteins and hence have a very high biological value. The chemical composition of fish muscle proteins varies greatly from one species to species as per environmental conditions and age. Proteins and various other cellular constituents are remained in a state of continuous turn over and this may significantly enhance the organism's ability to readily adapt to its changing environment [9].

Alteration in muscle Protein contents (mg/gm) in control, naturally infected and artificially infected fishes with Fungi were carried out to know the effect of fungal infection on the biochemical contents of the infected fishes, muscle proteins were estimated seasonally both in control as well as infected fishes. Muscle protein contents in control *C. punctatus* were highest ( $26.58 \pm 1.37$  mg/gm) during March and lowest ( $14.02 \pm 0.46$  mg/gm) during December (Table, 4.3). While in *Clarius batrachus* muscle protein contents were highest during February ( $26.60 \pm 1.16$  mg/gm) and lowest during November ( $16.86 \pm 2.61$  mg/gm). Similar studies on the total protein contents have already been carried out in *Garra mullaya* [10], *Channa orientalis* [11], *H. fossilis* [12] and *O. mossambicus* [13] in fish muscle. Protein is being estimated to know the health status of the fishes [14].

Muscle protein contents in naturally infected *C. punctatus* were also highest ( $19.58 \pm 2.17$  mg/gm) during March and lowest ( $9.45 \pm 0.46$  mg/gm) during December (Table, 4.3). While in *Clarius batrachus* muscle protein contents were highest during January ( $18.51 \pm 0.25$  mg/gm) and lowest during December ( $10.44 \pm 1.16$  mg/gm).

Muscle protein contents in artificially infected *C. punctatus* were also highest ( $14.06 \pm 2.74$  mg/gm) during March and lowest ( $6.29 \pm 0.37$  mg/gm) during December (Table, 4.3). While in *Clarias batrachus*, muscle protein contents were highest during January ( $15.50 \pm 2.22$  mg/gm) and lowest during December ( $7.80 \pm 1.72$  mg/gm) following more or less similar trends. The Present results were correlated with Biochemical studies of fish tissue are of considerable interest for their specificity in relation to the food values of the fish and for the evaluation of their physiological needs at different periods of life.

The seasonal variations in muscle protein content were observed in the present study also. The highest muscle protein content was recorded in summer and the lowest in winter season (Table, 4.1, 4.2 and 4.3). The present results revealed that muscle protein content was high from March to July and low during September to February. It is further observed that as fungal infection increased protein content decreased. The muscle protein content was thus positively exponential to fungal infection. The total protein values estimated in the corresponding period showed comparatively protein in the naturally as well as experimentally infected *Channa punctatus* and *Clarias* species (Table 4.1 to 4.3). Protein content was found to be more in summer which may be because of consumption of more food in comparison to other seasons resulting in higher percentage. Mycotoxins are found to be associated with reduced growth and health status of fishes. The most prevalent Fungi responsible for the occurrence of mycotoxins are *Aspergillus*, *Penicillium* and *Fusarium* sp.

Mycotoxins can enter the blood stream and lymphatic system; they inhibit protein synthesis, damage macrophage systems [15].

IV. CONCLUSION

The seasonal variations in muscle protein content were observed in the present study. The highest muscle protein content was recorded in summer and the lowest in winter season. The muscle protein content was high from March to July and low during September to February. It is further observed that as fungal infection increased protein content decreased. The muscle protein content was thus positively exponential to fungal infection. Mycotoxins were found to be associated with reduced growth and health status of fishes. The most prevalent Fungi responsible for the occurrence of mycotoxins are *Aspergillus*, *Penicillium* and *Fusarium* sp. Mycotoxins can enter the blood stream and lymphatic system; they inhibit protein synthesis, damage macrophage systems. Higher protein content in summer may be attributed to availability of more food in comparison to other seasons and subsequently more consumption. Thus, present investigation provides valuable information on seasonal variations in protein content of fish species studied in order to take necessary precautions from a fishing point of view.

V. REFERENCES

[1] FAO, (1997): Fisheries Department. Aquaculture development. FAO Technical Guidelines for Responsible Fisheries. No. 5. Rome, 40

[2] William, C. Frazier. and Dennis, C. Westerhoff. (1988): Food Microbiology, 4th edition, food science series. MacGraw-Hill Book Company, Singapore, pp. 243-252.

[3] World Bank (2010): India marine fisheries, issue opportunities and transition for sustainable developments Agriculture and rural development sector unit south Asia region. Report No. 54259.

[4] Nicola, F. (1969): Systemic mycosis in channel catfish. Bull. Wildlife Disease Assoc. Vol. 5: 109-110.

[5] Pickering, A.D. and Willoughby, L.G. (1982a): *Saprolegnia*, infections of Salmonid fish. In: 50<sup>th</sup> Annual Report, Institutes of freshwater Ecology, Windermere Laboratory, England, pp. 38-48.

[6] Hatai, K. and Hoshiai, G. (1992): Saprolegniasis in cultured Coho salmon. Fish Pathol. 11:233- 234.

[7] Lawry, O. H.; Rosebrough, N. J.; Farr, A. L. and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265.

[8] Fisher R.A. (1992) Statistical Methods for Research Workers.S. Kotz et al. (eds.), Breakthroughs in Statistics © Springer-Verlag New York, Inc.

[9] Goldberg, A.L.and Dice, J.F. (1974): Intracellular protein in degeneration in mammalian and bacterial cells.ANU.rev.Biochem.43.835-859.

[10] Khan, E.A. and Mehrotra, P.N. (1991): Variation in liver protein and RNA in relation to egg maturation in hill stream teleost Grana mullya (Sykkes). J.Reprod.Biol.Comp. Endocrinol.3, 47-52.

[11] Saxena, D.N. and Saxena, M.(1999):Event of biochemical integration during the reproductive cycle found in Murrel *Channa orietalis* (Lin)In Ichthyology Res.Advan.Oxford and IBH publishing Com.Pvt.Ltd.New Delhi.345-354.

[12] Bunge, T.R. and Balle, V.V. (2003): Annual variation in the protein, glycogen and cholesterol in liver and testes of the cat fish *Heteropneustes fossilis* (Biotech). Trends in Life Sci. (India), 18: 111-116.

[13] . Hunge, T.R. (2002): Studies on protein, lipids and carbohydrates of gonads in breeding cycle of the cat fish *Heterpneustes fossilis* (Bioch) Ph.D. Thesis Nagpur University Nagpur.

[14] Verma, P.; Chand, G.B. and Nath, A. (2008): Assessment of health status of air breathing fishes of North Bihar. J. Ecophysiol.Occup. Hlth. 8 :1-6.

[15] Godish, Thad. (2001): Indoor Environmental Quality. Chapter Ten: Source Control. CRC Press. pp: 325-326.

[16]

[17] Pickering, A.D. and Willoughby, L.G. (1982a): *Saprolegnia*, infections of Salmonid fish. In: 50<sup>th</sup> Annual Report, Institutes of freshwater Ecology, Windermere Laboratory, England, pp. 38-48.

Table 4.1: Alteration in muscle Protein contents (mg/gm) in control, naturally infected and artificially infected fishes during 2011-2012.

Months	<i>Channa punctatus</i>			<i>Clarius batrachus</i>		
	Control	Naturally Infected	Artificially Infected	Control	Naturally Infected	Artificially Infected
August 2011	19.41 ±0.16	9.642 ±0.46 (-0.03)	6.08 ±0.53 (-0.33)	17.81 ±0.66	13.55 ±0.27 (-0.09)	9.62 ±0.26 (-0.86**)
September 2011	17.81 ±0.66	11.89 ±0.61 (-0.48*)	6.36 ±0.29 (-0.11**)	17.43 ±0.27	12.51 ±0.22 (-0.40*)	9.48 ±0.27 (-0.05)
October 2011	18.51 ±0.19	16.78 ±0.13 (-0.13)	13.63 ±0.27 (0.28)	19.46 ±0.18	9.64 ±0.46 (0.02)	7.33 ±0.18 (0.32)
November 2011	19.36 ±0.23	16.78 ±0.13 (0.54**)	12.48 ±0.25 (0.36*)	14.36 ±0.25	9.78 ±0.24 (-0.35*)	6.19 ±0.33 (0.20)
December 2011	14.41	9.33	6.40	12.45	9.85	6.18

	$\pm 0.23$	$\pm 0.32$ (0.04)	$\pm 0.27$ (0.68**)	$\pm 0.25$	$\pm 0.20$ (-0.09)	$\pm 0.33$ (0.94**)
January 2012	25.06 $\pm 1.08$	18.57 $\pm 0.21$ (0.33*)	13.61 $\pm 0.21$ (0.33*)	22.37 $\pm 0.24$	18.51 $\pm 0.25$ (0.29)	13.55 $\pm 0.27$ (0.18)
February 2012	25.49 $\pm 0.66$	19.42 $\pm 0.16$ (0.42*)	14.42 $\pm 0.23$ (0.89**)	25.62 $\pm 0.28$	19.36 $\pm 0.22$ (0.06)	17.57 $\pm 0.25$ (-0.27)
March 2012	25.46 $\pm 0.57$	21.64 $\pm 0.17$ (-0.49**)	16.67 $\pm 0.27$ (0.67**)	27.88 $\pm 0.64$	22.31 $\pm 0.22$ (-0.50**)	17.40 $\pm 0.32$ (0.02)

Figures are average of 6 replicates in respective months during 2011-2012, ( $\pm$  SD),

\* and \*\* denotes significance at 5% ( $p < 0.05$ ) and 1% ( $p < 0.01$ ) level respectively.

**Table 4.2: Alteration in muscle Protein contents (mg/gm) in control, naturally infected and artificially infected fishes during 2012-2013.**

Months	<i>Channa punctatus</i>			<i>Clarius batrachus</i>		
	Control	Naturally Infected	Artificially Infected	Control	Naturally Infected	Artificially Infected
August 2012	18.51 $\pm 0.20$	11.59 $\pm 0.23$ (0.19)	9.47 $\pm 0.29$ (0.41*)	17.52 $\pm 0.26$	14.42 $\pm 0.23$ (-0.05)	9.57 $\pm 0.33$ (0.63**)
September 2012	16.78 $\pm 0.13$	9.37 $\pm 0.19$ (0.38*)	6.27 $\pm 0.21$ (0.11)	17.43 $\pm 0.27$	12.51 $\pm 0.22$ (-0.40*)	9.48 $\pm 0.27$ (-0.05)
October 2012	18.83 $\pm 0.41$	13.62 $\pm 0.21$ (0.19)	7.33 $\pm 0.18$ (0.67**)	20.66 $\pm 0.26$	16.75 $\pm 0.13$ (0.11)	11.54 $\pm 0.28$ (-0.001)
November 2012	19.31 $\pm 0.17$	12.42 $\pm 0.28$ (0.76**)	7.35 $\pm 0.16$ (-0.13)	19.35 $\pm 0.21$	16.78 $\pm 0.13$ 0.65**)	14.42 $\pm 0.13$ (0.45**)
December 2012	14.63 $\pm 0.22$	9.56 $\pm 0.32$ (0.48**)	6.18 $\pm 0.23$ (-0.30)	16.38 $\pm 0.23$	11.54 $\pm 0.28$ (-0.67**)	9.40 $\pm 0.42$ (-0.46**)
January 2013	16.78 $\pm 0.13$	9.34 $\pm 0.20$ (0.42*)	6.28 $\pm 0.21$ (0.11)	18.51 $\pm 0.31$	18.51 $\pm 0.25$ (0.15)	13.55 $\pm 0.27$ (0.72**)
February 2013	22.34 $\pm 0.23$	18.51 $\pm 0.20$ (-0.29)	11.59 $\pm 0.23$ (0.38*)	22.58 $\pm 0.78$	16.77 $\pm 0.12$ (0.45*)	13.43 $\pm 0.24$ (0.10)
March 2013	27.70 $\pm 0.88$	17.52 $\pm 0.26$ (0.004)	11.46 $\pm 0.30$ (-0.33*)	25.13 $\pm 0.74$	13.47 $\pm 0.28$ (0.11)	7.33 $\pm 0.18$ (0.62**)

Figures are average of 6 replicates in respective months during 2012-2013, ( $\pm$  SD),

\* and \*\* denotes significance at 5% ( $p < 0.05$ ) and 1% ( $p < 0.01$ ) level respectively.

**Table 4.3: Alteration in muscle Protein contents (mg/gm) in control, naturally infected and artificially infected fishes with Fungi.**

Months	<i>Channa punctatus</i>			<i>Clarius batrachus</i>		
	Control	Naturally Infected	Artificially Infected	Control	Naturally Infected	Artificially Infected
August	18.96	10.62	7.82	17.66	13.98	9.59
	±0.50	±1.07	±1.77	±0.51	±0.52	±0.29
	-0.88**	-0.91**	0.92**	-0.30	-0.28	-0.23
September	17.29	10.63	6.31	17.47	13.46	9.53
	±0.70	±1.38	±0.25	±0.26	±1.02	±0.30
	0.63*	0.08	0.29	0.12	0.33	0.09
October	18.66	15.19	10.48	20.06	13.19	9.43
	±0.35	±1.66	±3.29	±0.66	±3.72	±2.21
	-0.46	-0.45	0.99**	0.94**	0.94**	0.98**
November	19.33	14.60	9.91	16.86	13.12	10.30
	±0.19	±2.28	±2.68	±2.61	±3.82	±4.31
	0.17	0.13	0.99**	0.99**	0.97**	0.99**
December	14.02	9.45	6.29	17.41	10.44	7.80
	±0.46	±0.27	±0.37	±5.18	±1.16	±1.72
	0.43	0.37	0.26	0.97**	0.98**	0.96**
January	20.91	13.96	9.94	23.93	18.51	13.46
	±4.38	±4.82	±3.84	±1.66	±0.25	±0.27
	0.98**	0.99**	0.99**	0.03	0.07	0.34
February	23.91	18.96	13.00	26.60	18.06	15.50
	±1.71	±0.50	±1.49	±1.16	±1.36	±2.22
	0.91**	0.98**	0.92**	0.85**	0.87**	0.98**
March	26.58	19.58	14.06	26.51	17.89	12.36
	±1.37	±2.17	±2.74	±1.58	±4.63	±5.26
	0.85*	0.84**	0.98**	0.90**	0.90**	0.99**

Figures are average of 12 replicates in all the respective months during 2011 to 2013, ( ± SD)

\* and \*\* denotes significance at 5% ( p<0.05) and 1% (p<0.01) level respectively.