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***Research Article** (Open Access)*

PROTEIN CONTENT FROM BODY MUSCLES OF *WALLAGO ATTU* AND *SPERATA SEENGHALA* FROM VARIOUS HABITATS

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ABSTRACT
Investigations were carried out to study the seasonal variation of total protein content in body muscles of *Wallago attu* and *Sperata seenghala* in selected habitats during February 2010 to January 2011 and February 2011 to January 2012. The result depicted that there is significant difference (p<0.05) in total protein content of body muscles of *Wallago attu* collected from selected reservoirs in the selected study area in different seasons. Also, there was significant difference (p<0.05) in total protein content in body muscles of *Sperata seenghala* in all seasons during February 2010 to January 2011, but there was no significant difference (p>0.05) in total protein content of body muscles of *Sperata seenghala* collected from selected reservoirs during February 2011 - January 2012.

Keywords: *Total Protein - Wallago attu - Sperata seenghala - Habitats - Seasons.*

INTRODUCTION
The freshwater fish *Wallago attu* (Bloch & Schneider, 1801) commonly called as Freshwater shark belonging to Family- Siluridae and Giant River catfish *Sperata* (*Mystus) seenghala* (Sykes, 1939) belonging to Family- Bagridae are one of the common catfishes from rivers and reservoirs of India, Bangladesh, Afghanistan, Pakistan and Nepal [1, 2]. Fish are the important component in human diet as rich source of proteins. Fishes contains good quality, balanced and digestible protein. Protein content in fish meat varied between 16 to 21% [3]. In fish's two types of muscles are present i.e. dark muscles (red) and white muscles. Dark muscles contain low level of moisture and protein than the white muscle. Proteins are classified on the basis of solubility and chemical structure. On the basis of solubility in salt solution, Proteins are of three group's Sarcoplasmic protein, Myofibrillar protein and Stroma. Sarcoplasmic protein (Albumin and Globulin) constitutes 25-30 % of protein. Myofibrillar protein (myosin, actin, tropomyosin, troponin) are structural protein constitute 65-70 % of total protein. Stroma proteins are also known as connective tissue protein. It constitutes 3 % of the total protein.
Proteins are complex organic compounds and made up of prolong chain of amino acid bounded together by peptide bonds. Fresh fish meat provides good source of protein for human diet, about 90-95 % of fish protein is assimilated in human body. In the present investigation, it was essential to know that, how much protein is present in the selected sample or to measure the concentration or amount of protein in fish muscle samples.
Protein content in fishes was analyzed in *Sperata seenghala* [4], *Wallago attu* [5], *Arius arius* [6], *Amblypharyngodon mola, Gadusia chapra*, *Puntius sp., Chanda nama, Pseudeutropinus atherinoides* and in *Ailia coila* [7], *Mystus tengara*, *Mystus cavasius, Mystus gulio* [8], *Labeo gonius* [9], *Clarias gariepinus* [10], *Labeo bata* [11] in different regions. Protein content variation is affected by breeding capability, planktonic diet and climatic changes in year. The highest and lowest protein content associated to breeding, spawning and biological regression, resting stages, respectively [12].

MATERIALS AND METHODS

2.1. Sample collection

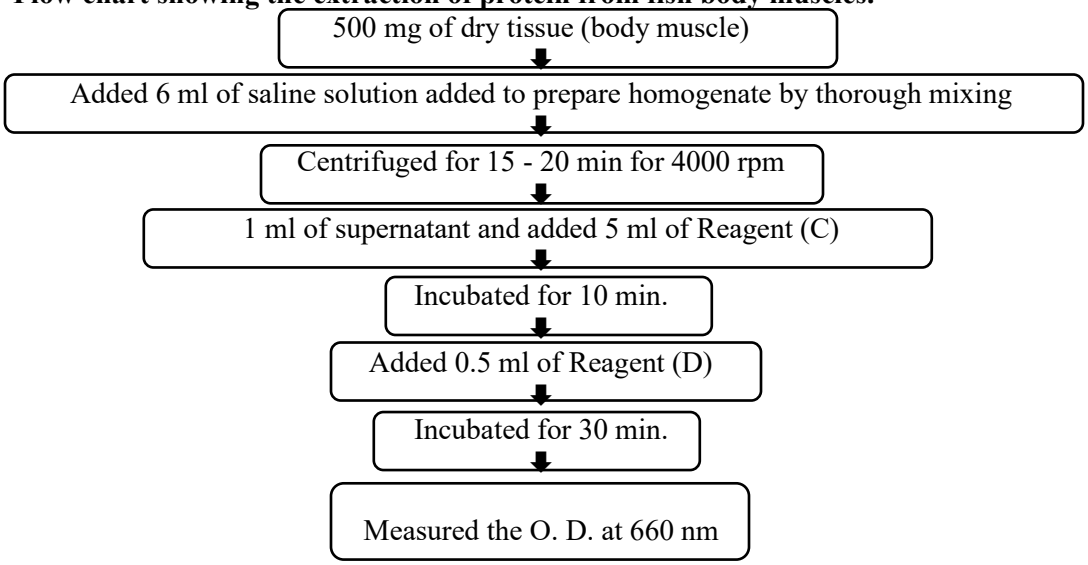
The selected freshwater fishes *W. attu* and *S. seenghala* were collected from four different reservoirs namely, Karpara Reservoir, Masoli Reservoir, Yeldari Reservoir and Siddheshwar Reservoir located in Godavari River basin, Maharashtra, India. Total 521 specimen of both the species of which 182 *S. seenghala* and 339 *W. attu* were collected monthly at the fishing sites. The body muscles of both selected fishes were used for analysis of total protein content during February 2010 to January 2011 and February 2011 to January 2012.

2.2. Reagents and Chemicals.

To estimate the total protein content from the selected fish species *W. attu* and *S. seenghala* Lowry's method [13] was used. This method is sensitive enough to moderate constant volume of sample. The blue colour developed by the reduction of the Phosphomolybdic-Phosphotungustic components in the Folin-Ciocalteau reagent by amino acids tyrosine and tryptophan present in the protein plus the colour developed by biuret reaction of protein with alkaline cupric tartrate are measured in the Lowry's method.

1) Reagent (A) -: 2 % Sodium carbonate ($Na_2Co_3.5H_2O$) in 0.1 N sodium hydroxide.
2) Reagent (B) -: 0.5 % Copper sulphate $CuSo_4.5H_2O$ in 1% Potassium sodium tartrate.
3) Reagent (C) -: Alkaline copper Solution - 50 ml of Reagent (A) and 1ml of Reagent (B) are mixed prior to use.
4) Reagent (D) -: Folin - Ciocalteau Reagent.
5) Protein Solution (Stock Solution) -: 50 mg of BSA was weighed accurately and dissolved in distilled water and made the volume up to 50 ml in a standard flask.
6) Working standard -: 100 ml of the stock solution was mixed with 50 ml of distilled water in a standard flask. 1ml of this solution contains 200 µg proteins.

2.3. Flow chart showing the extraction of protein from fish body muscles.



The selected fishes *W. attu* and *S. seenghala* were purchased in fresh condition from fish market of Parbhani and selected reservoirs (Study area). Washed the fishes in running tap water to remove the mucus from the outer surface of the bodies of fishes. Then, weights of the fishes were recorded with the help of weighing balance and the readings were noted. Body muscles were separated from fish body. 100 gm of muscles were weighed and dried in microwave oven. After drying, the dry muscles were weighed. Dried tissues made into fine powder using pestle and mortal. 500 mg of dry muscle powder was weighed in a test tube and added 5 - 6 ml of saline. Centrifuged for 15 - 20 min for 4000 rpm. After some time, 1ml of supernatant

was taken and added 5ml of reagent C in another test tube. This way the sample is ready for further chemical analysis. For 10-time dilution of sample, therefore pipette out 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 ml. sample solution and 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 ml of distilled water to make the volume up to 1ml. The samples were incubated at room temperature for 10 min. and shaked well 0.5 ml of reagent D in same test tube was added. Again, incubated at room temperature for 30 min. After 30 min O. D. at 660 nm was recorded using UV- Spectrophotometer (Shimadzu, UV - 1800).

2.4. Statistical Analysis

The results obtained for two freshwater fishes *W. attu* and *S. seenghala* are mean ± SD. The mean values of total protein content are submitted to two - way analysis of variance using software Microsoft Office Excel 2007 and statistically significant difference (p<0.05) was recorded. If the entire F-test was significant (p<0.05), then a Fishers T- test was performed to discern difference among the reservoirs and seasons.

RESULTS

In table - 1, 2, 3 and 4, the seasonal variation of total protein (mg/g) in body muscles of selected fishes *Wallago attu* and *Sperata seenghala* collected from selected habitats (reservoirs) during study period are shown.

The total protein content (mg/g) in the body muscles of *Wallago attu* collected from all selected habitats (reservoirs) in three seasons during February 2010 - January 2011 was in the range of 0.69±0.22 to 7.89±0.63 mg/gm. Highest total protein content was observed 7.89±0.63 mg/g in the body muscles of *Wallago attu* collected from Masoli Reservoir during summer season. While, lowest total protein content in the body muscles of *Wallago attu* was found 0.69±0.22 mg/g collected from Siddheshwar Reservoir during monsoon season as compared to all selected reservoirs from different seasons are presented in table 1 and Fig. 1. The total protein content in the body muscles of *W. attu* collected from all selected habitats in different seasons during February 2011 - January 2012 was 1.75±0.35 to 7.02±0.97 mg/g. Highest total protein content in the body muscles of *W. attu* collected from Masoli Reservoir during summer season was found 7.02±0.97 mg/g. While, lowest total protein content in the body muscles of *W. attu* collected from Siddheshwar Reservoir during winter season was found 1.75±0.35 mg/g as compared to all selected reservoirs in all seasons are as shown in table 2 and Fig. 2.

The total protein content in the body muscles of *S. seenghala* collected from all selected habitats during February 2010 - January 2011 was 1.14±0.53 to 6.48±0.84 mg/g. Highest total protein content in the body muscles of *S. seenghala* collected from Masoli Reservoir during summer season was 6.48±0.84 mg/g. While, lowest total protein content in the body muscles of *S. seenghala* collected from Siddheshwar Reservoir during winter season as compared to all selected reservoirs in three seasons was found 1.14±0.53 mg/g the details are as shown in table 3 and Fig. 3.

The total protein content in the body muscles of *S. seenghala* was obtained 1.79±0.45 to 6.73±0.91 mg/g in all selected habitats during February 2011 - January 2012. Highest total protein content in the body muscles of *S. seenghala* collected from Masoli Reservoir during summer season was found 6.73±0.91 mg/g. While, lowest total protein content in the body muscles of *S. seenghala* collected from Karpara Reservoir during monsoon season was observed 1.79±0.45 mg/g as compared to all selected reservoirs in all three seasons are presented in table 4 and Fig. 4.

By using Two-way ANOVA between the result obtained for the protein in the selected fish species from different selected reservoirs and seasons, it was observed that there is significant difference (p<0.05) in total protein content (mg/g) in body muscles of *Wallago attu* during February 2010 to January 2011 and February 2011 to January 2012. However, in case of *Sperata seenghala* significant difference was observed among seasons (p<0.05) and it was no significant among reservoirs (p>0.05) during February 2011 to January 2012.

Table 1: Seasonal variation in Total Protein content (mg/g) in body muscles of *Wallago attu* collected from different selected habitats during February 2010 - January 2011.

Seasons ↓ / Habitats →	Karpara Reservoir	Masoli Reservoir	Yeldari Reservoir	Siddheshwar Reservoir
Summer	6.52±0.67	7.89±0.63	6.47±0.97	5.44±0.77
Monsoon	2.03±0.89	4.12±0.75	2.19±0.45	0.69±0.22
Winter	1.79±0.25	2.14±0.43	2.36±0.99	1.61±0.68

The data is shown as mean±standard deviation (n=10).

Table 2: Seasonal variation of Total Protein content (mg/g) in body muscles of *Wallago attu* collected from different selected habitats during February 2011 - January 2012.

Seasons ↓ / Habitats →	Karpara Reservoir	Masoli Reservoir	Yeldari Reservoir	Siddheshwar Reservoir
Summer	6.30±0.57	7.02±0.97	6.57±0.81	4.97±0.62
Monsoon	4.54±0.97	4.04±0.83	4.31±0.97	3.09±0.71
Winter	2.28±0.66	4.20±0.76	3.49±0.96	1.75±0.35

The data is shown as mean±standard deviation (n=10).

Table 3: Seasonal variation of Total Protein content (mg/g) in body muscles of *Sperata seenghala* collected from different selected habitats during February 2010 - January 2011.

Seasons ↓ / Habitats →	Karpara Reservoir	Masoli Reservoir	Yeldari Reservoir	Siddheshwar Reservoir
Summer	4.92±0.67	6.48±0.84	5.60±0.70	4.70±0.72
Monsoon	3.27±0.66	4.15±0.90	3.22±0.84	2.16±0.42
Winter	2.49±0.36	3.68±0.53	3.06±0.88	1.14±0.53

Table 4: Seasonal variation of Total Protein content (mg/g) in body muscles of *Sperata seenghala* collected from different selected habitats during February 2011 - January 2012.

Seasons ↓ / Habitats →	Karpara Reservoir	Masoli Reservoir	Yeldari Reservoir	Siddheshwar Reservoir
Summer	6.14±0.87	6.73±0.91	6.37±0.62	5.51±0.28
Monsoon	1.79±0.45	3.91±0.41	3.88±0.57	2.49±0.67
Winter	2.15±0.29	2.46±0.82	3.03±0.68	2.07±0.85

The data is shown as mean±standard deviation (n=10).

Fig. 1 - Seasonal variation of Total Protein content (mg/g) in body muscles of *Wallago attu* collected from different selected habitats during February 2010 - January 2011 .

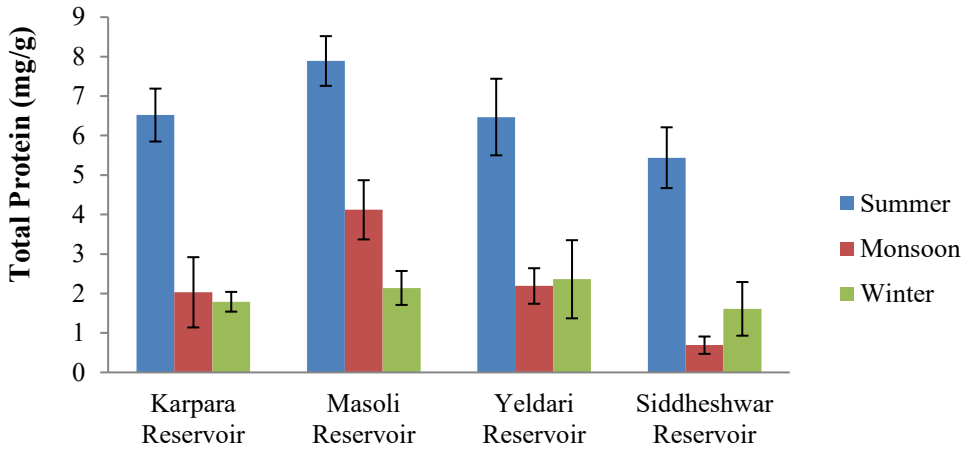
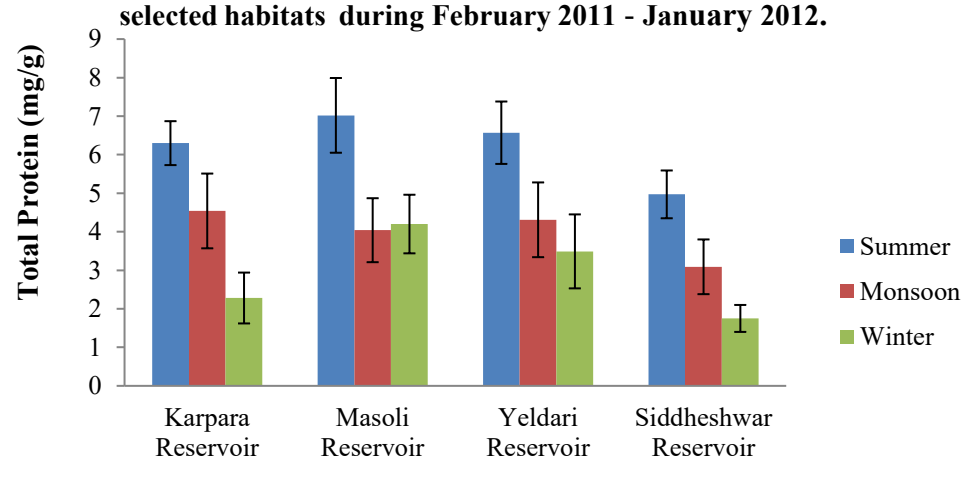


Fig. 2 - Seasonal variation of Total Protein content (mg/g) in body muscles of *Wallago attu* collected from different selected habitats during February 2011 - January 2012.



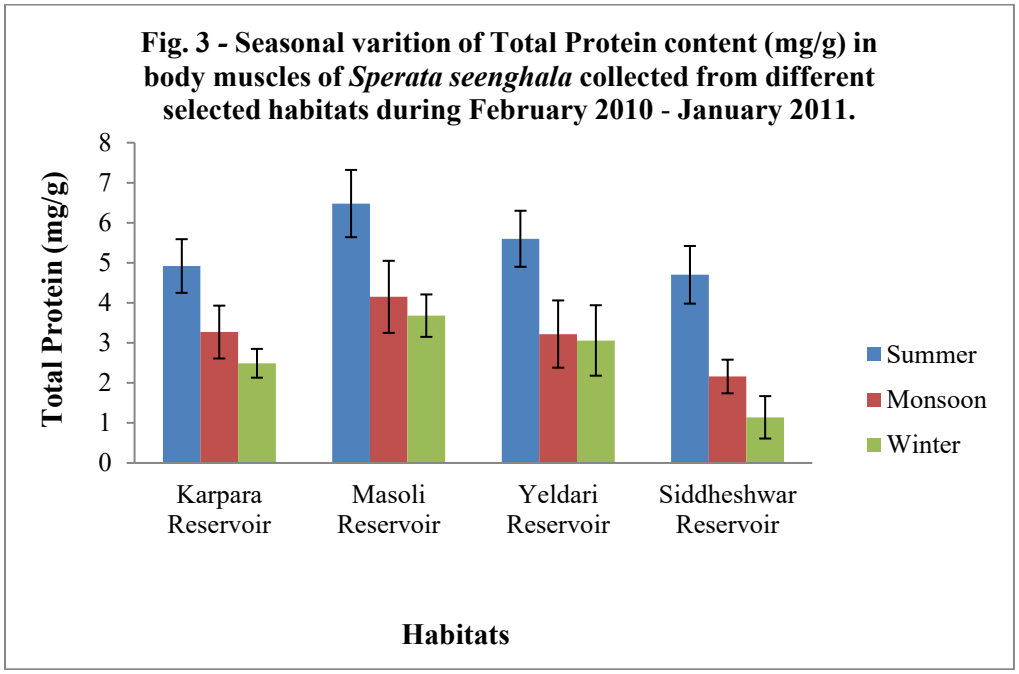


Fig. 3 - Seasonal varition of Total Protein content (mg/g) in body muscles of *Sperata seenghala* collected from different selected habitats during February 2010 - January 2011.

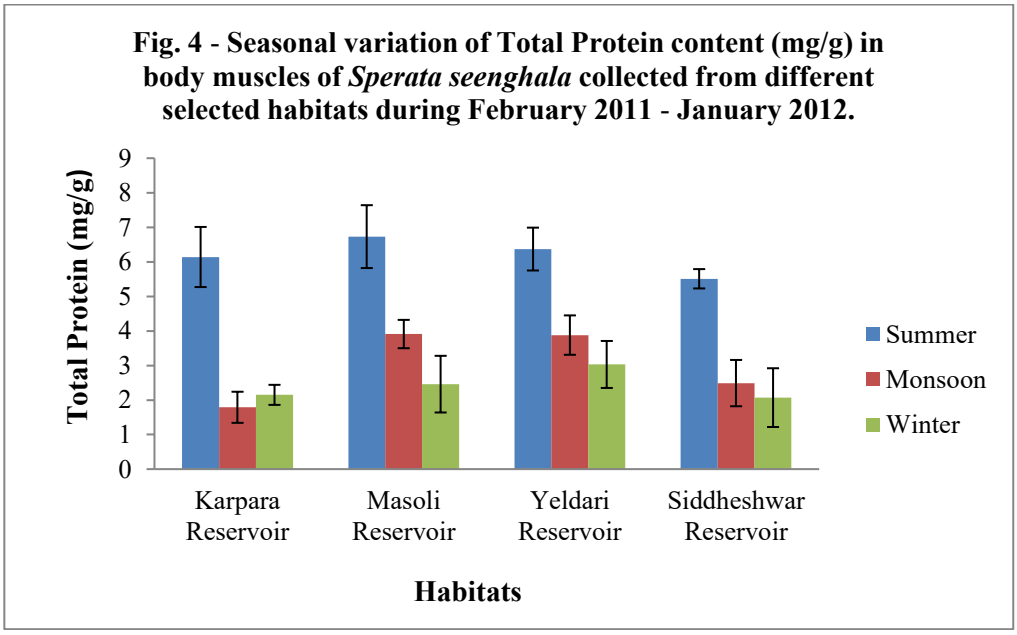


Fig. 4 - Seasonal variation of Total Protein content (mg/g) in body muscles of *Sperata seenghala* collected from different selected habitats during February 2011 - January 2012.

DISCUSSION

As per the results obtained on the total protein content in the selected fish species *W. attu* and *S. seenghala,* there is significant difference (p<0.05) in total protein content (mg/g) in body muscle of *Wallago attu* during February 2010 to January 2011 and February 2011 to January 2012 and *S. seenghala* during February 2010 to January 2011. There is significant difference (p<0.05) in total protein content (mg/g) in body muscle of *S. seenghala* in different seasons but, no significant difference (p>0.05) in total protein content (mg/g) in

body muscle of *S. seenghala* collected from different selected reservoirs during February 2011 to January 2012.

The total protein content in various tissues in fishes were investigated by various authors and correlated their results with different factors like seasons, habitat differences etc. which are summarized as below. There are very few reports on the variations in the total protein content in the fish tissues with difference in the habitat of fish.

The protein level of *Mystus tengara*, *Mystus cavasius, Mystus gulio* in fresh condition were 16.26 %, 15.52 % and 14.80 % examined [8]. After twenty days of freezing protein level decreased to 14.97 %, 13.91 %, 13.43 %. The amount of moisture for these fishes increased from 76.12 % to 78.02 %, 75.35 % to 77.25 % and 76.03 % to 78.23 % respectively. In fresh condition the lipid content level of *Mystus tengara*, *Mystus cavasius* and *Mystus gulio* was 6.15 %, 5.98 %, and 6.20 % and the lipid were 4.9 %, 5.37 % and 4.86 %. The reduction in protein level was corelated with denaturation of fish protein that is associated with freezing. Analyzed the fish species [14] *Thunnus albacares*, *Parupeneus bifasciatus*, *Hyporhamphus dussumieri* and maximum protein level was found in *Thunnus albacares* (13.69%) and minimum protein level was in *Hyporhamphus dussumieri* (10.51%). The lipid content was ranged from 2.96% in *Thunnus albacares* to 6.97% in *Hyporhamphus dussumieri*. The lipid was found negatively correlated with protein ($r = -0.765$, $p<0.05$).

Total lipid content from muscles of *Oncorhynchus myskiss* reached the lower level in May and increased from June to September studied [15]. The highest protein 17.61 % in May (pre-monsoon) and lowest in 16.0 % in September (post-monsoon) in catfish *Arius arius was also examined* [6]. The average protein value was 17.36 % during pre-monsoon, 16.9 % during monsoon (June and July) and 16.3 % during Post-monsoon months. The highest amount of lipid content was 6.26 % in April and lowest amount in August 3.7 %. The average lipid value was 6.1 % in pre-monsoon, 3.9 % in monsoon and in post-monsoon 5.6 %. The lipid from the body muscle of *Lepidocephalus guntea* (Ham.) was analyzed in three size group size-I (below 8.5 cm), size-II (8.5- 9.5 cm) and size-III (above 9.5 cm) [16], among these groups, highest amount of lipid was found in size-III i.e. 3.455 % and lowest in size-I i.e. 2.515 %.

It was reported that the higher level of total protein in the body muscles of *Labeo gonius* was observed in pre-monsoon season 138.22 in Lotic habitats and lowest level was found in retreating monsoon 42.8. Comparatively lower protein content was observed in all the seasons of Lentic habitat whereas in the present study the protein content in the muscles of *Wallago attu* has significant difference between seasons and reservoirs selected the study [9]. Monthly variation of the total protein content [18] reported ranged from 0.0947 to 0.220 gm/gm tissue in *Schizothorax niger*. Highest total protein was found in month of July and lowest was in January and December. Seasonal variation of total protein in the muscle tissue of *Schizothorax niger* was observed in summer season (19.25 %) and lowest in winter season, from Dal Lake of Kashmir.

The correlation between the absorbance obtained at 700nm and the concentration of curve is linear from 2.5 to 200µg/ml was also reported [19]. The microwave micro assay tolerates the presence of higher concentrations of various known substances to interfere with Lowry's method. The protein content in *Schizothorax esocinus* was higher in the month of July 0.358 gm/gm and lowest in the month of December 0.089 gm/gm was examined [20]. Seasonal variation in the protein content was observed in summer season (28 %) and lowest in winter season (10%) from Dal Lake of Hazratbal Shrinagar, Kashmir. Protein content in various fishes was examined [21] *Salmostoma sardinella* 25.64 %, *Cirrhinus reba* 24.19 %, *Garra mullya* 28.69 %, *Rasbora daniconius* 32.79 % and *Puntitus conchonius* 27.10%. The highest protein content was found in *Rasbora daniconius* 32.79 %. The amount of protein percentage in the muscles of *Macrones leucophasis* fluctuated between 22.09 to 26.47 in female and in male from 22.07 to 25.63 was reported [22]. It is that the Lowry method to be unreliable in the acidic and increasing vitamin C rich medium was reported [23]. It was suggested to evaporate organic solvents (compounds) when they are used to extract protein prior to protein measurement the Lowry method. The influence of phenolic compounds on the Lowry's method was found to be governed by the structure of their molecules while ammonium sulphate was a major agent

used in the enzyme purification process. The protein levels of muscle in male catfish *Mystus cavasius* varied from 0.42 to 5.27, highest in December and lowest in July was reported [24].

Mean value of protein 14.87 % in *Clarius batrachus*, protein 17.34 % in *Heteropneustes fossilis*, 19.63 % protein in *Anabas testudineus*, 18.30 % protein in *Notopterus notopterus*, 16.09 % protein in *Nandas nandas*, 19.13 % protein in *Channa punctatus*, 15.62 % protein in *Mystus vittatus* from river Mouri, Khulna, Bangladesh was revealed [26].

Protein content in *Glossogobius giuris* studied [27] it was ranged from 14.09 % (February) to 16.03 % (August) in male and in female 13.88 % (February) to 15.56 % in (November). The average protein content of female 14.61% *Glossogobius giuris* was lower than the male 15.23 %. The protein value was recorded higher in *Scomberoides commersonianus* 21.68 % and lower in *Scomberoides lysan* 19.47 % [28].

The biochemical composition of cultured and natural fishes *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* was studied [29]. The concentration muscle protein was maximum in March 19.920 gm/gm, 20.021 gm/gm, 19.560 gm/gm in natural fishes. But in Cultural fishes, maximum protein concentration in muscle was 18.516 gm/gm in *Catla catla*, 19.210 gm/gm in *Labeo rohita* and 18.327 gm/gm in *Cirrhinus mrigala*.

The protein content of *Cyprinus carpio* decreased from summer to spring season from 17.6- 15.9 % in the farmed carp samples [30]. Protein in *Amblypharyngodon mola* (18.46%), *Gundusia chapra* (15.23%), *Puntius chola* (14.08%), *Chanda nama* (18.26%), *Pseudeutropinus atherinoides* (15.84%) and in *Ailia coila* (16.99%) was analyzed [7].

The protein content concentration varied from 73.1 to 85.6 %, 73.0 to 87.5%, 73.5 to 88.0 % and 73.8 to 85.2% from four stations i.e. El- Kanates, Benha, Zefta and Talka. Lowest protein content (73.0%) was found in female during autumn from Behna and highest (88.0%) was from Zefta during spring season was examined [32].

The protein content was analyzed in *Chrysichthys walkeri* varied from 19.47 - 20.14 and 18.62 - 20.54 for *Chrysichthys nigrodigitatus* was reported [33]. The protein content in muscle tissues was studied [34] ranged from 66.00-84.00% and higher mean percentage of protein was found in muscle tissues of *Labeo niloticus* 66.00 % and *Protopterus annectens* 84.00 %.

The protein content was examined [35], it was 22 % in *Thunnus alalunga*, *Evynnis japonica* 13.02 %, *Caulerpa lentillifera* 26.9 %, *Orcynopsis unicolor* 22 % and *Euthynnus affinis* 24 %. The amount of protein was higher in *Caulerpa lentillifera* 26.9 % and lower in *Evynnis japonica* 13.02%. The protein content levels in Tiger Tooth Croaker (Otelithes ruber) and four Finger threadfin (*Eleutheronema thetradactylium*) were recorded [36] 21.524 % and 20.732 %. Protein content level in immature and mature group of *Harpodon nehereus* [37] were 7.10% and 7.43%.

The protein means percentage of muscle as 86.00% in Powan (*Coregonus lavaretus*) from Loch Lomond, Scotland was examined [42]. The protein content in muscle of *Lethrinus lentjan* [43] ranged from 18.75 to 23.84 mg/gm. Maximum level of protein 20.26% in catfish *Hemisynodontis membranacea* was studied [44]. The crude protein in *Clarias gariepinus* as 16.375% and 14.328% in *Oreochromis niloticus* was studied [45]. The crude protein 65.94%, 67.77% in wild and cultured *Clarias gariepinus* was examined [46].

Protein content was reported [47] that 16.78%, 17.31%, 18.17%, 16.69%, 16.73% in *Ailiichthys punctatus*, *Clupisoma psendeutropius atherinoides*, *Puntius sarana*, *Gundusia chapra*, *Corica soborna*. The crude protein level in freshwater fish was reported [48] varied from 20.26% in *Alestes nurse* to 21.52% in *Oreochromis gallilaeus*. The crude protein was19.30%, 17.37%, 16.86% was reported [49] in *Labeo rohita, Cirrhinus mrigala, and Catla catla.*

Lates niloticus (protein 74.2%), *Tetraodon lineatus* (protein 71.5%), *Heterotis niloticus* (protein 78.8%), *Mormyrus niloticus* (protein 73.4%), *Clarias lazera* (protein 59.9%), *Protopterus annectens* (protein 68.00%) were examined [50]. The highest level of protein 30.31% for young adult tail *Clarias gariepinus* and least value 23.06% Juvenile tail *Clarias gariepinus* was reported [10].

CONCLUSION

1. Total protein content in the body muscles of *Wallago attu* from Masoli Reservoir was higher in summer season and lower in winter season from Siddheshwar Reservoir.
2. Total protein content in body muscles of *Sperata seenghala* from Masoli Reservoir was higher in summer season and fluctuation in the result were found in lower total protein content in body muscles of the fish from Siddheshwar Reservoir in winter season and from Karpara Reservoir in monsoon season.
3. There is significant difference (p<0.05) in total protein content of body muscles of *Wallago attu* during February 2010 to January 2011 and February 2011 to January 2012 collected from different selected reservoirs in the selected study area in different seasons.
4. There is significant difference (p<0.05) in total protein content of body muscles of *Sperata seenghala* in all seasons during February 2010 to January 2011 and February 2011 to January 2012 but there is no significant difference (p>0.05) in total protein content of body muscles of *Sperata seenghala* collected from different selected reservoirs during February 2011 - January 2012.

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