



## Nutritional profile of climbing perch (*Anabas testudineus* Bloch, 1792) muscle tissue with emphasis on seasonal variations

Treesa Varghese<sup>1,2\*</sup> & Saleena Mathew<sup>2</sup>

<sup>1</sup>School of Biosciences, Mar Athanasios College for Advanced Studies Tiruvalla (MACFAST), Kerala, India

<sup>2</sup>School of Industrial Fisheries, Cochin University of Science and Technology, Cochin, Kerala

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Nutritional profile of Climbing Perch (*Anabas testudineus* Bloch, 1792) muscle tissue were analyzed with emphasis on seasonal variations. Climbing Perch muscle tissue had high lipid content, constituting about 12.87%. The mean value of protein, moisture and ash were found to be 15.46, 68.86 and 1.25%, respectively. Highest values were observed in the month of March and April for protein (17.02%) and lipid (14.06%), respectively, while lowest values were in the month of July for both protein (13.02%) and lipid (11.08%). Highest and lowest values for water content were noticed in the month of July (73.37%) and March (66.91%). Protein ( $P < 0.01$ ) and lipid ( $P < 0.01$ ) content showed a negative correlation with moisture content within the muscle tissue. Additionally, between protein and lipid a positive relation was observed ( $P < 0.01$ ). Ash content showed no significant correlation with protein, lipid or moisture content in the muscle tissue and its maximum value was noticed in the month of March (1.75%). The results suggested that the proximate composition of fish muscle tissue significantly varies during different seasons. The present studies thus provide information on variation in proximate composition of the Climbing Perch with seasons which is useful for the processing industry.

**Keywords:** Fishery, Muscle tissue

Local fish species, valuable resources of macro and micronutrients, play an important role in providing vital nutrients for humans. Fish muscle consists of protein, fat and moisture as the main components of food and carbohydrates, vitamins and minerals as minor components<sup>1</sup>. Because of its special nutritional qualities, fish is increasingly getting popular as a healthy food<sup>2</sup>. In this context, correct information on the biochemical constituents of fish has become a crucial necessity. In addition, its evaluation is important for better processing and conservation. The composition of the biochemical components of an organism changes depending on changes in the environment. In order to highlight the nutritional value of fish as a food product and the deterioration in its quality with various processing methods, it is necessary to analyze the chemical composition of the fish.

Proximate analysis of food sample determines the total amount of protein, fat, ash and moisture, which is presented as a percentage of food. In addition, carbohydrates, vitamins, nucleotides and non-protein nitrogenous components exist as minor components. Water is the main component of fish meat, its content is

about 70-80%, and its content varies widely. Water is present in tissues in two forms: in free form and associated with protein and have well-defined biological roles. Water is lost from the tissue in various ways during processing and this can affect the quality, especially the texture of the processed products. Water acts as a medium for transport of nutrients and metabolites and is essential for proper functioning of many biological molecules. The sum of lipids and water percentages is 75-85% of the total wet weight of the fish meat. The amount of lipids gradually increases with increasing fish size, and the water content decreases with increasing lipid content<sup>3</sup>. Lipids make up about 1-20% of total tissue mass and their diversity is wider than protein content. Lipids vary in different parts of the fish's body and show significant changes in different seasons<sup>4</sup>. Fish proteins are much richer in methionine, lysine, and tryptophan than mammals<sup>5</sup>. Fish muscle protein makes up about 10-22% of the total tissue structure. Protein content depends on the type of fish muscle, and dark muscles generally have lower levels of protein and water than light muscles. Nutritionally, fish primarily provide protein, from which 80-90% of food energy comes from, so it can be safely used in foods to supplement protein<sup>6</sup>. Ash is defined as the total mineral content of food and is about 0.5-5% of fish muscle.

\*Correspondence:  
E-Mail: treesav@gmail.com

The biochemical composition of fish muscles depends mainly on the time of year, size, age and habitat, migratory swimming, sex differences, hunger states, species and even individuals<sup>6</sup>. Composition analysis has traditionally been used as a good indicator of the nutritional value of food<sup>7,8</sup>. In addition, no data are available on changes in the approximate composition of climbing perch under different seasonal conditions. Here, we studied the seasonal variations in the proximate composition of the Climbing Perch caught in small freshwater streams and paddy fields in Thiruvalla, Kerala.

## Materials and Methods

### Collection and handling of the fish sample

The Climbing Perch (Fig. 1) for analysis were caught from the streams and paddy fields near Tiruvalla, Kerala and brought to the laboratory in live condition. Identification and conformation of fish species was done by Dr M Harikrishnan, School of Industrial Fisheries, CUSAT, Kerala. The fish were slaughtered without delay by a blow on the head and sampling of the tissue was done. The fish used for the study were of uniform size having weight of  $100 \pm 10$  g and length range of  $10 \pm 2$  cm. Muscle tissues from the dorsal side of the fish between the gills and the dorsal fins were used for analysis in triplicates. Similarly, samples were collected every month for seasonal variation studies.

### Determination of Moisture

Moisture content was estimated by the method of AOAC<sup>9</sup>. The moisture content was determined by drying 10 g sample at  $103^\circ\text{C}$  in a thermostatically controlled hot air oven. The samples were taken in a pre - weighed glass dish with cover and kept in oven till the weight became constant. The weight was checked for constant weight by repeatedly heating and then cooling the sample in desiccator. The percentage solid was determined from the above experiment by using the formula;

$$\text{Percentage solid} = \frac{\text{Weight of dry sample}}{\text{Weight of wet sample}} \times 100$$

The percentage moisture was calculated by subtracting solid weight % from 100.

### Determination of crude protein

Crude protein content was determined by the method of AOAC<sup>9</sup>. One gram homogenized sample was used for determining the crude protein content using Micro Kjeldahl method. About 2 g of digestion mixture ( $\text{CuSO}_4$  and  $\text{K}_2\text{SO}_4$  as catalyst in the ratio of 1: 8) and 10 mL of concentrated  $\text{H}_2\text{SO}_4$  were added to the sample taken in the digestion tube. The samples were digested to a clear solution in a Kjeldahl digestion unit, and 50 mL of distilled water was added to the cooled tube



Fig. 1 — Climbing Perch (*Anabas testudineus*)

slowly till no heat was generated on adding water. The solution was made up to 100 mL. Pipetted out 5 mL of the prepared sample into the Kjeldahl Micro Distillation Apparatus. The bottom end of the condenser was fitted to a delivery tube immersed in 10 mL of 2% boric acid with Tachiro's indicator. NaOH (40%) was added to the sample in the distillation unit to make it alkaline. The ammonia, produced on steam distillation was absorbed into the boric acid solution. The distillate collected was back titrated against N/70  $\text{H}_2\text{SO}_4$  and using the titer value, nitrogen content was estimated. Crude protein content in the sample was calculated by multiplying the nitrogen content by the factor of 6.25.

$$\text{Percentage of protein} = \frac{V \times 1 \times 100 \times 100 \times 6.25}{5 \times 5 \times \text{Weight of the sample}}$$

### Determination of crude lipid

Fat content of the moisture free sample was determined by extracting the fat by Soxhlet extraction method<sup>9</sup>. About 2 g of moisture free sample was accurately weighed into an extraction thimble (Whatman No.1) and placed in the extractor. The extractor was connected to a pre-weighed dry receiving flask and a water condenser. Petroleum ether (B. P.  $40-60^\circ\text{C}$ ) was used as solvent. The unit was heated in a water bath and temperature was maintained at  $40-60^\circ\text{C}$  so that solvent boiled continuously and siphoned at a rate of 5-6 times/h. The extraction was continued till the solvent in the extractor became colourless and fat free. The solvent in the receiving flask was evaporated completely and weighed for fat content.

$$\text{Percentage of crude lipid} = \frac{\text{Weigh of fat}}{\text{Weight of sample}} \times 100$$

### Determination of Ash

The ash content was estimated by the incineration of the sample according to AOAC<sup>9</sup>. Two grams of moisture free sample taken in a pre-weighed clean dry silica crucible was charred on low heat. Then it was kept at  $550^\circ\text{C}$  in a muffle furnace to get a white ash, cooled in the desiccator and weighed.

$$\text{Percentage of ash} = \frac{\text{Weigh of ash}}{\text{Weight of sample}} \times 100$$

### Histochemical evaluation of muscular pattern

The fish muscle sample was cut into small piece of blocks and fixed overnight using formalin fixative,

followed by dehydration in serially diluted alcohol (70-96%). Then the dehydrated samples were treated with xylene to remove alcohol content and embedded in paraffin wax (melting point 60°C). Paraffin embedded sample was cut into sections of 5 micron using rotary microtome (Leica RM2155, Germany). Double staining was performed using Haematoxylin stain and Eosin stain. The myofibrils and collagen were stained yellowish orange and blue respectively, which was then observed under compound light microscope and photographed. (Leica microsystems, CH-9435, Heerbrugg, Switzerland)

#### Statistical analysis

All statistical calculations were performed using IBM SPSS Statistics 20.0 Software. Data analysis was performed using one-way analysis of variance (ANOVA) with post-hoc with multiple comparison analysis performed using Duncan test. *p* values less than or equal to 0.05 were considered as significant. Data are represented as mean  $\pm$  standard deviation. Correlation analysis between parameters analyzed was done using Pearson Correlation.

#### Results

Proximate composition of the climbing perch muscle tissue for twelve months was analyzed to obtain an estimate of the effect of seasonal variation on the nutritional status of climbing perch muscle tissue in terms of protein, lipid, moisture, and ash. The results obtained for the twelve months (June to May) are shown in Table 1. The data obtained in this study were statistically analyzed using SPSS. ANOVA was performed to compare differences in crude protein content, lipid content, moisture, and ash. Pearson Correlation analysis also conducted to in order to get an idea regarding level of correlation between moisture, protein and lipid content present in the perch muscle tissue.

The results obtained from the analysis show that the muscle tissue of the climbing perch had a high lipid content that represented around 12.87%. The mean values of protein, water and ash were 15.46, 68.86 and 1.25%, respectively. The highest was observed in March and April for proteins (17.02%) and lipids (14.06%), respectively, and the lowest was observed in July for both proteins (13.02%) and lipids (11.08%). The highest and lowest water contents were found in July (73.37%) and March (66.91%), respectively. Protein ( $P < 0.01$ ) and lipid ( $P < 0.01$ ) content showed a negative correlation with moisture content within the muscle tissue. Additionally, between protein and lipid a positive relation was observed ( $P < 0.01$ ). Ash content

Table 1 — Seasonal changes in the proximate composition of Climbing Perch muscle tissue

Month	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
June	15.06 $\pm$ 0.20 <sup>c</sup>	13.84 $\pm$ 0.29 <sup>c,e</sup>	69.09 $\pm$ 0.49 <sup>d,e</sup>	0.91 $\pm$ 0.12 <sup>a</sup>
July	13.02 $\pm$ 0.35 <sup>a</sup>	11.08 $\pm$ 0.22 <sup>a</sup>	73.37 $\pm$ 0.20 <sup>b</sup>	1.22 $\pm$ 0.01 <sup>e</sup>
August	13.60 $\pm$ 0.26 <sup>ab</sup>	11.66 $\pm$ 0.19 <sup>a</sup>	71.98 $\pm$ 0.28 <sup>g</sup>	1.07 $\pm$ 0.05 <sup>b,c,d</sup>
Sept.	14.11 $\pm$ 0.26 <sup>b</sup>	12.71 $\pm$ 0.13 <sup>b</sup>	70.41 $\pm$ 0.23 <sup>f</sup>	1.29 $\pm$ 0.06 <sup>e,f</sup>
October	15.46 $\pm$ 0.37 <sup>c</sup>	12.85 $\pm$ 0.12 <sup>b</sup>	69.37 $\pm$ 0.54 <sup>f</sup>	1.48 $\pm$ 0.03 <sup>g</sup>
Nov.	16.63 $\pm$ 0.62 <sup>e</sup>	13.25 $\pm$ 0.11 <sup>b,c,d</sup>	67.41 $\pm$ 0.31 <sup>ab</sup>	1.40 $\pm$ 0.01 <sup>g</sup>
Dec.	16.37 $\pm$ 0.77 <sup>d,e</sup>	12.53 $\pm$ 0.48 <sup>b</sup>	68.10 $\pm$ 0.52 <sup>b,c,d</sup>	1.09 $\pm$ 0.04 <sup>c,d</sup>
January	15.21 $\pm$ 0.46 <sup>c</sup>	12.77 $\pm$ 0.24 <sup>b</sup>	67.83 $\pm$ 0.73 <sup>ab,c</sup>	1.18 $\pm$ 0.03 <sup>d,e</sup>
Feb.	16.53 $\pm$ 0.19 <sup>e</sup>	13.72 $\pm$ 0.13 <sup>c,d,e</sup>	68.36 $\pm$ 0.13 <sup>b,c,d,e</sup>	0.95 $\pm$ 0.02 <sup>ab</sup>
March	17.02 $\pm$ 0.44 <sup>e</sup>	12.93 $\pm$ 0.23 <sup>b</sup>	66.91 $\pm$ 0.56 <sup>a</sup>	1.75 $\pm$ 0.04 <sup>i</sup>
April	17.01 $\pm$ 0.13 <sup>e</sup>	14.06 $\pm$ 0.02 <sup>e</sup>	66.71 $\pm$ 0.52 <sup>a</sup>	1.60 $\pm$ 0.05 <sup>h</sup>
May	16.53 $\pm$ 0.13 <sup>e</sup>	13.07 $\pm$ 0.74 <sup>b,c</sup>	68.79 $\pm$ 0.63 <sup>c,d,e</sup>	1.03 $\pm$ 0.06 <sup>b,c</sup>
Average	15.46	12.87	68.86	1.25

[All values are expressed as mean  $\pm$  standard deviation, n=3. Different superscripts in the same column indicates significant difference ( $P < 0.01$ )]

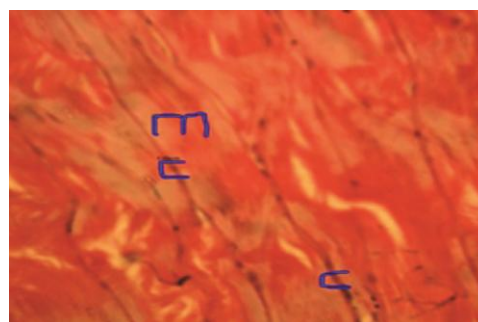


Fig. 2 — Histochemical image of Climbing Perch muscle tissue showed no significant correlation with protein, lipid or moisture content in the muscle tissue and its maximum value was noticed in the month of March (1.75%). ANOVA results showed that there was a significant variation between months ( $P < 0.01$ ).

#### Histochemical observations of muscle tissue

Evaluation of structural and textural parameters is important for determination of the nutritional quality of fish. Textural profile provides vital information on post-mortem tissue deterioration which significantly correlates with the storage period. Fig. 2 shows the histochemical observations of the muscle tissue. The musculature was regular and well closely packed myofibrillar bundles as indicated in the figure as M. The cellular structural design was compact and observed few extra cellular spaces. Collagen present in connective tissue (marked as C) formed myocommata sheath, which cover and hold each myotome bundle together. Presence of both connective tissue collagen and myofibrillar protein content could attribute a

significant level of textural integrity and toughness to the climbing perch fish muscle.

### Discussion

Influence of seasonal variation on proximate composition of fish and fishery products are equally important as the nutritional changes that occur in each component during various processing techniques used. The proximate composition of Koi (*Anabas testudineus*) collected from a rice field from Mymensingh, Bangladesh was analyzed and the mean content of moisture, protein, lipid and ash of raw fish in wet basis was found to be 70.07, 16.97, 13.01, and 0.95%, respectively<sup>10</sup>. Also, the proximate composition in *Anabas testudineus* muscle in Bangladesh and the mean content were found to be 70.26, 18.05, 8.64 and 1.30% for moisture, protein, fat and ash content respectively<sup>11</sup>. Nargis<sup>12</sup> noticed that in *Anabas testudineus* the protein content was higher in medium sized fishes and gradually decreased with the increase of age. Further, it reported that the larger-sized males had higher lipid content than females.

The analysis of results reveal that highest protein content was observed during summer season (March to April), which is in line with previous reports<sup>13,14</sup>. During the winter (January), there was a slight decrease in protein content possibly due to lack of feed, which led to low growth. Increasing the amount of food in February and March can be beneficial in creating a protein store of energy that can be useful for gonadal maturation. During the late summer and early rainy season (July), the protein content in the muscles gradually began to decrease, and this may be due to the translocation of protein into the ovaries to meet its energy needs of the fish for egg formation. The spawning peak in perch is compatible with peak rainfall, *ie.*, from June to July. Changes in protein content during the spawning period were related to changes in the endocrine system, which monitored the supply of nutrients to the gonads from all parts of the body, including the liver and muscles<sup>15</sup>. When the fish is mature, the proteins accumulate in the gonads and during the process they are disguised as eggs or milt, which carry the protein along with the eggs, resulting in a reduction of the protein level. Over time, the protein content increased significantly due to the restoration of fish to the normal life.

The maximum value of lipid content in muscles was observed during the summer season (April). The second highest lipid value was also observed in the post-monsoon season. In the case of *Euthynnus affinis*, a high average fat concentration was found in the postseason

(November)<sup>14</sup>. Consequently, the highest fat content was recorded in September in males and females of the golden mullet *Liza aurata*<sup>16</sup>. This could be due to optimal food availability and active feeding. Post monsoon is marked by algal blooms and plankton peaks<sup>17</sup>. A sharp decrease in lipid content was observed at the start of the monsoon season, and this season is marked as the spawning season. This may be due to the use of stored lipids as an energy source to compensate for the high energy requirements during ovulation and spawning. The decrease in fat content may be related to the low intensity of the diet and the low availability of the food *Paratelphusa masoniana*<sup>18</sup>. Several studies have discussed about a good reduction in muscle lipids for gonad development and maturation<sup>19-23</sup>. A rapid decrease in the lipid content of *Sillago sihama* (Forsskal) has been noted during spawning<sup>24</sup>. The lipid content of fish is highly variable and is associated with food consumption, migratory swimming, or sexual changes associated with spawning<sup>25</sup>.

The seasonal variation of various biochemical components in fish tissue depend on the cycles of maturation and depletion of food and gonads<sup>26-28</sup>. The gonadosomatic index (GSI) values, which correlate well with high levels of protein and lipids in the pre-breeding and breeding stage of the three major carps, probably require higher levels of lipoprotein in the sperm and spermatogenesis in the ovary<sup>29</sup>. Protein content decreased with age and fat content increased accordingly, but other factors such as Cu, Zn and Fe had no effect. They reported a negative correlation between protein and lipid levels with age and size<sup>30</sup>. Ironically, as the protein increased with age/size, the lipids decreased<sup>4</sup>.

The average water content of perch muscle tissue reached its maximum in July. Moisture content was also shown to be inversely related to the fat and protein content of perch muscle tissue. The opposite relationship may be due to lower atmospheric temperature, lower food intake, decreased food availability, and increased energy demand for homeostasis the body temperature during monsoon and winter season. A similar inverse relationship was reported in Bib (*Trisopterus luscus* L.) (Pisces, Gadidae) and anchovy (*Engraulis encrasicolus* L.)<sup>31,32</sup>.

Ash content showed no significant correlation with other component in the perch muscle tissue, proving there is no any direct relationship between the ash with protein, lipid and moisture content in response to their feeding or spawning activities. On the contrary, an increase in the ash content was reported in the female crabs during the post spawning period<sup>18</sup>.

## Conclusion

The results suggest that the proximate composition in Climbing Perch muscle tissue significantly varies during different seasons. High protein and lipid content were detected during non-spawning period and minimum during spawning months, which was inversely related to the moisture content.

## Conflict of interest

The authors declares no conflict of interest

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