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Seasonal changes in proximate composition and textural attributes of farm raised chocolate mahseer (*Neolissochilus hexagonolepis*)

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Abstract
Neolissochilus hexagonolepis, a potential candidate species for aquaculture, have suffered from severe declines. The year round harvested fresh fish samples were graded into small (<50 g), medium (51-150 g) and large fish (>150 g) and sampled according to the stages i.e. I (Sept-Dec), II (Jan-Feb), III (March-May) and IV (June-Aug). Analysis of seasonal variation showed that small fish dominated in terms of moisture content (80.08±0.69%) and hardness values (3277.73±101.03g) while the large fish contains highest fat (8.18±0.58%) and ash; highest protein content (20.15±0.65%) was seen in the medium size group. Though the medium fish had properties deeming to be presented to the consumers in its expected state, the breaking down of the fish flesh while eating being same within the groups due to insignificant (p>0.05) values of springiness ranging 0.86±0.00 and 0.88±0.03 for the samples is also a remarkable finding of the study.

Keywords: Cold water fish, *Neolissochilus hexagonolepis,* hardness, size groups, seasonal stages

Introduction
The composition of major groups of species of farmed aquatic animals varies greatly across the world. Finfish farming is the most important type of aquaculture operation in volume terms on all continents. In 2015, finfish farming accounted for 67.8% of total aquaculture output of aquatic animals. Aquaculture has made steadily increasing contribution to the world fish supply. Within 15 years in the new millennium, the aquaculture contribution to the world production of aquatic animals (captured and farmed combined) has increased from 25.7% in 2000 to 45.3% in 2015 [1]. Mahseers were considered as carnivorous and slow growing and thus, unsuitable for fish culture. However, a careful study of the feeding habits of mahseer indicating their omnivorous nature has dispelled the notion that mahseer is carnivorous. Studies on the anatomical adaptations of the alimentary canal system also confirm that mahseer is omnivorous. Tripathi [2] suggested the inclusion of mahseer in polyculture, cage culture and for river ranching and has stated that mahseer would not compete with mrigal (*Cirrhinus mrigala*) and the common carp. Despite their abundance at one time, the mahseer population has been declining in number and size in natural waters and is in danger of extinction. Environmental stresses coupled with the increase in fishing activity, destruction of brood fishes and juveniles during monsoon, low fecundity and higher predation during its longer hatching and semi-quiescent periods have reduced the abundance and availability of natural stock of mahseers to a very low level [3]. The adverse effect of the river valley projects was extensively surveyed and detailed biological investigation on this alarming situation was presented [4]. Protein, fat and water content of fish is critical to consumers, scientists and manufacturers from many aspects including nutritional value, seasonal variations and considerations regarding processing [5]. Commonly Mahseer is the name used for the genera *Tor*, *Neolissochilus*, and *Naziritor* in the family Cyprinidae [6]. Mahseer fetches a high market price, and are potential candidate species for aquaculture [4]. Several of the bigger species have suffered severe declines, and are now considered threatened due to pollution, habitat loss, and overfishing.
Neolissochilus hexagonolepis (Family-Cyprinidae) is locally known as “Katli” in Kalimpong and it is commonly known as Chocolate Mahseer. The Darjeeling Himalaya has a pronounced seasonal climate and lies north of the tropical belt between 27°13’05”–26°17’10”N and 88°53’00”–87°59’30”E. The spring-fed torrential hill streams of the region represent a unique

lotic water ecosystem characterized by rocky and gravely bottoms, high transparency, low temperature, high oxygen level and water velocity, complex flood regimes and seasonal variations in volume of flow [7]. In Kalimpong, fish intake is increasing day by day. The demand of the fish is either fulfilled by the fishes coming from Andhra Pradesh or Kolkata. Thus, the need for the local fresh fish is always high which is unfortunately not met in the region due to setbacks relating to the aquaculture practices. The hindrance is mostly due to the unavailability of the area for aquaculture and the inadequate knowledge among the people of the region relating to its vast scope. The fish "Katli" is a common game fish in the region and are caught using various traps, angling, electricity, nets and sometimes even poisoning and are even highly esteemed food fish. Flesh is considered delicious, and market demand is great. One ray of light for the demand of the local fish related matter is that the fish "Katli" is introduced into the aquaculture system in the region. Though the culture practice is just in an infant stage, still it awaits a great future ahead of it. The culture is not only crucial for the region but also can be marketed to the areas where the fish is considered as a delicacy. This inclusion relates to the current work of analysis of the proximate composition of the fish. The seasonal variation study of the proximate composition will also enlighten about the correct time for the harvest of the species after it has been introduced in the system. Now since the fish has high market value and is considered as a delicacy, its marketing, and preservation during this period is also an equally important aspect of the holistic approach. Thus, the objective of the study is to determine the seasonal variation of nutrient profiles by analyzing the proximate composition as well as variation in textural profile parameters of *Neolissochilus hexagonolepis* cultured in farms of Kalimpong, West Bengal.

Materials and Methods

Experimental Design

For the study, the whole year was split into four seasons/stages based on post-monsoon, winter, pre-monsoon and monsoon as in Darjeeling Himalayan region, the winter and monsoon seasons being the longest. Thus, the sampling was done according to these stages or prominent seasons i.e. Stage I (Sept-Dec), Stage II (Jan-Feb), Stage III (March-May) and Stage IV (June-Aug). Fresh fish samples were randomly harvested year-round from the farms in Kalimpong district and graded into three size grades viz., small (<50 g), medium (51-150 g) and large fish (>150 g). After weighing the fishes were gutted and gilled. The fishes were then kept in ice boxes and transported to the laboratory of Fish Processing Technology, Faculty of Fishery Sciences, WBUAFS, Kolkata. The fishes were instantly deiced on arrival at the laboratory and assessments were carried out on these fishes. Five fishes belonging to each size grade were harvested every month evaluated for every parameter in triplicate.

Chemical composition or proximate composition of the fresh fish muscles was determined. Moisture of the experimental samples was measured by Moisture Balance (Precisa, Dietikon, Switzerland). Total nitrogen was estimated by Kjeldahl method [8]. Crude protein value was calculated by multiplying the total nitrogen value by a factor of 6.25. Estimation of total lipid was done by the method described by Bligh and Dyer [9]. The ash content was measured by the method of AOAC [8]. All the results were expressed on wet weight basis.

Textural Profile Analysis (TPA) of fish flesh was performed at ambient temperature with TA-XT plus texture analyzer (Stable Micro System, Surrey, UK) and a 50 kg load cell. The attributes evaluated was hardness, springiness, cohesiveness, resilience, chewiness, and gumminess. Gel preparation and analysis were carried out by the method as described by Rawdkuen *et al.* [10] with minor modifications. Gels were cut in cylinders of 18 mm diameter x 18 mm length and was compressed vertically in two consecutive cycles of 50% compression, 5 seconds apart using a flat plunger (SMS-P/75) and a heavy-duty platform. This test was done according to the specifications used by Mao *et al.* [11]. The adopted test settings for this experiment were pre-test speed of 1.5 mm/second, test speed of 0.5 mm/second, post-test speed of 1.5 mm/second, strain at 50% compression, interval (time) of 5 seconds, Trigger type was auto (Force) and trigger force was 25 gm.

Statistical Analysis

All the proximate analyses data were checked for normal distributions with normality plots prior to one-way analysis of variance (ANOVA) using statistical tools of Microsoft Excel and R software. For the texture profile parameters values, a two-way ANOVA with replication was carried out followed by Tukey's HSD. Significant level (p) of 0.05 was used for all analyses. Data are presented as mean ± standard deviation (SD).

Results and Discussion

The proximate composition of fish species greatly varies due to physiological reasons and changes in environmental conditions, i.e., spawning, migration, and starvation or heavy feeding. Species-specific physiological characteristics might greatly affect proximate composition [12]. According to Huss [13], the chemical composition of fish varies depending on species and even within the same species, based on the starvation and intensive food intake periods. Water temperature and salinity are also known to influence the chemical composition [14]. The sampled Chocolate Mahseer belonging to the small size group (<50 g) was recorded to have a consistently higher moisture content ($p>0.05$) during stages II, III, IV as compared to larger sized fish (Figure 1). The highest moisture content was observed at stage II (80.08±0.69%) for <50 g fishes. It is clear from the values of the moisture content that during stages II, III and IV prominently low values were recorded for the large fishes (>150 g). According to Huss [13], percentage of moisture and fat generally constitute 80% of the fillet. Boran and Karacam [12] opined that lipid and moisture contents are inversely related. A similar observation is reflected in the present study with the large size group (>150 g) fishes exhibiting corresponding high fat content during the different season. Higher moisture content was observed in small size groups for the maximum duration over the entire study period which may be due to the fact that, unlike larger size groups, there is the lesser scope of fat development due to insufficient maturity/age. The values of moisture content are reasonably consistent with the values recorded for several other fish species like sardine (70.79-78.16%) [15].

In the present study, the protein content of the medium (51-150 g) and large size (>150 g) fishes were found to be highest during stage IV (June-August). The highest protein content (20.15±0.65%) was seen in the middle-sized group (51-150 g) as compared to that of the larger group (>150 g) and the smaller group (<50 g) with values ranging from 18.28±0.42% and 17.44±0.56% respectively (Figure 2). The variations in protein content among the seasons were not significant (p>0.05) but higher values during stage IV for both medium and larger groups may be attributed to the monsoon season, which gradually decreased during post-monsoon (stage I) and spent/winter season (stage II). Similar season wise variation of proteins of chocolate mahseer was reported by [16], who recorded the highest protein content during the post-breeding season and lowest during the spent season. Love [17], also reported that amino acid related to depletion in protein at the end of the spawning time to show the selection of materials for building up the gonads for further reproduction. The findings of the present study for protein content have been similar to that of Devi and Sarojnalini [18] for chocolate mahseer stating that protein content was 18.51±0.09% and 24.64±0.07%, in 14.2 cm and 15.7 cm long fish respectively. Protein content is said to be high when it is greater than 15% [15]. Hence it may be concluded that *N. hexagonolepis* is rich in protein content and can be an excellent source of protein for human diet. The highest fat content was recorded for Chocolate Mahseer >150 g size consistently over all stages compared to other size groups (p<0.05) (Figure 3). The fat content for >150 g size group was found to be 8.18±0.58%, 7.38±0.27%, 7.58±0.39%, and 7.98±0.48% for stages I, II, III and IV respectively. The small-sized fishes (<50 g) was found to contain least (p<0.05) fat ranging from 5.31±0.28% to 5.63±1.75% among all size groups. The variation in fat content (p>0.05) within the size groups may be attributed to the season of harvest, food availability, and sexual maturity. High-fat content for large group in stage IV (June-August) and stage I (Sept-Dec) coincides with the reports of Jyrwa & Bhuyan [16] and Deka *et al.* [19]. Jyrwa and Bhuyan [16] showed an increase in fat content of *N. hexagonolepis* during the breeding season. The lower fat content during stage II and stage III may be because fishes tend to reduce their feed intake during sexual maturation. As a result, essential fatty acid and other nutrients needed for ovarian growth are taken from the reserves in their body. On the contrary, the medium-sized fishes showed an increase in fat content in stage II which coincides with the winter season in the area. It has been reported that some fishes tend to increase their lipid concentration to survive in low temperature [15]. The changes in the lipid concentration within the groups may be due to the various level of breeding activity and the quality of feed that the fishes were introduced with at different farms. The mineral content of a fish is indicated by its ash content. Ash may be defined as the residue that lacks water and volatile constituents containing carbon dioxide, oxides of nitrogen, etc. The highest ash content was seen in the post-breeding (September to December) stage and the spent phase (January to February) for all the size groups (Figure 4). The highest ash content recorded was 1.13± 0.10% in large fish and 0.83 ± 0.04% in the medium-sized fish. Azim *et al.* [20] stated that the value of ash in *Sillaginopsis panijus* in December, April and July was estimated as 15.64±0.03%, 11.50±0.09% and 14.13±0.06% in dry weight basis which supports the findings of the present study that highest ash content was observed in the post-breeding stage.

Texture Profile Analysis, for determining the textural properties of foods, is a famous double compression test, quantifying multiple textural parameters in just one experiment. The highest force that occurs during the first compression is the hardness value. In the present study, the hardness values increased in stage II and then consequently decreased for during remaining stages with the exception of small-sized fishes. The <50 g fish gel had higher hardness values followed by gels of 51-150 g and >150 g fish. Hardness value for small fish was found to be the highest reaching a force of 3277.73±101.03 g during stage III and lowest was for the larger group with a value of 2593.92±221.56 g during stage I. The changes in hardness values were found to be significant (p<0.05) within the various stages and amongst the groups. Hatae *et al.* [21] reported a similar toughness in the abalone meat in winter and lowest collagen content in summer leading to tenderness in meat. According to Gökoğlu and Yerlikaya [22], fish that are spent after spawning have low reserves of energy and are in a poorer physical condition which shortens the time taken to go into rigor. They also stated that small fishes usually go into rigor faster than larger species of the same species. Upon rigor onset, muscle elasticity decreases, and at its completion, the tissue reaches its maximum toughness [23]. This might be the plausible reason for an increase in the hardness values in stage II which coincides with the spent phase of the species. It also provides a base for concluding the fact that small fishes (<50 g) had the highest hardness values as they might have entered the rigor the fastest amongst the size groups.

The cohesiveness indicates the strength of internal bonds making up the body of food and the degree to which food can be deformed before it ruptures [24]. Cohesiveness, being defined as the ratio of the positive force area during the second compression to that of the first compression, can be measured as the rate at which the material is disintegrated under mechanical action. The way to experience cohesion is the energy it takes to break down the product until it is palatable to be swallowed in case of food. Stronger the cohesion, more tolerant the product is towards manufacturing, packaging and delivery stresses and hence overall presented to the consumers in its expected state [25]. In the present study, the cohesiveness values (Table 1) were significantly (p<0.05) higher in the medium-sized groups deeming it to be presented to the consumers in its expected state. Minimum cohesiveness values (0.41±0.02 and 0.41±0.03) were obtained for gels from small and large fishes in stages IV and II respectively. A highly significant (p<0.05) difference was seen in cohesiveness values amongst the groups, during various stages. Though thoroughly chewed foods generally do not have sufficient structural integrity to spring back, TPA was designed to emulate the sensory chewing experience. Naturally enough lower springiness values will be exhibited for a destroyed product. In instances, where the objective is to evaluate the relative spring back of different formulations, it is recommended to have less destructive compressive distances so that the product retains enough geometric stability to exhibit relative differences [25]. The non-significant (p>0.05) differences in springiness values (Table 1) between the size grades and even so during the various stages of the samples implies that the breaking down of the fish flesh while eating had similar properties within the groups. The product of hardness and cohesiveness is defined as Gumminess. Gumminess is of more important textural parameter of semisolids than solids like gelatin. Chewiness is a measure of energy that is required to masticate the food and is reported for solid foods. Chewiness is defined as the product of gumminess and springiness which is equal to product of hardness x cohesiveness x springiness. Precise measurement of Chewiness is the most difficult to measure because mastication involves compressing, shearing, piercing, grinding, tearing and cutting along with adequate lubrication by saliva at body temperatures [26]. Resilience is a measurement of how the sample recovers from deformation both in terms of speed and force [27], i.e., the elastic recovery of the sample. From the Table 1, it can be seen that higher gumminess, resilience, and chewiness value were found in medium fish during stage I and lower values were found in large fish during stage II. The higher gumminess has arisen from higher hardness value. The chewiness value of samples varied from 1489.04±199.01 g and 1027.28±135.35 g for medium and large fish during stages I and II respectively. The interaction between the variables concerning the parameters were found insignificant ($p>0.05$), i.e., the changes in parameters during various stages were not affected by the size grade of the species.

Table 1: Changes in texture parameters of chocolate mahseer gel sampled at different stages of the year

Size groups	Stage I	Stage II	Stage III	Stage IV
TPA- Hardness (g)				
<50 g	2675.17±425.61^{a1}	3055.09±545.10^{a1}	3277.73±101.03^{a1}	3143.29±155.9^{a1}
51-150 g	2857.02±78.54^{a1}	2894.74±180.05^{a1}	2825.86±102.25^{b1}	2806.21±116.69^{b1}
>150 g	2593.92±221.56^{a1}	2891.08±183.09^{a1}	2810.41±193.03^{b1}	2787.41±224.17^{b1}
TPA- Springiness				
<50 g	0.87±0.02^{a1}	0.86±0.01^{a1}	0.86±0.00^{a1}	0.86±0.00^{a1}
51-150 g	0.87±0.02^{a1}	0.86±0.03^{a1}	0.87±0.01^{a1}	0.87±0.01^{a1}
>150 g	0.88±0.03^{a1}	0.86±0.01^{a1}	0.86±0.01^{a1}	0.87±0.01^{a1}
TPA- Cohesiveness				
<50 g	0.46±0.07^{b2}	0.45±0.03^{b12}	0.38±0.04^{c1}	0.41±0.02^{c12}
51-150 g	0.60±0.06^{a1}	0.56±0.06^{a1}	0.57±0.05^{a1}	0.57±0.05^{a1}
>150 g	0.50±0.09^{ab1}	0.41±0.03^{b1}	0.46±0.03^{b1}	0.49±0.04^{b1}
TPA- Resilience				
<50 g	0.13±0.05^{b2}	0.13±0.03^{b12}	0.08±0.03^{c1}	0.1±0.02^{c12}
51-150 g	0.23±0.04^{a1}	0.2±0.04^{a1}	0.21±0.03^{a1}	0.21±0.03^{a1}
>150 g	0.16±0.06^{ab1}	0.1±0.02^{b1}	0.13±0.02^{b1}	0.15±0.03^{b1}
TPA- Gumminess				
<50 g	1219.66±133.70^{b1}	1354.65±193.94^{ab1}	1235.35±130.93^{b1}	1295.93±106.47^{b1}
51-150 g	1718.39±201.20^{a1}	1565.98±224.07^{a1}	1613.69±178.43^{a1}	1585.28±131.71^{a1}
>150 g	1290.57±183.28^{b1}	1194.44±150.39^{b1}	1285.95±114.97^{b1}	1354.36±47.79^{b1}
TPA- Chewiness				
<50 g	1059.84±118.63^{b1}	1171.81±173.82^{ab1}	1064.1±117.84^{b1}	1118.62±95.83^{b1}
51-150 g	1489.04±199.01^{a1}	1335.47±226.41^{a1}	1400.51±165.91^{a1}	1385.76±108.85^{a1}
>150 g	1133.83±177.81^{b1}	1027.28±135.35^{b1}	1109.64±103.47^{b1}	1178.52±52.96^{b1}

Values are mean of five (n=5) determination with s.d.
Values with different alphabetical superscripts within a column are significantly ($p<0.05$) different
Values with different numerical superscripts within a row are significantly ($p<0.05$) different

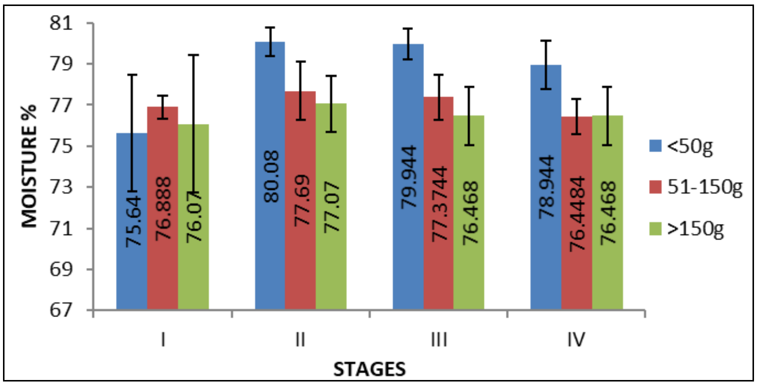

Fig 1: Moisture content of different size groups of chocolate mahseer during different stages.

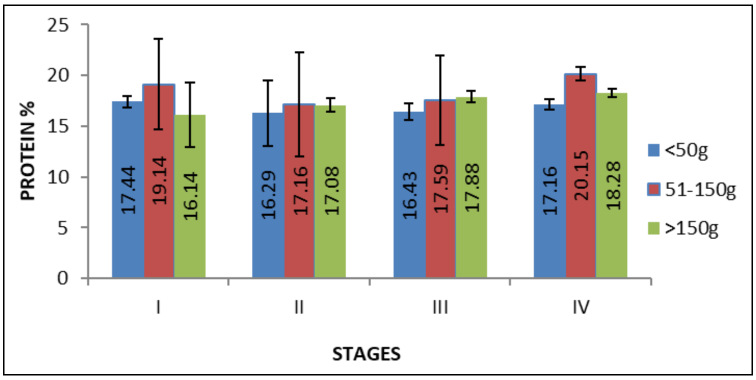

Fig 2: Protein content of different size groups of chocolate mahseer during different stages

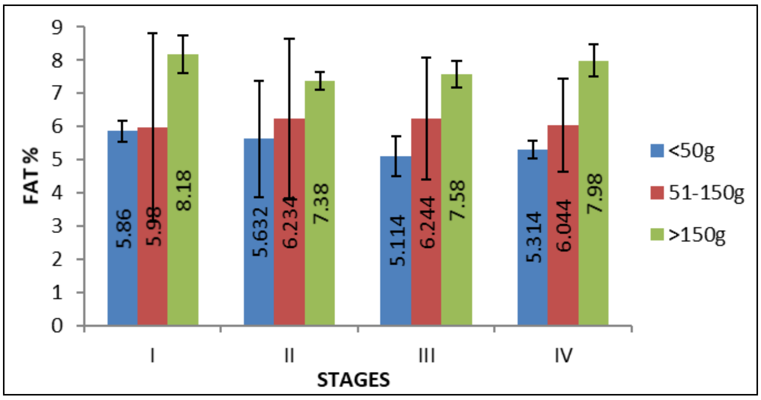

Fig 3: Fat content of different size groups of chocolate mahseer during different stages

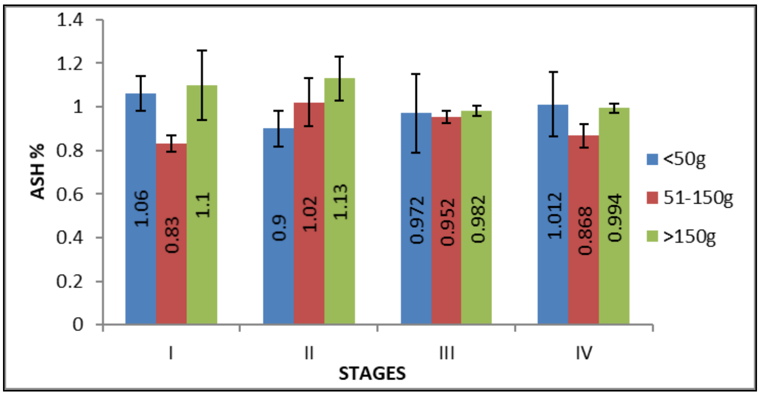

Fig 4: Ash content of different size groups of chocolate mahseer during different stages.

*Results are mean of three determinations (n=3) with s.d.
Values of moisture% vary non-significantly ($p>0.05$) between the stages & within the size group

Conclusion

After the thorough study of assessing the seasonal variation in proximate composition and the instrumental textural attributes of flesh of chocolate mahseer, *Neolissochilus hexagonolepis* popularly known as 'Katli' in Darjeeling and Kalimpong, it may be concluded that chocolate mahseer (*Neolissochilus hexagonolepis*) are very rich source of protein and ideal size group for harvest is above 50 g. Throughout the year harvesting is also possible as the protein content is fairly consistent year round with the highest protein content during the post breeding season (Sept-Dec). The small fishes (<50 g) had the highest hardness values than the groups above 50 g throughout the study period, whereas the cohesiveness values were significantly ($p<0.05$) higher in the medium-sized groups deeming it to be presented to the consumers in its expected state along with the deduced fact that the breaking down of the fish flesh while eating within the groups due to insignificant ($p>0.05$) values of springiness for the samples.

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