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Analysis of biochemical and nutritional constituents of different size groups of *Macrobrachium malcolmsonii* (Milne-Edwards, 1844) (Decapoda: Palaemonidae) for the identification of its nutritional requirements

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Abstract. Prawns have been contributing almost 15% by value in global aquaculture production. In this study, different size groups (4-19 cm) of fresh water prawn, *Macrobrachium malcolmsonii* (Milne-Edwards, 1844) (Decapoda: Palaemonidae) were collected from the Cauvery River, Tamil Nadu, India, and estimated to identify the protein (P), lipid (L) and carbohydrate (CHO) level. The adult prawns exhibited highest values 61.63% P, 6.95% L and 5.40% CHO. The gas chromatography of small size groups of prawns showed increased amount in various fatty acids such as palmitic acid, stearic acid etc. The tissues result determined by high performance liquid chromatography showed that the amino acids such as Aspartic acid, Lysine etc., are high in adult groups. The protein profile of the muscle samples displayed various polypeptides ranging from 200 to 20 kDa. Results of this study clearly implied the biochemical and nutritional constituents of freshwater prawn, *M. malcolmsonii*. It would be useful to design the required composition of feed in order to get higher yield at lower cost production.

Keywords: *Macrobrachium malcolmsonii*; SDS-PAGE; HPLC; Fatty acid profile; Gas chromatography.

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Introduction

Aquaculture is the unique and proven technology to produce proteinaceous animal food for the teeming world population. Prawn constitutes an important aquatic resource for human consumption and its commercial exploitation is on the increase. The FAO (2012) report indicated that the contribution of freshwater aquaculture production has gradually increased up to 65.6% in 2010 from around 60% in the 1990s. Asian aquaculture is predominated by crustaceans (9.7%) and others as well. The contribution of China to the world aquaculture production volume in 2010 was 61.4%. Other major producers in Asia (India, Viet Nam, Indonesia, Bangladesh, Thailand, Myanmar, the Philippines, and Japan) are among the world's top producers. In India, fish culture has gained phenomenal significance in recent years. The culture of Penaeid prawns in coastal farms has received considerable attention in many countries due to their high demand and price in markets world over. The freshwater prawns of the genus *Macrobrachium* are the most suitable species for aquaculture in many parts of the world. The *Macrobrachium* species are distributed throughout the tropical and subtropical zones (Laxmappa and Savalla, 2015). The common species of freshwater prawns available in India are the giant freshwater prawn *Macrobrachium rosenbergii*, *M. malcolmsonii*, *M. idae*, *M. nobilii*, *M. rudae*, *M. lamarrei lamarrei*. Among these species, *M. rosenbergii* and *M. malcolmsonii* are the larger sized fast growing species, widely considered for soft meat production. Both *M. rosenbergii* and *M. malcolmsonii* command the same price in export market (New, 2002). Their good characteristics such as the fast growth rate, high tolerance to a wide range of hydrological parameters and less cannibalistic tendency makes them the appropriate species for aquaculture. *M. malcolmsonii* is widely distributed throughout India, especially in the rivers draining into the Bay of Bengal. This species grows fast and has good taste (Kanaujia et al., 1997).

Various endogenous and exogenous factors play a dynamic role in the composition of crustaceans. To better understand nutritional requirements of *M. malcolmsonii*, the biochemical composition of their body tissues at different growing stages and its variation in relation to the environment will be very useful. The protein, lipid, and carbohydrate levels of prawn tissue would indicate the basic energy-giving nutrient profiles. The ash content of the body suggests the mineral needs of prawns. Furthermore, a probe into the amino acid and fatty acid profiles will lead us to understand the more specific and balanced nourishment to optimize the requirement of the prawns and improve their healthy growth.

The purpose of this study was to obtain information on protein, amino acids, and fatty acids composition of muscle tissues in fresh water prawn *M. malcolmsonii* during different growth stages, which may be helpful in better understanding the dietary requirements of *M. malcolmsonii*.

Materials and methods

The different size of *M. malcolmsonii* were collected from the River Cauvery at lower Anicut (latitude 11º 15' N; longitude 79º 30' E) near Kumbakonam, Tamil Nadu, India. The prawns were brought in oxygenated polyethylene bags to the laboratory and maintained in 50 L, well-aerated plastic tanks. The prawns were separated based on its size, group of juveniles (up to 5 cm), sub adult groups (5-12 cm) and adult groups (above 12 cm). The different size groups of prawns used in this study are presented in Table 1.

The prawns (n = 10) from each stage of I to VI, were sacrificed and the muscles were segregated. Collected tissues were pooled, weighed separately and used for the analysis of individual parameters protein (P), lipid (L), carbohydrate (CHO), fatty acid (FA), and amino acid (AA).

Table 1. Different size groups of prawns used for biochemical studies.

Size groups	Length groups (cm)	Mean length (cm)	Mean weight (g)
I	4.0-4.9	4.26±0.251	0.7229±0.041
II	5.0-7.0	5.83±0.305	1.7006±0.250
III	7.1-11.0	7.43±0.404	3.6342±0.433
IV	11.1-12.0	11.33±0.251	14.7179±0.677
V	12.1-14.0	12.43±0.404	21.9579±2.652
VI	17.0-19.0	17.76±0.642	68.8674±7.865

Protein estimation

Total protein content in the muscle tissue was estimated by Lowry et al. (1951). Samples were ground with 10% TCA. Homogenate was centrifuged at 3,000 rpm for 15 min. The precipitate was taken in a test tube and reagents were added and left incubation for 20 min in dark. The developed blue color was measured at 720 nm in UV-Visible Spectrophotometer. Bovine serum albumin (BSA) was used as standard. The protein concentration was expressed in mg/g of muscle tissue.

Lipid estimation

The concentration of total lipids from the prawn tissues was evaluated by gravimetric method (Folch et al., 1957). The tissues were extracted using chloroform and methanol (2:1). The extract was filtered and the extraction procedure was repeated to ensure maximum removal of lipids and vortexed thoroughly to remove water soluble impurities. The moisture present in the lipid was removed by addition of anhydrous sodium sulphate crystals. The filtrate was dried by keeping it in an oven at 60 ºC. The final weight and initial weight were taken for calculation to estimate the lipid. The values were expressed as mg/g of the sample.

Carbohydrate estimation

Total carbohydrate content of the muscle was measured by Anthrone method (Dubois et al., 1956). The tissues were homogenized with TCA and centrifuged at 4000 rpm for 15 min. Anthrone reagent was added and placed in boiling water bath for 10 min. Then it was cooled in dark at room temperature for 30 min. The optical density (OD) was measured at 620 nm in UV-Visible Spectrophotometer. The amount of carbohydrate present in the sample was found out using glucose as standard.

Electrophoretic analysis of protein

The muscle tissue was homogenized with refrigerated phosphate buffer (50 mM; pH 7.2) in the ratio of 1:5. The homogenate was then centrifuged at 11,200 g/min for 20 min at 5 ºC. The supernatant was separated and stored at -80 ºC until analysis. Equal amount of protein (120 µg) was loaded for separation. Muscle samples were subjected to linear SDS-PAGE using 6% stacking gel and 10% separating gel (Laemmli, 1970). The gels were stained with 0.2% coomassie brilliant blue R-250 in 50% methanol and 7% acetic acid for 6 h. The gel was then de-stained with 30% methanol and 7% acetic acid solution until the background was clear.

Amino acid analysis

The tissue samples were analyzed for amino acid composition using High Performance Liquid Chromatography (HPLC). The filtered derivative amino acid sample was injected into the C-18 reverse phase column at the temperature of 40 ºC and analyzed using sodium acetate buffer and acetonitrile as solvent. The samples were compared with a chromatogram of standard amino acids.

Fatty acid profile

Lipids were extracted from prawns and fatty acids were saponified and methylated. From each vial, the fatty acid methyl ester (FAME) was injected into the Gas Chromatography (GC) column (Miller and Berger, 1985) fused with DEGS

(Diethylene Glycol Succinate) column equipped with FID (Flame Ionization Detector). The sample was injected at 200 ºC with nitrogen as the carrier gas. The column was operated isothermally at oven temperature of 180 ºC and a detector temperature of 210 ºC. The individual fatty acids were identified and quantified by using the FAME standard under similar conditions. The results were expressed in mg/g of lipid sample.

Results

The biochemical compositions of body muscles in different size groups of *M. malcolmsonii* are presented in Figure 1. The higher amount of protein was found in the size group of VI (61.63%) and very lower amount was observed in the size group of V (50.83%). Lipid content showed wide fluctuations among the size groups. The higher amount of lipid was present in the size group of VI (6.95%) and lower amount was noticed in the size group of II (4.93%). The highest amount of ash content was present in the size group VI (17.28%) and lowest amount in size group I (8.05%). The ash content gradually increased from group I to group VI (juveniles to adult prawns). The moisture content also showed increasing values from group I to group VI. The higher amount of moisture in group VI (83.57%) and lower amount in group I (70.21%) were recorded. Carbohydrate was maximum in the size group V (5.40) and minimum in the size group I (2.85%).

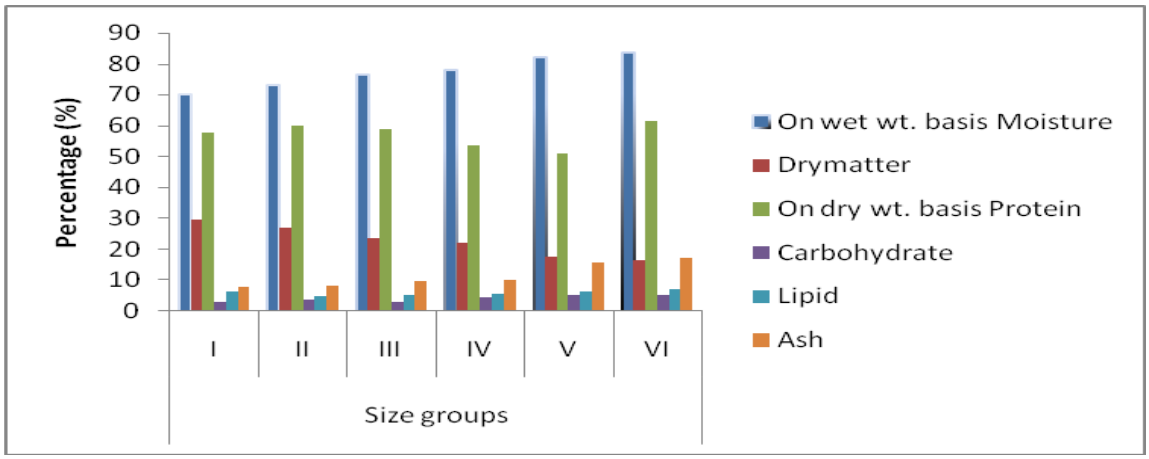

Figure 1. Biochemical constituents of muscle tissues in different size groups of *Macrobrachium malcolmsonii*

The SDS-PAGE (10%) for muscle showed several polypeptides between 200 and 20 kDa (Figure 2). The maximum numbers of 16 bands were found in size groups of II-VI and minimum number of 12 bands were recorded in size group of I. In group I, the 170, 140, 80, 30 and 25 kDa proteins were found to be thick and therefore deeply stained. In other groups, the staining intensity of 170, 140, 30, 25 and 20 kDa polypeptides were found to be thinner when compared to group I. However, the band size of 80 kDa protein in group II-VI was found to be more in comparison to that of group I. The 200 and 90 kDa proteins were found to be absent in group I, when noted with the other groups. The 70 kDa protein was found to be presented in group I and group II only.

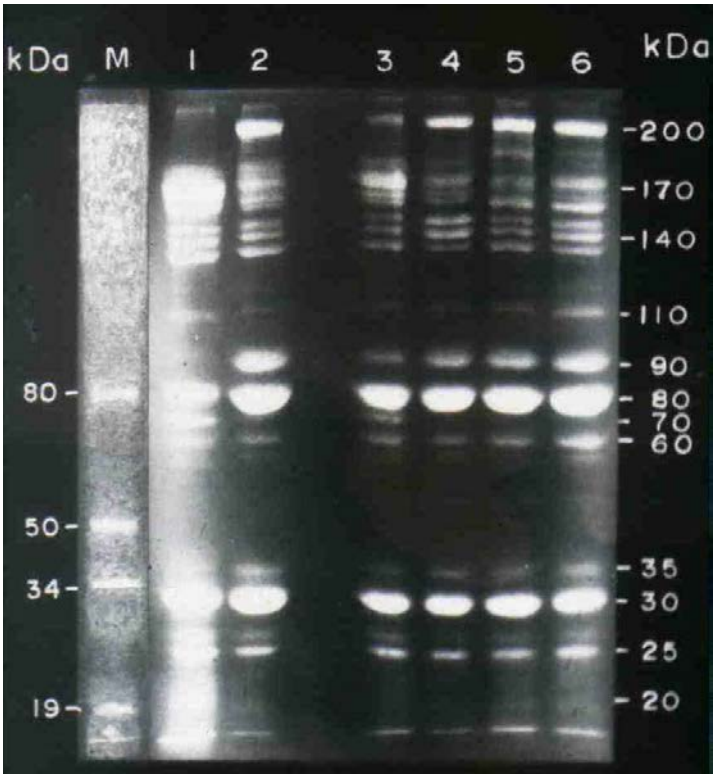

Figure 2. Protein profile of the muscles of *Macrobrachium malcolmsonii* (M-Marker; 1 to 6-Different size groups).

The amino acid compositions of the muscles are presented in Table 2. The essential amino acids arginine, lysine, and threonine are presented in higher amounts in all size groups. The higher amount of lysine noted in the group VI (26.09%) and the lowest amount in the group V (11.18%). The maximum amount of arginine noted in the size group VI (20.40%) and minimum amount in the size group IV (10.19%) and lowest amount in VI group of prawns (5.82%). Leucine ranged from 0.61% to 4.93% in group VI to group I. The high amount of phenylalanine is present in the II group (14.45%) and low amount in VI group of prawns (1.00%). Histidine was not found in group I and it is present in the remaining groups of prawns. The aspartic acid was available in all size groups of *M. malcolmsonii*. The glutamic acid occurred in all size groups of prawns. The maximum amount of serine was noted in the VI group (17.53%) and minimum amount in the V group of prawns (1.90%).

Fatty acid composition of different size groups of prawns is presented in Table 3. Myristic acid, pentadecanoic acid, palmitic acid, heptadecanoic acid, stearic acid, and arachidic acid are found almost in all size groups. Cis linoleic acid was found only in the II size group of prawns (0.005 mg/g lipid). The maximum amount of lauric acid was presented in I size group of prawns (0.0312 mg/g lipid) and the minimum amount in the II size group of prawns (0.008 mg/g lipid). The higher amount of heptadecanoic acid was presented in the group II (0.0688 mg/g lipid) and lower amount in the group VI (0.0204 mg/g lipid). The maximum amount of stearic was recorded in group I (0.3724 mg/g lipid) and minimum amount in group II (0.1185 mg/g lipid). The highest amount of nondecanoic acid was noticed in group III (0.1028 mg/g lipid) and lowest amount in group II (0.346 mg/g lipid).

Table 2. Amino acid composition of different size groups of prawn *M. malcolmsonii* (expressed in mole percentage).

Amino acids	Size groups					
	I	II	III	IV	V	VI
Alanine	10.93	7.47	6.10	8.48	6.68	10.23
Arginine	18.51	12.53	11.14	13.97	10.15	20.40
Aspartic acid	8.54	9.13	8.01	9.54	8.73	10.034
Cy2	17.84	7.03	6.52	16.14	11.25	7.60
Glutamic acid	4.53	3.96	4.85	5.01	4.98	5.24
Glycine	1.24	2.15	1.88	2.05	1.68	3.15
Histidine	ND	4.82	4.07	4.80	3.60	4.33
Isoleucine	2.49	1.75	2.06	1.86	2.54	3.13
Leucine	4.93	3.18	1.65	0.77	1.39	0.61
Lysine	18.96	13.97	14.24	11.54	11.18	26.09
Methionine	1.37	11.11	9.17	5.88	ND	ND
Norvaline	ND	ND	ND	ND	ND	ND
Phenylalanine	2.54	14.45	14.62	1.98	12.36	1.00
Serine	3.83	3.15	3.46	2.63	1.90	17.53
Threonine	6.43	8.26	7.28	10.19	7.54	5.82
Tryptophan	ND	6.33	7.43	6.16	14.82	ND
Tyrosine	8.11	7.68	5.89	5.92	ND	ND
Valine	4.05	9.75	8.45	7.64	17.46	1.13

Table 3. Fatty acid composition in the muscle (mg/g of lipid) of different size groups of prawn *M. malcolmsonii*.

Fatty acids		Size groups					
		I	II	III	IV	V	VI
Saturated fatty acids							
Heptanoic acid	C7:0	ND	ND	ND	ND	ND	ND
Caprylic acid	C8:0	ND	ND	ND	ND	ND	ND
Nonanoic acid	C9:0	ND	0.0585	ND	ND	ND	ND
Capric acid	C10:0	ND	ND	ND	ND	ND	ND
Undecanoic acid	C11:0	ND	ND	ND	ND	ND	ND
Lauric acid	C12:0	0.0312	0.0008	0.0157	0.0100	0.0195	0.0187
Tridecanoic acid	C13:0	0.0002	ND	0.0002	ND	ND	ND
Myristic acid	C14:0	0.0588	0.0661	0.0497	0.038	0.0417	0.0419
Pentadecanoic acid	C15:0	0.0121	0.0431	0.0102	0.0008	0.0008	0.0007
Palmitic acid	C16:0	0.3630	0.1644	0.1324	0.1865	0.2249	0.2537
Heptadecanoic acid	C17:0	0.0555	0.0688	0.0418	0.0235	0.0213	0.0204
Stearic acid	C18:0	0.3724	0.1185	0.2966	0.1424	0.1968	0.1978
Nondecanoic acid	C19:0	0.0902	0.0346	0.1028	0.0496	0.0599	0.0610
Arachidic acid	C20:0	0.0723	0.0194	0.0215	0.0168	0.0004	0.0004
Heneicosanoic acid	C21:0	0.0005	ND	ND	0.0005	ND	ND
Behenic acid	C22:0	0.2533	0.8664	0.1800	ND	0.1270	0.1300
Tricosanoic acid	C23:0	ND	ND	ND	ND	ND	ND
Lignoceric acid	C24:0	ND	0.0275	0.0196	0.0815	0.1100	0.1109
Unsaturated fatty acids							
Palmitoleic acid	C16:1	ND	ND	ND	ND	ND	ND
Oleic acid	C18:1	ND	ND	ND	ND	ND	ND
Cis Linoleic acid	C18:2	ND	0.0005	ND	ND	ND	ND
Linolenic acid	C18:3	ND	ND	ND	ND	ND	ND
Arachidonic acid	C20:4	ND	0.0414	ND	ND	ND	ND
Eicosapentaenoic acid	C20:5	ND	ND	ND	ND	ND	ND
Decosahexaenoic acid	C22:6	ND	ND	ND	ND	ND	ND

Discussion

In the current study, higher amount of protein was found in the size group VI and low protein was found in the size group V. From the group III to V the protein level is slightly decreasing. Joshi and Diwan (1996) stated that gradual depletion of muscle protein was noticed with the advancement of maturation. The number of polypeptide bands resolved in subadult (groups II to IV) and adult (groups V and VI) stages were found to be higher when compared to juvenile prawns (group I). This reflects the synthesis of different proteins during growth and maturation. In all life forms, proteins are essential to grow, repair and maintenance of cells (Kaushik and Seiliez 2010; Gamble et al., 2015). Protein syntheses are specific to species and developmental stage and in crustaceans it is a major energy demanding process (Carter and Mente, 2014).

The muscle proteins are referred to as acidic proteins because of the presence of large amounts of aspartic acid and glutamic acid (Shamasunder and Prakash, 1994). The sweetness of the prawn muscle has been attributed to free glycine (Konosu and Yamaguchi, 1982). Cathetic proteases (Pan and Yeh, 1993) and other enzymatic proteins are also constituents of muscle proteins. Nor Faadila et al. (2013) found that in tiger prawn, *Penaeus esculentus* the protein is the main energy source because of changes in lipid and protein during starvation. The dry matter content of *M. malcolmsonii* was noted between 16.34% and 29.79% in different size groups. The ash content gradually increased from group I to group VI.

Generally the ash content denotes the presence of mineral element in the body tissue. The observation of increasing trend of ash content from the size groups I to VI reflects the continuous accumulation of mineral elements in their body tissue. Previous studies found that the changes are noted in the proximate chemical composition, fresh mass, water content, ash content, organic constituents, lipid and protein contents and energy levels of penaeid prawn, *Penaeus monodon* during reproductive stages (Suneetha et al., 2009). Hill et al. (1992) and Correia et al. (2003) reported that the content of total lipid was higher in adults than in juveniles of many crustaceans. During growth and maturation there is an increase in lipid and fatty acid content (Rosa et al., 2005).

Our results are agreed with Alava and Pascual (1987) and Sarac et al. (1993) findings of the dry matter content of whole prawn. In female prawns the polyunsaturated and saturated fatty acids levels were higher than the males (Bhavan et al., 2010). Augusto and Masui (2014) identified that in the Amazon River prawn, *Macrobrachium amazonicum* the male prawns showed accelerated growth than the female and they suggested that the female prawns might have utilized the energy into other pathways such as reproduction. Additionally, Ekpenyong et al. (2013) showed that the mineral elements are distributed in different parts of the prawn disproportionately.

The higher amount of carbohydrate in *M. malcolmsonii* was observed in group VI (5.36%) and lower amount in group I (2.25%). One possible explanation for these finding is that the lipid can be converted rapidly when prawn is growing. This agrees with a study by Wenjuan and Xueliang (1994), who stated that linolenic acid is used rapidly for energy, or converted into higher unsaturated fatty acids and is not retained in the tissues. The fatty acids, linolenic, alpha-linolenic and arachidonic acid are present in low concentrations.

Proteins are digested or hydrolyzed and releases free amino acids, absorbed from intestinal tract and distributed to the organs. Amino acids are used either to form new proteins or replacing the existing proteins for balancing the protein level in the body (Robert, 2002). Farmanfarmaian and Lauterio (1980) reported that, after glutamic acid, and aspartic acid the third major amino acid in the tail muscle of the freshwater prawn, *M. rosenbergii* was arginine. In this work, arginine was found in higher amount in all size groups of *M. malcolmsonii*. Arginine is associated with the flat flavor of the prawns (Weng et al., 1997). It has already been reported that

arginine is involving in growth related processes (Hird, 1986).

As different lipids vary in their fatty acid composition, the nutritive value of prawns depends upon the quality and quantity of polyunsaturated fatty acids. In the present work, the variations in the amino acids and some fatty acids of freshwater prawn, *M. malcolmsonii* may be due to several factors such as age, moulting stage, climatic conditions, and food composition.

Conclusion

The experimental data obtained may have practical utility in understanding the body nutrient levels of *M. malcolmsonii* and also the amino acid and fatty acid profiles of the body tissues. The biochemical and nutritional data of the prawn *M. malcolmsonii* given above might be helpful in understanding the dietary requirements of freshwater prawn. This in turn will help in formulating the balanced diets *vis-a-vis* the nutritional requirements of the prawns.

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Conflict of interest statement

Authors declare that they have no conflict of interests.

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