



Biological parameters, culture potential and nutritional quality of freshwater mussel *Lamellidens marginalis* (Lamarck, 1819)

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ABSTRACT

Lamellidens marginalis (Lamarck, 1819), is a widely distributed mussel species in India and it is an important bivalve that can be used for the production of pearl. In the present study, *L. marginalis* were collected from low lying areas around Patna and analysed for length-weight relationship, proximate composition, amino acid concentration and fatty acid profile. Growth study was also conducted for a period of 160 days to record the performance of this species, when reared at subsurface and bottom of a fish pond. The length-weight relationship was found to be $Y=3.856X-12.81$ and growth coefficient “b” was significantly higher indicating allometric growth pattern. Crude protein, fat, ash and moisture content in the flesh recorded were 7.19, 1.64, 3.70 and 83.92% respectively. Among essential amino acids, lysine was most abundant and low levels of isoleucine was recorded, while among non-essential amino acids, glutamate was dominant. Moreover, 32 fatty acids were recorded in the mussel including omega-3-fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Off-bottom culture in the pond performed better than on-bottom culture. Percentage body weight gain (BWG%) and specific growth rate (SGR%) were also higher for off-bottom grown mussels.

Keywords: Amino acid, Growth, *Lamellidens marginalis*, Length-weight relationship, Protein, Survival

Introduction

Molluscs are filter feeding benthic organisms widely distributed around the world and perform many important functions in aquatic ecosystems. The importance of molluscs in contribution to food security, employment and economy, especially for the poorer segments of the society is well documented (Balian *et al.*, 2008; Strong *et al.*, 2008; Kohler *et al.*, 2012; Ngor *et al.*, 2018). It is also playing an important role in ecosystem services (Ngor *et al.*, 2018; Vaughn, 2018). Though freshwater mussels are not commercially very important as a source of food, these mussels support small-scale fisheries in some parts of India and are potential candidate species for freshwater pearl production (Ramakrishna and Dey, 2007; Thippeswamy *et al.*, 2014). It is also used as a bio-indicator for monitoring the health of aquatic ecosystems because they are extremely sensitive to a wide range of environmental factors including the levels of dissolved oxygen in water (Wayker *et al.*, 2012; Ray *et al.*, 2013; Mundhe *et al.*, 2015; Ramesha and Sophia, 2015). India has more than 50 species of mussels distributed in various freshwater bodies and among them, the genus *Lamellidens* is represented by nine species and two sub-species (Rao, 1989). *Lamellidens marginalis* (Lamarck, 1819), the most commonly available freshwater mussel, is

widely distributed in ponds and derelict water bodies in Bihar and substantially contributing as a source of protein and income for local people. Thus, changes in the abundance and biomass of this species can directly or indirectly influence the ecosystem functioning and the livelihood of local people. It is well recognised that length-weight relationship is an important tool that explains the changes of shell proportions in bivalves found in natural water bodies or from culturing in various environmental conditions; water depth, currents, turbulence, wave exposure, types of bottom and sediments (Akester and Martel, 2000; Lajtner *et al.*, 2004). The length-weight data are also employed in physiological investigations and to obtain estimates of seasonal variations in the growth of individual and whole populations (Gosling, 2003). Though *L. marginalis* is listed under the category of “least concern” by IUCN (Madhyastha *et al.*, 2010), in recent times there is large scale decline in many mussel species, leading to large losses in mussel-provided ecosystem services (Haag and Williams, 2014; Vaughn, 2018). Hence, it is high time to have a detailed study of different biological and nutritional characteristics of *L. marginalis*, especially from eastern states of India. Hence, the present study attempted to develop baseline information on the different biological parameters and nutritional quality of *L. marginalis*. An attempt was also made to evaluate

the comparative growth performance of mussels grown off-bottom and on-bottom in pond conditions.

Materials and methods

Sample collection

In the present investigation, a total of 230 individuals of *L. marginalis* were collected from low lying areas of Patna from April to August 2018 and subjected to morphometric measurements. The collected samples were transported to the laboratory with ice in an insulated box. Total weight of individual mussels was taken after surface cleaning and removing the surface excess moisture. The total shell lengths (maximum antero-posterior distance) of the specimens were measured to the nearest 0.5 mm using Vernier caliper and the mussels were weighted using an electronic balance with 0.10 g accuracy. Samples of the mussels were opened up using stainless steel scalpel blades, removed the fresh meat, which was then blotted and weighed individually. The meats were dried in a hot air oven at a constant temperature of 60°C for 2 days and homogenised in a mechanical blender. Subsequently, the samples were stored in desiccators for analysis of chemical composition.

Length-weight relationship

The length-weight relationships were established using linear regression analysis (least-squares method) proposed by Le Cren (1951):

$$W = aL^b$$

After logarithmic transformation of length-weight data, the equation is expressed as:

$$\text{Log}W = \log a + b \log L$$

where, W is the weight of the mussel in grams, L is the total length of mussel in mm, a is the intercept of the regression curve and b is the regression coefficient. The degree of association between variables was calculated by the determination coefficient (r^2). To test the b value against the isometric value of $b = 3$, Student's t -test ($H_0: b=3$) was employed to predict any significant deviation (Snedecor and Cochran, 1967). The t -statistic was calculated as:

$$t = (b-3)/S_b$$

where, S_b is the standard error of b . The t -value was compared with t -table value of t -test which allowed the determination of statistical significance of b value.

Growth studies

Growth studies were attempted to elucidate the weight gain of this species in the culture condition. The study was carried out for 160 days in a perforated tray (51 x 32 x 29 cm) where two different strata were used, one set at the bottom of the pond (on-bottom) and another set about 30 cm below the surface (off-bottom). The water

depth of the pond was maintained at around 0.8-1.0 m. In the tray, 8 numbers of mussels were placed in triplicate. During each sampling, all the mussels were taken out, removed the dirt and algae on the top of the shell and weighted. The plastic trays were covered with mosquito nets to prevent escape of mussels.

Proximate composition and amino acid analysis

The crude protein, fat, moisture and ash content were determined following the methods described in AOAC (1995). For estimation of minerals, tissue samples were prepared by the nitric acid digestion method (APHA, 1998). The final samples were analysed using Atomic absorption spectroscopy and phosphorous was estimated spectrophotometrically (APHA, 1998). Amino acid profile of the samples was determined by the method described by Elkin and Wasynczuk (1987).

Fatty acids profile

The samples (dried form) of freshwater mussel (50 mg) was placed into labeled test tubes, then 1 ml of saponification reagent was added (900 mg NaOH dissolved in 3 ml methanol and 3 ml deionised water) and heated in a water bath at 100°C for 5 min. Subsequently the mixture was cooled at room temperature, then added 2 ml of methylation reagent (3.25 ml of 6N HCl mixed with 2.75 ml methanol) and reheated to a temperature of 80°C in water bath for exactly 10 min. It was then cooled and shaken until at 30°C and then 1.25 ml of extraction reagent (2 ml hexane mixed with 2 ml methyl *t*-butyl ether) was added. After that vortexed for 10 min, then the bottom layer was removed by pipetting; 3 ml of basic wash mixture (0.108 g NaOH dissolved in 9 ml deionised water) was added and again mixed for 5 min. Subsequently, the top layer was removed and transferred into an auto sampler vial of gas chromatography to analyse the fatty acid profile using Gas chromatograph.

Statistical analysis

All the data were subjected to ANOVA and t -test using the statistical software, Statistical Package for the Social Sciences (SPSS) version 22.0. Duncan's multiple range tests were used to determine the differences among treatment means at 5% level of significance.

Results and discussion

Analysis of length-frequency

Length-frequency distribution of *L. marginalis* is illustrated in Fig. 1, which is represented by a single cohort with a sample size that varies from 23 to 103 mm. The dominant size of mussel was found to be within 60-65 mm (22.6%), followed by 40-45 mm size group (13.5%). In terms of individual weight, majority of the

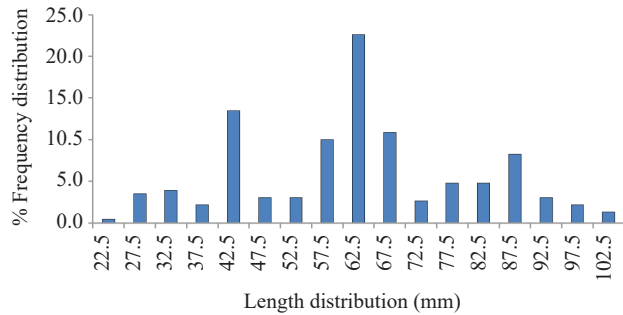


Fig. 1. Length frequency distribution of *L. marginalis*

samples were found to lie between 58-78 g which was almost similar to the observation made by Costa and Indrashena (1984) on the same species from the reservoir in Kurunegala District of Sri Lanka. In the present study, only a few species were found to weigh above 110 g (2.6%).

Length-weight relationships

Length-weight relationships are useful tools in fisheries research which can be used in converting length to weight or the estimation of biomass from length observations. It provides a mathematical expression between length and weight, indicating the general well-being of an organism. In the present study length-weight relationship of *L. marginalis* was found to be $Y=3.856X-12.81$ and regression coefficient (r) estimated was 0.99 ($R^2=97.67\%$). The growth coefficient b was found significantly higher indicating allometric growth pattern with a standard deviation of 'b' being 3.79 to 3.94 (Fig. 2, 3). The b value was found more than 3 indicating that the mussels are heavy to their length and in good condition. The present finding corroborated the report of Nahar *et al.* (2019) where they got high b value (3.87) for the same species from Bangladesh. Ansa and Allison (2008) also reported that 'b' for *Senilia senilis*, *Tagelus adansonii* and *Tellina nymphalis* were 2.942, 3.395 and 2.633, respectively. Wilbur and Owen (1964) stated that in most of the bivalves, values of b lies between 2.5 to 3.5. In isometric growth patterns when the age increases, the body weight also increases. However, some individuals of the same age showed different weights due to maturation of their gonads (Haag and Station, 2003; Ravera *et al.*, 2007; Ramesha and Thippeswamy, 2009; Nahar *et al.*, 2019).

Growth performance

To record the weight gain of mussels, a growth study was conducted, where *L. marginalis* was cultured at the bottom of the ponds (on-bottom) (T1) as well as in the subsurface waters (off-bottom) (T2). The details of weight gain against time are presented in Fig. 4. The study revealed that until 100 days of rearing, there was no

significant difference ($p>0.05$) in weight gain between the two treatments. But, the difference in the weight gain was significantly higher in off-bottom culture (T2) compared to on-bottom (T1) at 160 days ($p<0.05$). Percentage body weight gain (BWG%), survival percentage and specific growth rate (SGR%) of *L. marginalis* under two different

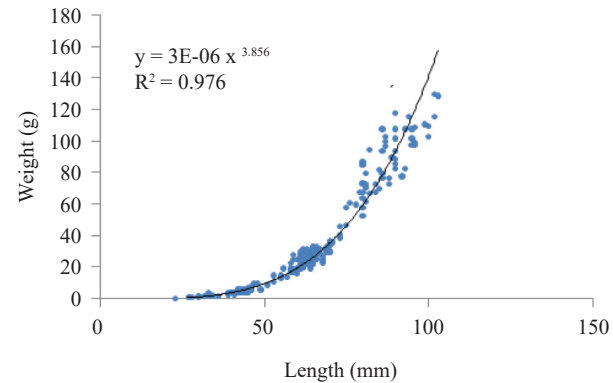


Fig. 2. Length-weight relationship in *L. marginalis*

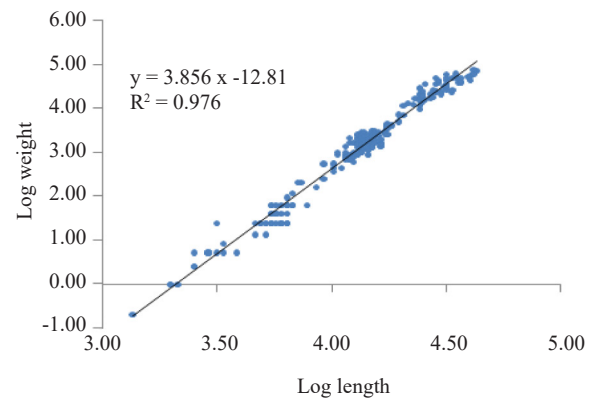


Fig. 3. Length-weight relationship in *L. marginalis* (logarithmic form)

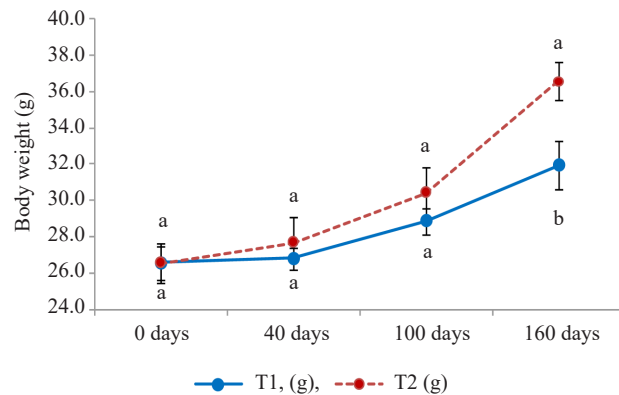


Fig. 4. Growth of *L. marginalis* under two different culture conditions

culture conditions are illustrated in Fig. 5. It was found that changes in culture conditions affect BWG%; survival percentage and SGR% of *L. marginalis*. The BWG% and SGR% were higher when cultured off-bottom. This indicated that the weight gain is better when they are cultured off-bottom. Similar to the present study, Clarke (2010) reported about 20% higher growth rate of mussels in one pool when cultured in cages compared to un-caged (bottom) mussel.

The higher growth rate in subsurface water, in the present study, may be due to better environment in terms of water quality parameters like higher dissolved oxygen, temperature and food (higher phytoplankton and zooplankton density) availability in the water column. The higher growth rate of mussels in subsurface waters can also be attributed to differences in temperature (Clarke, 2010). Comparatively poor growth of mussels in on-bottom culture could be attributed to the fact that silt clogs the gills of mussels, interfere with filter-feeding mechanism (Clarke, 2010) and the indirect effect by reducing light availability for phytoplankton. Lower survival rate was recorded in off-bottom culture compared to on-bottom culture (Fig. 5). At the end of 160 days, survival rates of T1 and T2 were 91.7 and 70.8% respectively. The maximum mortality in T2 was recorded in the first month of culture. This might be due to acclimation issues in the subsurface water, which was not their natural ground or habitat. Clarke (2010) reported that mussel growth could be strongly correlated with water temperature which only weakly and non-significantly correlated with habitat.

Proximate composition

The proximate composition and important mineral constituents of edible parts of *L. marginalis* are given in Table 1. The percentage of crude protein, fat, ash and moisture in the flesh of *L. marginalis* collected during February-March 2019 were 7.19, 1.64, 3.70 and 83.92%, respectively. Baby *et al.* (2010) reported that meat of *L. marginalis* from Bangladesh contained 6.46% crude protein, 0.51% fat, 2.18% ash and 85.90% moisture which are almost as per the present study. Similar results have also been reported by Carvalho *et al.* (2007); Ersoy and Sereflisan (2010) and Sereflisan and Altun (2018) in other mussel species. The marginal variation within the species and also between species in the proximate composition of molluscs may be due to variations in feeding regimes,

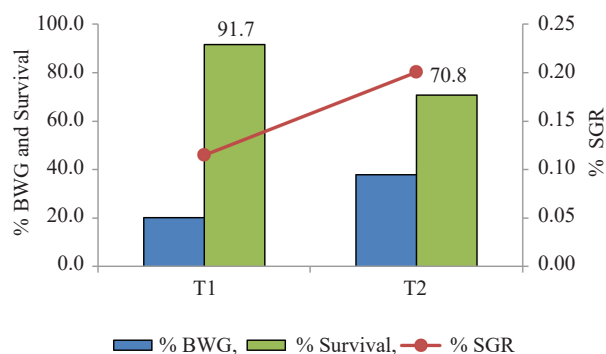


Fig. 5. Percentage body weight gain, survival percentage and SGR% of *L. marginalis* under two different culture conditions

physical and environmental conditions (Nurnadia *et al.*, 2011). In the present study, the meat yield of *L. marginalis* was found to be 16.92%, which was slightly lower than 21% as reported by Costa and Indrashena (1984) on the same species from Sri Lanka. Moreover, meat yield in general in mussel vary from 13.4 to 25.2% (Vernochhi *et al.*, 2007). Meat yield percentage generally varies due to water temperature, food availability, gametogenic cycle of organism (Orban *et al.*, 2006; Delgado *et al.*, 2007) as well as seasonal variations (Vernochhi *et al.*, 2007). All aquatic animals require inorganic elements or minerals for their normal life processes and they have the ability to absorb some inorganic elements from their diets as well as from the aquatic environment they live in. Manganese, iron, phosphorous, copper and zinc are essential elements for all living organisms and acts as a cofactor for numerous enzymatic reactions (Lall, 2002; Sarma *et al.*, 2013; Basuini *et al.*, 2017; Dominguez *et al.*, 2019). The analysis of minerals in the whole tissue of *L. marginalis* showed that it is a good source of minerals. Manganese concentration was found higher and zinc was least in the meat of the mussels. Our results concerning Mn, Cu and Zn are in close agreement with the study reported by Ravera *et al.* (2003) in freshwater mussel (*Unio pictorum*), while Fe and P were relatively lower in the present study compared to concentration reported by Moniruzzaman *et al.* (2021) in *L. marginalis*. Marginal variations in mineral accumulation in soft tissues of mussels may be due to species-specific factors, feeding behaviour, fish size and age, feeding habit, interaction

Table 1. Proximate composition and mineral content of *L. marginalis*

Parameter	Crude Protein%	Fat%	Ash%	Moisture%	Meat yield %
Proximate composition	7.16±0.99	1.64±0.21	3.70±0.62	83.92±1.03	16.94±0.75
Mineral content (mg 100 g ⁻¹ of dry meat)	Mn	Fe	P	Zn	Cu
	486.78±1.88	284.460±18.07	173.26±26.65	30.32±3.42	1.05±0.13

Data represent mean±SE values of three analytical measurements (n=3)

among various elements and the physiological needs of each specimen (Pourang, 1995; Ravera *et al.*, 2003, 2007; Daka *et al.*, 2006; Bawuro *et al.*, 2018). Proximate and mineral analysis clearly indicated that *L. marginalis* can be a good source of protein and minerals for consumers.

Amino acid profile

Amino acids are the most important nutritional component and building blocks of proteins (Sarma *et al.*, 2015). They play a vital role in various metabolic pathways and serve as precursors for the synthesis of biologically important substances (Babu *et al.*, 2011). All amino acids in foods have different roles; essential amino acids are required for nutrition, promotion of normal growth and maintenance of nitrogen balance while the non-essential amino acids are physiologically important to take part in the general metabolic reactions (Jayaprabha, 2016). The amino acid composition of edible parts of *L. marginalis* is presented in Table 2.

Essential amino acids like Lysine (5.11%), Threonine (2.52%), Arginine (1.37%), Methionine (1.27%), Phenylalanine (1.24%), Valine (0.86%), Histidine (0.35%) and Isoleucine (0.05%) were recorded in the mussel in substantial amount, while, Glutamate, the non-essential amino acid constituted the major share (67.62%), followed by Aspartate (13.77%), Serine (4.26%), Glycine (0.57%), Tyrosine (0.46%), Proline (0.33%) and Cysteine (0.22%). Similar to the present study, Jamaluddin *et al.* (2016) and Moniruzzaman *et al.* (2021) also reported the highest concentration of Glutamic acid in *Batissa violacea* and *L. marginalis*, respectively. However, the results of the amino acid composition have been found to vary marginally with the previous reports of Baby *et al.* (2010) and Haldar *et al.* (2014) in *L. marginalis*. It is reported that amount and compositions of amino acids are generally directly dependent on size, seasonal conditions, temperature, food availability, water pollution and geographical conditions (Wesselinova, 2000; Leiwakabessy and Lewerissa, 2017). As such mussels are good sources of proteins especially essential amino acids and essential minerals (Moniruzzaman *et al.*, 2021) and can play important roles in achieving nutritional security.

Table 2. Amino acid composition (mg kg⁻¹) of *L. marginalis*

Amino acid	Concentration		Amino acid	Concentration	
	mg kg ⁻¹	%		mg kg ⁻¹	%
L-Arginine	415.0	1.37	L-Methionine	386.5	1.27
L-Aspartate	4179.5	13.77	L-Phenylalanine	375.5	1.24
L-Cysteine	67.0	0.22	L-Proline	100.0	0.33
L-Glutamate	20519.5	67.62	L-Serine	1293.0	4.26
L-Glycine	173.0	0.57	L-Threonine	766.0	2.52
L-Histidine	106.5	0.35	L-Tyrosine	140.5	0.46
L-Isoleucine	14.0	0.05	L-Valine	260.5	0.86
L-Lysine	1550.5	5.11			

Fatty acids profile

Table 3 presents the fatty acids composition recorded in *L. marginalis*, which shows slight variation with the previous report of Baby *et al.* (2010) and Haldar *et al.* (2014) in the same species. This may be due to changes in geographical conditions, pollution and availability of nutrients. Mussels are sedentary animals that feed on drifting phytoplankton, thus differences in location and nutrient availability could quite easily influence the lipid composition. Molluscs show variations in lipid profile; depending on temperature, weather conditions, amount of nutrients in the environment and reproductive cycle (Murphy *et al.*, 2002; Lachance *et al.*, 2008). The lipid profile is also strongly related to the season and geographical origin (Ventrella, 2008). Presently in India, freshwater mussels are not very important commercial species, except in some parts of the country where it supports small-scale artisanal fisheries. However, due to low cost, easy availability, potential for pearl production as well as the ecosystem services it provides, the importance of these mussels is gaining momentum in recent times. In the present study, an attempt was made to develop a comprehensive database on the biology, growth performance and nutritional quality of this species. Results of the present study indicated that *L. marginalis* follow allometric growth pattern and from the nutritional point of view, it was found to be a good source of protein, fat and minerals apart from the presence of many essential and non-essential amino acids and fatty acids.

Hence, this mussel can be a good source of human food, especially for underprivileged people of underdeveloped and developing countries. As *L. marginalis*

Table 3. Fatty acid composition of the flesh of *L. marginalis*

Name of fatty acid	
Cyclopropanebutanoic acid	Nonadecanoic acid
Cyclopropaneoctanoic acid	Octadecadienoic acid
Docosatetraenoic acid	Octadecanoic acid
Docosahexanoic acid	Octadecynoic acid
Dodecanoic acid	Octadecatetraenoic acid
Eicosanoic acid	Octadecatrienoic acid
Eicosapentaenoic acid	Octadecenoic acid
Eicosatetraenoic acid	Octanoic acid
Eicosatrienoic acid	Pentadecanoic acid
Eicosenoic acid	Tetracosanoic acid
Heptadecanoic acid	Tetradecanoic acid
Hexacosanoic acid	Tetradecynoic acid
Hexadecadienoic acid	Tridecanoic acid
Hexadecanoic acid	Undecanoic acid
Hexadecatrienoic acid	Undecynoic acid
Hexadecenoic acid	(z,z,z)-octadeca-9,12,15-trienoic acid
Hydroxypentadecanoic acid	5,8,11,14,17-eicosapentaenoic acid

is a pearl producing species, the present study also revealed that *L. marginalis* can be cultured in the water column to achieve better growth, which will have practical importance. However, freshwater mussels are very sensitive and imperil groups of organisms and selective exploitation, habitat destruction, pollution are some major known threats to this species. More systematic studies need to be undertaken to have a database on the present status and distribution of this species as well as growth and reproduction competencies for future inclusion as a candidate species for aquaculture.

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