Evaluation of Proximate and heavy metals in Twelve Edible Freshwater Macroinvertebrates of Poba Reserve Forest Assam, India

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The present study focuses on proximate and mineral compositions on 12 freshwater edible macroinvertebrate species under phylum Arthropoda and Mollusca mostly preferred by the ethnic communities around the Poba reserve forest of Assam, India. The analysis revealed protein was the most abundant nutrient for all the species; followed by carbohydrates and fats. The highest protein content was in Lobothelphusa fungosa (50.50%), total carbohydrate in Bellamya bengalensis (22.54%) and fats in Sartoriana spinigera (16.32%). Ash, fibre and moisture were highest in Corbicula assamensis (12.46%), Sartoriana spinigera (11.41%) and Pila globosa (63.72%) respectively. Among the minerals, Calcium, Copper, Iron, Manganese, and Zinc were recorded highest in Bellamya bengalensis (138.62 mg/100g) Macrobrachium assamense (2.73 mg/100g), Sartoriana spinigera (35.02 mg/100g), Macrobrachium assamense (11.42 mg/100g) and Lethocerus indicus (3.71 mg/100g) respectively. Heavy metals (Lead, Cadmium, Molybdenum and Mercury) were absent in all the species under study. The freshwater macroinvertebrates analyzed could form a baseline for future non-conventional food resources of considerable nutritive value.

Keyword: Entomophagy, macroinvertebrate, non-conventional food, Poba reserve forest.

Aquatic macroinvertebrates refer to the small organisms that have no internal skeletal system and live part or all of their lives in water. They comprise a diverse group of organisms that includes insects, molluscs, annelids and crustaceans. They show their presence in aquatic bodies by swimming, clinging or burrowing in the bottom substrate. Many aquatic insects and molluscs species are consumed worldwide due to their taste and seasonal availability.

This practice of consumption was an old and well-established tradition in many parts of the world and recently has gained popularity and acceptance due to the promising source of nutrition challenges facing the world. The trend has gradually been increasing from tropical countries to Western societies. Generally, it is used as ingredient in many cooking recipes in countries like Africa, Asia, South America, and Australia. It is reported that the majority of edible macroinvertebrates contains a rich nutrient concentration. As a part of their daily diet, it may be an important component in eliminating deficiency diseases related to malnutrition. With the rapid increase in human population size, there would be tremendous pressure on animal source foods. It is projected that the world population would exceed 9 billion around 2050 and there will be a food crisis due to a reduction in food production. To combat this crisis, one of the alternatives of a protein-rich source is edible insects. It has been speculated that a 10%
increase in the global animal protein supply through mass production of insects would largely eliminate the malnutrition problem and also decrease the pressure on other protein sources. Therefore, insects and other forms of terrestrial and aquatic macroinvertebrates may offer an important resource for humans nutrition and are worthy of development in various bio-prospecting aspects. Although there is a considerable amount of information on the entomophagy and nutrient contents of terrestrial insects, the information on aquatic macroinvertebrates is scanty. The proliferation rate of insects is very high and many of them are pest in crops causing economic damage to farmers. The consumption of such insects is part and partial of the traditional pest management practices. However, many edible terrestrial and aquatic macroinvertebrates are disease vectors. So understanding these issues and refinement of traditional food habits is a major concern of an entomophagous community.

Poba reserve forest (PRF) situated in the eastern part of Assam is a repository of a variety of aquatic fauna distributed heterogeneously in the aquatic bodies of the reserve forest. Many species of aquatic macroinvertebrates are used as non-conventional food by the ethnic communities inhabiting around the reserve forest. So far no detailed exploration on diversity and proximate nutrient analysis on aquatic macroinvertebrate fauna had been carried out. Realizing the paucity of information the present study was undertaken on 12 commonly preferred species by the consumers under phylum Arthropoda and Mollusca collected from different aquatic bodies of PRF. The inventory of proximate nutrients composition may help the communities for the ideal quantity of intake of nutritiously valuable invertebrate resources. Further, this information may encourage sustainable use of these alternative cheap sources of non-conventional food resources and for possible domestic and commercial uses.

**MATERIALS AND METHODS**

**Sample Collection**

The samples were collected from different perennial and ephemeral water bodies of PRF (latitude 27°50′11″N and Longitude 95°17′45″E) of Assam, India covering an area of about 102.21 km² of land. Some specimens were collected from local markets during the seasons of their availability. Collected specimens were brought to the laboratory, sorted and preserved in 80% ethanol for future uses. Identification of the recorded specimens was done following standard taxonomic keys and available updated literature from IUCN along with the technical support of the Zoological Survey of India, Kolkata, India.

**Proximate Analysis**

Moisture, Ash, and fibre contents were carried out following standard methods. Nitrogen was determined by the micro-Kjeldahl method. Crude protein content was subsequently calculated by multiplying the nitrogen content by a factor of 6.25. Fat content was determined by using the Soxhlet extraction method. Carbohydrate content was calculated by subtracting the sum of the weights of protein, fibre, ether extract and ash from the total dry matter and reported as nitrogen-free extractives.

**Estimation of Minerals**

Selected minerals were determined in an Inductively Coupled Plasma-Atomic Emission Spectrometry (ARCONS, Simultaneous ICP Spectrometer, SPECTRO Analytical Instruments GmbH, Germany) after acid digestion of the tissue samples with nitric acid and perchloric acid (5:1v/v) respectively.

**Statistical Analysis**

Data of the analysis are presented as means ± standard error (SE). Mean differences in proximate and minerals concentrations among the twelve freshwater macroinvertebrates were determined with one-way ANOVA using LSD in SPSS (Version 21) Software. Significance was accepted at P ≤ 0.05 levels.

**RESULTS**

The systematic position and taxonomic keys of the twelve recorded freshwater macroinvertebrates are listed in Table 1. Analysis of the proximate composition of the species depicts a fair amount of nutrients (Table 2). There were significant differences in the means of the proximate compositions among the analyzed species (P < 0.05). The highest amount of moisture content was observed in *Pila globosa* (63.72%) and least in *Sartoriana spinigera* (45.72%). The ash content of the recorded species ranges from 3.29% to 12.46% highest in *Corbicula assamensis* and least in *Lamellidens corrinanus*. The highest fibre content was recorded in *Sartoriana spinigera* (11.41%), *Cybister tripunctatus* (11.00%) and *Leiocerus indicus* (11.31%) against the *Lamellidens corrinanus* (0.28%) having the least fibre content. For most of the species, crude protein was the most abundant substance ranged from 18.10% (*Macrobrachium assamense*) to 50.50% (*Lobothelphusa fungosa*). Lipid content was recorded highest in *Sartoriana spinigera* (16.32%) and least in *Lamellidens marginalis* (2.05%). The mean value of carbohydrate content was 11.32%, being highest in *Bellamya bengalensis* (22.54%) and lowest in *Macrobrachium assamense* (5.25%).

The results of the analysis of selected minerals are given in Table 3. Calcium (Ca) content was highest in *Bellamya bengalensis* (138.62 mg/100g) and lowest in *Parreysia corrugata* (5.44 mg/100g). The highest...
amount of Copper (Cu), Iron (Fe), Manganese (Mn) and Zinc (Zn) contents were observed in *Macrobrachium assamense* (2.73 mg/100g), *Sartoriana spinigera* (35.02 mg/100g), *Macrobrachium assamense* (11.42 mg/100g) and *Lethocerus indicus* (3.71 mg/100g) and lowest in *Cybister tripunctatus* (0.08 mg/100g), *Lobothelphusa fungosa* (0.76 mg/100g), *Pila globosa* (1.07 mg/100g) and *Parreysia corrugata* (0.93 mg/100g) respectively. The presence of selected heavy metals Lead (Pb), Cadmium (Cd), Molybdenum (Mo), and Mercury (Hg) were not detected in any of the specimens.
Table 1. Systematic position and taxonomic keys of analysed freshwater Macroinvertebrates

| Class        | Order    | Family   | Species                          | Taxonomic Keys                                                                                                                                 |
|--------------|----------|----------|----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| Insecta      | Coleoptera | Dytiscidae | Cybister tripunctatus (Olivier, 1795) | Head transverse, slightly convex above, subrounded and slightly sloping in front, antennae filamentous, mandibles are heavily sclerotized, triangular and strongly toothed on the inner side lie closely above the lacinia and galea, the joint between the cardo and stipes is elbow-shaped, mesoscutellum composed of two lateral chitinised sclerites. |
| Hemiptera    | Belostomatidae | | Lethocerus indicus (Lepeletier and servile, 1825) | Body narrowly elliptical, Antennae small, hidden inside a groove ventrally, Rostrum sharp and stout, the intercerebral region is little wider than the eye width, Scutellum little broader than hemelytra, sharp claws, Mid and hind legs provided with thick sets of swimming hairs on the ventral side. |
| Malacostraca | Decapoda | Potamonidae | Lobothelphusa fungosa (Alcock, 1909) | The carapace is almost square, cervical groove is hard to make out, outer orbital angle and the lateral epibranchial tooth prominent, antero-lateral borders of the carapace are subcristiform. The epigastric crests are not so continuous with the postorbital crests, and the latter is sharper, especially in the outer half. |
| Malacostraca | Decapoda | Sartoriania spinigera (Wood-Mason, 1871) | | Carapace wide, gently convex and smooth with a slightly concave free edge. |
| Malacostraca | Decapoda | Paleomonidae | Macrobrachium assamense (Tiwari, 1958) | The abdomen has six body segments with five pair of walking legs, last segments contain telson with two pairs of dorsal spines, Rostrum is slightly slender reaching to the distal end of the epiphrebral, rostral tip slightly upturned; |
| Malacostraca | Decapoda | Gastropoda | Architaenioglossa | Bellamya bengalensis (Lamarck, 1822) | Shell thin, whorls gradually increasing, shallow sutures, rather straight sides. Spire and body whorl of almost equal height, less rounded, with straight sides, aperture sub-circular with narrow black margin. |
| Malacostraca | Decapoda | Anguillidae | Pila globosa (Swainson, 1822) | Shell globose shaped, spacious, the upper surface of whorls obliquely flattened, suture not deep, spire depressed. |
| Malacostraca | Decapoda | Bivalvia | Sorbecoconcha | Pachychilidae | Brota costula (Brandt, 1847) | Shell elongated, regularly increasing, prominent axial ribs with spines, sculpture with spiral ridges. |
| Malacostraca | Decapoda | Unionoida | Lamellidens coriarius (Lea, 1834) | Shell elliptical to oval, umbones prominent, ventral margin convex, cardinal teeth strong, not lamellar, greenish. |
| Malacostraca | Decapoda | Veneridae | Corbicula assamensis (Prashad, 1928) | Shell obliquated and ovoid-shaped, periostracum blackish-brown with a light brown border along the ventral margin, ventral margin slightly contracted in the middle. Shell ovate, dorsal margin regularly arched convex, anterior side short and rounded, posterior broad and truncate, striae regular concentric, distinct, but not deep. |
Table 2. Percentage Proximate composition (% per 100 g Dry tissue).

| Macroinvertebrates | Moisture  | Ash       | Fibre       | Crude Protein | Total Carbohydrate | Crude Lipid |
|-------------------|-----------|-----------|-------------|---------------|---------------------|-------------|
| Sartoriana spinigera | 45.72±0.45 | 8.61±0.39 | 11.41±0.40 | 35.24±1.38    | 9.05±0.32          | 16.32±0.39  |
| Macrobrachium assamense | 54.65±0.58 | 11.00±0.26 | 6.04±0.31  | 18.10±0.70    | 5.25±0.11          | 9.11±0.48   |
| Parreysia corrugata | 52.37±0.50 | 5.28±0.14  | 4.37±0.13   | 27.62±1.02    | 7.56±0.26          | 11.22±0.19  |
| Lamellidens corriatus | 48.87±0.58 | 3.29±0.14  | 0.28±0.02   | 25.54±0.86    | 11.12±0.48         | 3.58±0.27   |
| Lamellidens marginalis | 58.62±0.46 | 8.55±0.20  | 1.90±0.25   | 38.35±0.88    | 7.45±0.39          | 2.05±0.04   |
| Corbicula assamensis | 63.50±0.27 | 12.46±0.53 | 4.86±0.32   | 20.90±0.46    | 7.89±0.31          | 4.09±0.33   |
| Pila globosa | 63.72±0.50 | 8.28±0.24  | 0.95±0.04   | 33.31±1.01    | 12.11±0.32         | 3.12±0.29   |
| Bellamya bengalensis | 56.80±0.27 | 10.52±0.32 | 6.47±0.15   | 35.67±1.03    | 22.54±0.60         | 2.53±0.15   |
| Cybister tripunctatus | 47.00±0.57 | 3.58±0.21  | 11.00±0.27  | 26.54±0.99    | 19.54±0.36         | 12.35±0.50  |
| Brotia costula | 62.86±0.83 | 8.50±0.40  | 1.85±0.10   | 44.42±0.96    | 21.89±1.03         | 2.90±0.27   |
| Lethocerus indicus | 46.76±0.48 | 3.53±0.30  | 11.31±0.30  | 22.67±0.74    | 5.86±0.29          | 13.21±0.35  |
| Lobothelphusa fungosa | 53.03±0.58 | 7.22±0.12  | 2.83±0.26   | 50.50±1.30    | 5.59±0.35          | 6.52±0.37   |

± Standard Error
Values with different alphabets in each column are significantly different at P ≤ 0.05 level.
Table 3. Mineral composition (mg/100g).

| Freshwater Macroinvertebrates | Ca    | Cd | Cu     | Fe     | Mn     | Pb     | Zn | Mo | Hg |
|-------------------------------|-------|----|--------|--------|--------|--------|----|----|----|
| *Sartoriana spinigera*        | 125.44±1.48 | ND | 1.32±0.01 | 35.02±0.50 | 8.95±0.32 | ND | 3.52±0.12 | ND | ND |
| *Macrobrachium assamense*     | 109.20±2.17  | ND | 2.73±0.22 | 28.65±0.24 | 11.42±0.34 | ND | 3.64±0.13 | ND | ND |
| *Parreysia corrugata*         | 5.44±0.45    | ND | 0.13±0.01 | 6.85±0.26  | 7.35±0.09  | ND | 0.93±0.02 | ND | ND |
| *Lamellidens corrianus*       | 113.25±0.08  | ND | 0.56±0.01 | 8.64±0.23  | 2.86±0.14  | ND | 1.52±0.08 | ND | ND |
| *Lamellidens marginalis*      | 98.43±0.84   | ND | 1.32±0.09 | 4.24±0.10  | 7.95±0.27  | ND | 2.31±0.03 | ND | ND |
| *Corbicula assamensis*        | 114.62±1.19  | ND | 0.87±0.04 | 8.52±0.15  | 2.38±0.10  | ND | 2.13±0.04 | ND | ND |
| *Pila globosa*                | 19.87±1.82   | ND | 1.90±0.02 | 3.61±0.17  | 1.07±0.03  | ND | 1.14±0.04 | ND | ND |
| *Bellamya bengalensis*        | 138.62±1.03  | ND | 0.38±0.32 | 5.91±0.24  | 1.24±0.05  | ND | 3.61±0.19 | ND | ND |
| *Cybister tripectatus*         | 11.87±0.43   | ND | 0.08±0.72 | 31.08±0.32 | 8.34±0.20  | ND | 1.68±0.15 | ND | ND |
| *Brotia costula*              | 19.87±0.95   | ND | 1.94±0.83 | 3.61±0.17  | 1.09±0.08  | ND | 1.14±0.01 | ND | ND |
| *Lethocerus indicus*           | 125.21±1.30  | ND | 0.59±0.02 | 26.05±0.04 | 10.21±0.10 | ND | 3.71±0.20 | ND | ND |
| *Lobothelphusa fungosa*       | 129.62±0.85  | ND | 0.41±0.64 | 0.76±0.08  | 6.37±0.09  | ND | 2.06±0.03 | ND | ND |

ND- Not detected
± Standard Error
Values with different alphabets in each column are significantly different at P ≤ 0.05 level.
DISCUSSION

Studies on nutrient compositions of edible macroinvertebrates around the world show that most of them had a satisfactory amount of energy, protein, fats, fibres, minerals and vitamins\(^\text{22-25}\). Although, some edible species such as *Macrotermes subhyalinus* is not rich in amino acids like tryptophan and lysine for compensating the nutritional gap\(^\text{46}\). Reports on the nutritional aspects of some aquatic and terrestrial macroinvertebrates of the north-eastern region of India suggested the presence of many important nutrients\(^\text{37-39}\). In the present study results of the analysis of proximate composition shows that there was a significant difference (\(P<0.05\)) in the analyzed specimens (Table 2). The Variations in nutrient contents of edible macroinvertebrates are due to the life stages, the prevalence of various biotic and abiotic factors in their habitats and diet\(^\text{13-14}\). For example, the fatty acid composition of edible grasshopper *Ruspolia differenssis* reported to be influenced by the plants on which they feed\(^\text{15}\). Methods of food processing also influence the nutrient content to a greater extend\(^\text{31-33}\).

The present study revealed that the moisture content in *Pila globosa* was higher compared to other macroinvertebrate species under study. The high amount of moisture content in food materials makes them a risk of microbial deterioration and spoilage \(^\text{36, 37}\). The values (58.62%, 56.80%, 63.72%, 62.86%) obtained in this study were lower than the value (85.9%, 82.1%, 85.5%, and 83.2%) reported earlier \(^\text{38}\) for *Lamellidens marginalis*, *Bellamya bengalensis*, *Pila globosa* and *Helix* sp. respectively. Analysis of ash content of the studied specimens revealed 7.56% on average which corroborated with the ash content for *Lethocerus indicus* and *Cybister tripunctatus* \(^\text{40}\) and higher than *Lamellidens marginalis*, *Bellamya bengalensis*, *Pila globosa* and *Helix* sp. \(^\text{41, 42}\). In general, the ash content of biological material is a reflection of the minerals contents in them and may be beneficial for the human body \(^\text{39, 40}\). The present findings of mean protein content ranged from 18.10 to 50.50 % (Table 2). The protein content of *Lamellidens corrianus* and *Lamellidens marginalis* in the present study slightly varied with the findings of other reports \(^\text{27, 30}\). The possible cause of variation in protein content may be associated with the reproductive stages of the specimens \(^\text{41-42}\). The carbohydrate content of the studied specimens ranges from 5.25 to 22.54 % which is higher than the report of Shantibala et al. (2014) \(^\text{30}\). Carbohydrates serve as a source of energy for aquatic fauna and perform many major structural roles in cells \(^\text{43}\). Generally, the ratio for carbohydrate content is less compared to other nutrients in aquatic organisms and fluctuates widely in response to changes in its habitat conditions \(^\text{44}\). The lipid content of the studied specimens ranges from 2.05 to 16.32 (Table 2) which is higher compared to the studies on mollusc and edible arthropods species \(^\text{24, 27}\), *Sartoriania spinigera* (16.32%), *Cybister tripunctatus* (12.35%) and *Lethocerus indicus* (13.21%) had the highest crude lipid content species which could contribute a significant source of dietary lipids. The crude fiber contents of the macroinvertebrate species in this study were quite similar with the other insect species such as termites and *Componotus* sp. \(^\text{44, 46}\). Crude fibre has a significant physiological role in the organism’s body in maintaining internal distension for proper peristaltic movement of the intestinal tract \(^\text{47}\). A diet with low fibre content is associated with constipation. Food materials with high fibre content have been used for weight control and fat reduction, as they give a sense of satiety even when a small amount of food is consumed \(^\text{48}\).

Minerals are the most important factors for the human body in maintaining several physiological processes and are constituents of bones, teeth, tissue, blood, muscles and nerve cells. Many insect species contain a satisfactory amount of minerals such as iron which boast almost equal iron contents to beef containing about 6 mg per 100 g of dry weight \(^\text{33}\). In the present study, the most abundant mineral was calcium ranged from 5.44 to 138.62 mg/100g (Table 3). The calcium content of *Pila globosa* (19.87 mg/100g), *Bellamya bengalensis* (138.62 mg/100g), *Helix* sp (19.87 mg/100g) and *Lamellidens marginalis* (98.43 mg/100g) was lower than the studies reported earlier \(^\text{38}\). Calcium is an important nutrient that has a vital role in neuromuscular functioning, enzyme-mediated processes and blood clotting. Therefore a constant concentration of calcium in the body is necessary. The second most abundant mineral in the present study was iron which is a component of haemoglobin and myoglobin that acts as a carrier molecule and as a cofactor of various enzymes \(^\text{39}\). Most of the analyzed specimens have a considerable amount of iron which boast almost equal iron contents to beef containing about 6 mg per 100 g of dry weight \(^\text{33}\). In the present study evaluation of minerals depicted devoid of heavy metal contents suggesting the studied species can be used for consumption. The absence of heavy metal alone...
can’t be considered for safe consumption. Some edible insects contain naturally content toxic substances such as cyanogenic glycosides[54]. Consumption of grasshopper and locust if consumed without removing their feet can cause intestinal blockage. Similarly, Chitin covering of the exoskeleton of many insects is generally indigestible and causes allergies[55]. There is also a risk of microbial disease transmission as intestinal microbiota of many terrestrial and aquatic insects could be a favourable growth medium for undesirable microorganisms[10, 11].

In different parts of the world aquatic insects such as mayflies are well harvested and eaten in countries like China, Vietnam, Japan and New Guinea[56]. In Thailand, many aquatic species of Nepidae, Hydrophilidae, Dytiscidae, Nepidae, Notonectidae and Belostomatidae are reported to be commonly used edible insects[57]. In India, about 255 insect species are used as non-conventional food by different ethnic tribes during the seasons of their availability. Among these, the most preferred species belong to the family of Coleoptera followed by Orthoptera, Hemiptera, Hymenoptera, Odonata, Lepidoptera and Isotera[58, 59].

Many reports on entomophagy revealed that it is practiced in all seven states of the north-eastern region of India[60-62]. More recently the enormous diversity of insects and other macroinvertebrate fauna are speculated as one of the major sources of future therapeutic agents. Ironically the poor section of the society across various communities consumes a good number of insects and mollusc species available around their localities as traditional food habits to meet their therapeutic and nutritional requirements during the lean period.

However, detail scientific knowledge on this food practice is quite scanty among the user communities. Therefore, the present study is a kind of scientific validation of these traditional food habits which may lead to the inventorization and utilization of bio-resources having nutritional and therapeutic potentials in a sustainable approach.

CONCLUSION

The result of the present work reflects that most of the analyzed freshwater macroinvertebrates have a fair amount of crude protein, carbohydrate, lipids and minerals. The studied macroinvertebrate species represented the most preferred and the cheapest source of protein among the ethnic communities of the study area. This practice of entomophagy should be encouraged as many people can’t afford protein-rich food such as fish or meat. At the same time more exploration and its identification, as well as proper scientific research on the nutritional and anti-nutritional composition, are needed to study the prevailing freshwater macroinvertebrate consumption in the study area which would prove beneficial as a local source of nutrients. Further, the information on nutritional aspects could be effectively used for better utilization of freshwater macroinvertebrates to combat malnutrition and undernourishment of the poor people of the society.

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Conflict of Interest

The authors have no conflict of interest.

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