PROXIMATE COMPOSITION OF SMALL INDIGENOUS FISH SPECIES OF BUDHI RAPTI RIVER, CHITWAN, NEPAL

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Submitted to:

Central Department of Zoology

Institute of Science & Technology

Tribhuvan University Kirtipur, Kathmandu, Nepal

September, 2022

DECLARATION

I hereby declare that the work presented in the thesis entitled "**PROXIMATE COMPOSITION OF SMALL INDIGENOUS FISH SPECIES OF BUDHI RAPTI RIVER, CHITWAN, NEPAL**" has been done by myself and has not submitted elsewhere for the award of any degree. All sources of the information have been specifically acknowledged by the reference to the author(s) or institution(s).

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RECOMMENDATION

This is to recommend that the thesis entitled "**PROXIMATE COMPOSITION OF SMALL INDIGENOUS FISH SPECIES OF BUDHI RAPTI RIVER, CHITWAN, NEPAL**" has been carried out by Mrs. Tara Sapkota for the partial fulfilment of Master of Science in Zoology with special paper **Fish Biology and Aquaculture**. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of the supervisor, "**Prof. Dr. Kumar Sapkota**, this thesis submitted by Mrs. Tara Sapkota entitled "**PROXIMATE COMPOSITION OF SMALL INDIGENOUS FISH SPECIES OF BUDHI RAPTI RIVER, CHITWAN, NEPAL**" is approved for the examination for the partial fulfilment of the requirements for the degree of Master of Science in Zoology with special paper Fish Biology and Aquaculture.

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CERTIFICATE OF ACCEPTANCE

The thesis work submitted by Mrs. Tara Sapkota entitled "**PROXIMATE COMPOSITION OF SMALL INDIGENOUS FISH SPECIES OF BUDHI RAPTI RIVER, CHITWAN, NEPAL**" has been accepted as partial fulfilment for the requirements of the degree of Master of Science in Zoology with special paper Fish biology and aquaculture.

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
AOAC	Association of Official Analytical Chemists
ASF	Intake of animal source foods
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Organization
IMC	Indian Major Carps
NARC	National Animal Nutrition Research Centre
PUFA	Polyunsaturated Fatty Acids
RAE	Retinol Activity Equivalents
SIFFS	Small Indigenous Freshwater Fish Species
SIS	Small Indigenous Species

ABSTRACT

Small indigenous fish are delicious than other fish used for food. An attempt has been undertaken in the current study to determine the proximate composition in small indigenous fish species of Budhi Rapti River Chitwan Nepal. The goal of the current study is to determine the proximate composition of the five local small indigenous fish species (*Puntius ticto, Puntius conchonius*, *Mystus tengara, Barilius bendelisis*, *Acanthocobitis botia*) found in Budhi Rapti River Chitwan Nepal. Samples were collected between January to March 2022. The samples were cleaned, gutted, dried, and grinded to obtain a homogenized powder that was then maintained in plastic containers for chemical analysis. Proximate analysis was performed in the laboratory of National Animal Nutrition Research Centre (NARC). For proximate analysis Association of Official Analytical Chemists (AOAC) method was used. Major proximate compositions such as, moisture, protein, lipid, ash, and fiber were estimated. Proximate compositions were found varied among the species. Protein was estimated in *Puntius ticto* (54.77 %), *Puntius conchonius* (63.95 %), *Mystus tengara* (70.68*%),* *Barilius bendelisis* (69.82), *Acanthocobitis botia* (67.98%) respectively. The results show the highest moisture content in *Mystus tengara* (99%) and lowest in *Puntius ticto* (98.17%). The Lipid content in the present study varied between 9.05 - 15.65% with the highest in *Acanthocobitis. botia* (15.65%) and lowest in *Puntius. chonchonius* (9.05%). The crude fiber content varied between (1.17 -7.42) with the highest in *Barilius bendelisis* (7.42%) and lowest in *Puntius ticto*. The ash content ranged between 16.54 -24.02% with the highest in *Puntius conchonius* (24.02%) and lowest in *Barilius bendelisis* (16.54%). Proximate analysis was performed in National Animal Nutrition Research Centre, Khumaltar. Analysis was carried out in triplicate. These findings demonstrate that all these fishes have a significant amount of nutrient. At the end of the study, no significant difference was observed in moisture, but protein, lipid, Fibre, and ash content shows significant differences. It is clear from the current study that these SIS are a reliable supply of macronutrients, protecting both dietary and financial security of Chitwan, Nepal.

1. INTRODUCTION

1.1 Background

1.1.1 Fish Nutrition Facts

One of the most significant sources of animal protein is fish, which is also a wonderful source of other nutrients for the upkeep of a healthy body. Since the beginning of time, fish has been a significant source of nutrition for people all over the world. It is now widely recognized that fish is a valuable source of polyunsaturated fatty acids, vitamins, and high-quality, balanced, readily digested protein (Andrew 2001). Fishing is a crucial component of food security in developing and undeveloped nations, where 22% of the world's total intake of animal protein is consumed, where there are many starving people (Mohanty et al. 2013). For around one billion people worldwide, fish serves as their primary source of animal protein. Fisheries play a significant role in ensuring food security, especially for many underprivileged people in emerging nations. Fish is a good source of protein, micronutrients, and vital fatty acids, which is a welcome addition to the diets of many impoverished people in developing nations, which are often high in carbohydrates (FAO 2003). Because fish is a good source of important elements, particularly high-quality proteins, and lipids, it is sometimes referred to as "rich food for poor people" (Sujatha et al. 2013). Fish protein is easily digestible. Additionally, it is an important source of both essential and non-essential amino acids (Astawan 2004). When compared to meat from goats, sheep, buffalo, and chicken, fish is safer and healthier to eat. Fish are well-known for being great sources of protein when compared to other protein sources, as evidenced by their amino acid composition and high protein digestibility (Louka et al. 2004). Fish and items obtained from fish must be included in the human diet since they are important sources of x-3 PUFA and include a wide variety of nutritious components. Fish has a higher protein to fat ratio than the flesh of goat, lamb, buffalo, and chicken, making it safer and healthier to eat as a complete meal. The most significant micro and macronutrient components that contribute to fish meat's nutritional value are moisture, protein, lipids, ash, and minerals (Steffens 2006). Other advantages include its hypolipidemic actions, a lower risk of prostate cancer, a lower incidence of renal-cell carcinoma in women, and under some circumstances, a lower risk of dementia (Musaiger & Souza 2008).

1.1.2 Small Indigenous Fishes and proximate composition

The term "small indigenous fish species" (SIS) refers to species of fish that can reach a maximum length of 25 to 30 cm when they reach adulthood (Felts et al. 1996, Mohanty et al. 2013). Small, indigenous fish are particularly important for nutrition because they are eaten whole, with bone, head, and eye, thereby providing a source of calcium and other micronutrients (Kongsbak et al. 2008). Principal composition of fish is 16-21% protein, 0.2-25% fat, 1.2-1.5% mineral, 0-0.5% carbohydrate and 66-81% water (Love 1970).

The measurement of the fish's water, fat, protein, and ash levels is called proximate body composition. The fish’s live weight typically consists of water (70–80%), protein (20–30%), and fat (2–12%) (Love 1980). Due to factors including age, sex, habitat, food, season, and physical activity, the proximate composition differs widely between species. Fish's differences in proximate composition are also directly correlated to the feed they consume and the water they inhabit. The protein composition of muscle tissue increases modestly during times of heavy meals, then there may be a substantial and quick increase in fat content. On the other hand, fish may experience starving periods for physiological or natural causes such as spawning or due to outside influences such a lack of food (Huss 1995). A good indication of physiology is proximate composition which is required for routine analysis (Cui & Wootton 1988). These variations in the nutritional make-up of various species can be related to dietary composition, eating, and drinking habits, feeding rate, habitats, sex, age, size, genetic characteristics, and season/migration (Dawson & Grimm 1980). It is crucial to gather data on the biochemical composition of fish since it will reveal the specific nutritional richness of various species and assist nutritionists and dieticians in developing "dietary guidelines" for the benefit of society (Mohanty et al. 2011). The nutritional composition and functioning of each individual fish are more important as people become more aware of their health and the advantages of eating fish. Consumers may better understand the nutritional benefits of fish that can ensure their improved health, fitness, and immunity against LDs by learning about its nutritional makeup (Gormley 2006). The high demand for fish is not only because of its taste but also largely due to its nutritional values which is a function of its proximate composition (Njinkoue et al. 2016).

Nepal is home of great diversity of fish species and includes 232 native fish in the country (Husen et al. 2019). In the early 1990s, fish consumption in Nepal's regular diet was extremely low, however fish protein consumption in the diets of ordinary people has increased (Nestel et al. 2015). The nutritional composition of same species has been found to vary by region, season, age of the fish and sex etc (Sikandar et al. 2020).

1.2 Objectives of the study

1.2.1 General Objective

General objective of this study was to find the proximate composition in small indigenous fish species of Budhi Rapti Chitwan, Nepal.

1.2.2 Specific objectives are:

- To estimate the moisture, crude fat, crude protein, Crude fibre and crude ash of small indigenous fish species.
- To compare the difference in proximate composition of macro-nutrients in small indigenous fish species of Budhi Rapti River Chitwan.

1.3 Rationale of the study

In the field of research in fisheries, proximate composition of small indigenous fish species of Budhi Rapti River Chitwan is still unexplored. This study gives the knowledge on nutrient composition of small indigenous fish species. Hence, it helps consumers in selecting the fish depending on their nutritional value. Understanding the chemical composition of fisheries products is essential to determine their nutritional content and the advantages of consuming them. This study shows that the investigated fish species are good sources of many major nutrients and essential elements. From the perspective of the customer, this study offers useful information on the nutrient content of the fish species so that one may discern their nutritional worth and decide based on that knowledge.

2. LITRETURE REVIEW

2.1 The nutrient content of fish

Since the beginning of time, fish has been a key source of food for people all over the world, contributing a significant amount of animal protein to people's diets (Agusa et al. 2007). Fish is generally accepted in the world's cuisine because of its flavour, low cholesterol content, and softness of its flesh (Eyo 2001). The live weight of most fish normally consists of about water (70-80%), protein (20-30%) and lipids (2-12%) (Love 1980). Compared to land-based species, aquatic animals have a higher proportion of omega-3 long chain polyunsaturated fatty acids (n-3 LC PUFA), are a richer source of protein, and have a lower caloric density (Tacon & Metian 2013). In addition to its deliciousness, fish is in high demand because of its high nutritional content, which is a result of its proximate composition (Njinkoue et al. 2016). Epidemiological research showed that those who regularly ingested fish or fish oil containing tiny levels (0.4 g) of n-3 series fatty acid saw a reduction in the number of mortalities brought on by coronary heart disease (Dolecek 1992). Fish protein is simple to digest. Furthermore, it serves as a significant source of both essential and non - essential amino acids (Astawan 2004). Fisheries are a crucial component of nutrition and food security, especially for people living in rural areas. It can make up roughly 22% of the world's consumption of animal protein, particularly in developing and underdeveloped nations (Mahanty et al. 2014). Fisheries have a significant role in nutrition and food security, especially for rural residents. The bulk of meats from terrestrial animals do not provide as much protein as those derived from aquatic creatures. Aquatic protein is also very digestible and rich in many peptides and essential amino acids, such as methionine and lysine, which are lacking in animal proteins from terrestrial sources (Tacon & Metian 2013). Fish intake has a beneficial composition of proteins, minerals, vitamins, and important fatty acids, which contributes to its nutritional value (Lund 2013). In addition to its deliciousness, fish is in great demand because of its high nutritional content, which is a result of its proximate composition (Njinkoue et al. 2016). By geography, season, fish age, sex, and other factors, it has been discovered that the nutritional makeup of the same species varies (Sikandar et al. 2020, Islam et al. 2020). The information on the chemical composition of fish in respect to the nutritive value is important to compare with other sources of animal protein, foods such as meat and poultry products (Stansby 1954).

A fish meal, in addition to providing essential nutrients, contains significant amounts of fatty acids, amino acids and some of the most important vitamins and minerals, which serve as a source of energy for healthy living and are sometimes referred to as 'rich food for poor people' (Balami et al. 2019). Fish from freshwater sources is a healthy food that is rich in nutrients. They are an excellent source of animal protein, particularly the crucial polyunsaturated fatty acids for human nutrition. Regular meals of fish (both freshwater and marine fish species) can aid in preventing heart problems. Additionally, freshwater fish can fulfil the requirements for several vitamins and minerals. The most significant macro and micronutrient components that contribute to the nutritional value of fish meat are moisture, protein, lipids, ash, and minerals (Steffens 2006). The assessment of moisture, protein, fat, and ash content in the fresh fish body constitutes the proximate composition. About 96–98% of the fish's total tissue constituents are made up of these constituents (Nowsad 2007). Micronutrients and macronutrients are two categories of nutrients that are essential for a healthy body. The fish include macronutrients such as proteins, lipids, ash, and carbohydrates (Lilly et al. 2017). Lipid is regarded as one of the most important food reserves and has led to the use of fat indices as a measure of relationship between percentage of water and fat (Salam and Davies, 1994). Lipids from fish are well known as a rich source of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which cannot be synthesised by humans and commonly obtained from the diet (Alasalvar et al. 2002).

Polyunsaturated fatty acids from fish have been reported to have preventive and/or curative effects for several diseases including arterial hypertension cancers and inflammatory diseases (Türkmen et al. 2005). Variation of biochemical composition in the fish body relates closely to feed intake (Oyelese 2006). An excellent measure of the relative energy, protein, and lipid contents is the percent of water content; the lower the water content, the higher the lipid and protein content and the higher the energy density of the fish (Aberoumad & Pourshafi 2010). Fish lipids are crucial for human nutrition because they provide both the necessary fatty acids (FA) and energy needed to meet the body's diverse physiological demands (ARAS et al. 2003). Additionally, the differences in the proximate composition of fish are tightly correlated with the amount of food they consume and the water in which they live. During times of heavy feasting, muscle tissue's protein content may initially rise just modestly, but later may experience a substantial and quick rise in fat content. On the other hand, fish may experience hunger periods due to external events, such as a food scarcity, or natural or physiological causes (such as spawning or migration) (Huss 1995). When deciding whether fish is suitable for processing and whether fish meat is suitable as a protein supplement, knowledge regarding the proximate composition and amino acid profiles of fish meat is crucial. These studies may help dieticians prescribe diets for patients with certain medical issues or for those who are health conscious (Ssali 1988).

In many Asian countries over 50% of the animal Protein intake comes from fish while in Africa, the proportion is 17.50% (Willmann et al. 1998). Nepal's total fish production from captured and aquaculture together is estimated to be about 64,900 metric tons in year 2013/2014 with the share of 33.17 % to capture fisheries (Durbar 2014). Communities of fishermen, like the Tharu, Chaudhary, and Jalari, engage in fishing in Nepal. Their primary source of nutrition is small indigenous species (SIS), which they catch from adjacent rivers, lakes, marshy plains, reservoirs, and tiny bodies of water. This gives the populations of fishermen who fish in Nepal, such as the Tharu, Chaudhary, and Jalari, a significant source of protein for their diets. Their main source of food is small indigenous species (SIS), which are harvested from nearby reservoirs, marshy plains, rivers, and lakes. This provides them with a diet high in protein (Sonawane 2013, Mohanty 2015). In terms of food and nutrition, Nepal is among the least developed nations. 41% of Nepalese children are thought to be stunted or chronically malnourished (Faruk et al. 2012).

Because of their large inhabitants and high abundance, they comprise a significant assemblage of the total finfish population in the inland capture and culture fisheries production. People in the villages consume more SIS than the urban counterpart as these fishes do not have good market demand as compared to the major carps and other large fishes but plenty available from local water bodies may serve as an alternative source of quality dietary proteins in rural food and nutrition. Thus, the SIS can play a key role in preventing the widespread micronutrient deficiencies and allied diseases (Mahanty et al. 2014).

2.2 Nutritional contribution of small indigenous fish species

The proximate composition of twenty - one small indigenous fish species of the Paschim Medinipur District of West Bengal, India, was studied by Jana et al. (2018). They concluded from that study that the SIS are the most affordable source of high-quality protein and important micronutrients, protecting both nutritional and financial security as well as protein hunger. The Proximate Analysis of ten small indigenous species (SIS) of Tripura, India, was studied by Jena et al. (2018). They discovered that the protein concentration varied from 12.89 to 16.75 percent. The moisture level also differed from 70.65% to 76.95%, while the lipid content ranged from 1.84% to 6.19%. According to the study's findings, the SIS are an excellent supply of macronutrients ensuring both nutritional and livelihood security.

In 2014, Debnath et al. (2014) studied the protein and mineral makeup of a few local fishes in Tripura, India. After analysis of the chosen fish species - *Amblypharyngodon mola, Esomus danricus, Puntius sophore, Channa fasciata, Labeo bata, Catla catla, Labeo rohita,* and *Cirrhinus mrigala* - it was found that every fish species was nutritionally competitive even when it was dried. According to the study, small indigenous fish species are incredibly nutritious and can ensure the impoverished classes nutritional security because they are so inexpensive and widely available. The small indigenous fish *Puntius sophore* was researched by Mahanty et al. (2014) with his colleagues to determine its proximate composition, amino acid, fatty acid, and micronutrient profiles. Their research showed that the fish species under study included high levels of nutrients and proteins. Histidine was the most prevalent of the necessary amino acids in the species. Additionally, *Puntius sophore* was a fish that was high in oleic acid and other unsaturated fatty acids. The nutritional makeup of important fish species in Bangladesh and their potential contribution to required nutrient intakes were studied by Bogard et al. (2015). The research found that mothers who are pregnant or breastfeeding can benefit significantly from eating small indigenous fish that have high nutritional profiles. Aquatic animal foods are a rich source of protein and have a lower caloric density and have a high content of omega 3 long chain polyunsaturated fatty acids (n-3 LC PUFA) compared to land living animals (Tacon & Metian 2013). In many European nations, the intake of fish and fish products has significantly expanded over the past several decades as a method of reducing cardiovascular disease Cahu et al. (2004).

3. MATERIALS AND METHODS

3.1 Study area

Chitwan district is in Nepal's Bagmati Province's south-western region. The Trisuli, Gandaki/Narayani River systems, which eventually empty into the Ganga River in India, drain this district, which has a subtropical climate and a monsoon climate. The Rapti and Rew Rivers, as well as a sizable number of eastern Chitwan tributaries including Khageri, Kayar, Ladari, Pampa, Dhongre, Martal, and Lothar, are all collected by the Narayani River, which is quite long and broad. The Budhi Rapti River is in the north-eastern region of Nepal's Chitwan province. The tropical monsoon climate zone encompasses the research region. The inhabitants of Northeast Chitwan are greatly benefiting from Budhi Rapti River. It is located at 27°37' 30' ' North and 84°31' 30'' East.

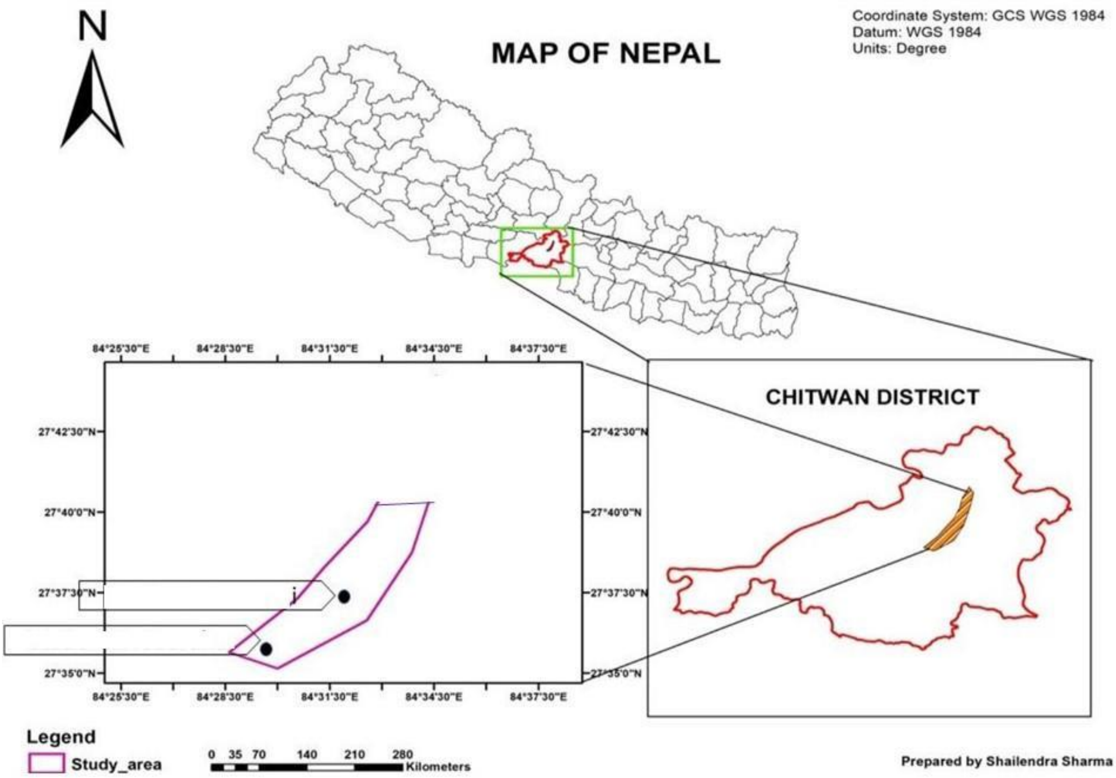


Figure 1: Study area map

3.2 Study period

The field research was carried out from January to March 2022AD.

3.3 Materials

Dissecting box, scissors, gloves, ruler, weighing balance, aluminium foil, tissue papers, trays, drying oven, homogenizer, blender mixture, tongs, zipper bags, refrigerator, cast net, gill net, Ghamka, Analytical balance, Hot air oven, Micro kjeldahl's digester and distiller, Digestion and distillation tube, Muffle furnace, Soxhlet apparatus.

3.4 Chemical reagent used for proximate analysis

Sodium hydroxide (NaOH), Catalyst: Sodium sulphate (Na_2SO_4) and Copper sulphate ($CuSO_4$), Concentrated Sulphuric acid (H_2SO_4), Boric acid (H_3BO_3), Methyl red indicator, Petroleum benzene ether.

3.5 Collection and identification of fishes

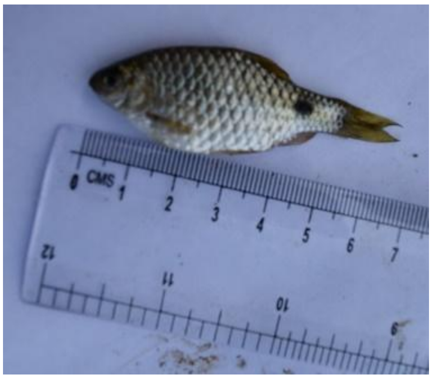
3.5.1 Collection

With the help of neighbourhood fishermen, samples of small indigenous fish species were taken from Budhi Rapti River in Chitwan using gillnet, cast net, and Ghamka. The fish samples were transported to the Agriculture and Forestry University (AFU) Rampur, Chitwan laboratory in an icebox. The keys provided in the book "Ichthyology of Nepal" were used to identify the various kinds of fish that were caught. Following fish identification, measurements of length in (cm) and total body weight in gram (g) were made.

3.5.2 Identification

Table 1: Local name, scientific name, order, and family of fish species collected from Budhi Rapti of Chitwan

S. N	Local Name	Scientific Name	Order	Family
1.	Punti	*Puntius ticto*	Cypriniformes	Cyprinidae
2.	Bhitte/Rato pothi	*Puntius chonchonius*	Cypriniformes	Cyprinidae
3.	Tengra	*Mystus tengara*	Siluriformes	Bagridae
4.	Tilawa	*Barilius bendelisis*	Cypriniformes	Cyprinidae
5.	Botia	*Acanthocobitis botia*	Cypriniformes	Cyprinidae



a. *Puntius ticto* b. *Puntius chonchonius*

c. *Mystus tengara* d. *Barilius bendelisis*

e. *Acanthocobitis botia*

Figure 2: Small indigenous fish species for proximate analysis.

a. *Puntius ticto* (Hamilton, 1822)

It has a small mouth. Absence of barbels; colour is silvery. On the incomplete lateral line, there are two black marks. body depth that is less than one-third the length of the average. It feeds on diatoms, algae, crustaceans, rotifers, insects, and other surface-feeding organisms. It is a little freshwater fish that lives in ponds, rivers, and streams.

b. *Puntius conchonius* (Hamilton, 1822)

It has deep bodied and silvery in colour. It is a common freshwater fish found in lakes and streams. Moderate mouth without barbels and upper jaw slightly longer. Pectoral if head excluding snout. Lateral line system is incomplete, and scales medium. and black bases on all scales. Reddish fins have rows of black markings on them. The middle of the dorsal rays is marked by a band of black. The paired fins have dark tips, and the caudal fin is transparent.

c. *Mystus tengra* (Hamilton, 1822)

During the rainy season, this species is typically found in weedy, sandy, and muddy areas of pools, streams, and rivers. (Bhuiyan 1964). The fish has a black patch on its shoulder and is yellow or brown in colour. Lengthened and somewhat compressed body. Head lowered. Barbels come in four pairs. The mouth is the terminal. Adipose is not long. The caudal fin's upper lobe is longer.

d. *Barilius bendilisis* (Hamilton, 1807)

Body is compressed and elongated, with a somewhat cleft mouth. There are two little pairs of barbels. (Maxillary and Rostral) less convex in the dorsal than the ventral profile. Fins are bigger, more developed, and evenly spaced. On the snout there are several pores. Scarlet-red side scales are brownish above, bluish below, and silvery between.

e. *Acanthocobitis botia* (Hamilton, 1822)

This fish is from freshwater. Adults inhabit swiftly flowing streams with stony, pebbly, or sandy substrates. The dorsal soft rays are 14 –17. 8 gentle anal rays. and the lateral line at least extends to the anus.

3.6 Preparation of samples for analysis

The individual fish length (in centimetres) and weight (in grams) were recorded once the fish species were identified. Samples were physically cleaned, degutted, and their weight after gutting was calculated. They were also dried for 24 hours at 75 degrees Celsius in an oven, and the dry weight was noted. Finally, using a homogenizer and a grinder mixture, the homogenization process was carried out, and the homogenised weight was recorded. After being homogenised, the final minced powdered samples were put in sealed plastic airtight containers and chilled for a while before examination. For proximate analysis, the dried samples were used.

Figure 3: Process of sample preparation of small indigenous fish species for proximate analysis.

3.7 Proximate analysis

Proximate composition of fishes was determined using AOAC methods (1990). All analyses were done in triplicate. The proximate composition of the minced samples for their nutrient analysis were performed in National Animal Nutrition Research Centre, Khumaltar, Lalitpur, Nepal. The analysis included Proximate Analysis i.e., Crude Protein, lipid, Total Ash, Moisture, and Crude fibre. The proximate analysis was done by methods of:

- determination of moisture content (hot air oven method),
- crude protein (Micro-Kjeldahl),
- lipid (ether extraction method),
- total ash based on AOAC (2000) standard methods.

3.7.1 Determination of moisture

Approximately 5 g of a representative clean, dry sample were weighed in an aluminium tray. The weighted sample was dried for 24 hours at 100^0 c in a hot air oven to achieve a constant weight. After cooling at room temperature, the dry sample was quickly weighed and maintained in a desiccator to prevent moisture absorption. The difference in the weight is calculated.

$$\text{Moisture (\%)} = \frac{\text{Initial sample weight} - \text{Final sample weight}}{\text{Sample weight}} \times 100$$

3.7.2 Determination of crude protein

Three distinct procedures, including digestion, distillation, and titration, were used to determine crude protein. A sample of 0.5 –1 gm was weighed and placed in a digestion tube. And about 5 gm of digestion mixture (Na2SO4 and CuSO4 in ratio 4:1) was added to the digestion tube and 20 ml of concentrated Sulphuric acid was added very carefully to the tube. The tube was then fixed in a digestion chamber and heated for two hours at 400^o C before being cooled. The solution turns green after heating. 100 ml of volume (Sample + distil water) in a volumetric flask was prepared and the colour of the solution turned in blue after the addition of distil water and left overnight. For distillation 10ml of solution was taken in a tube and fixed in a distillation chamber for the distillation process. During the distillation process the sample is mixed with 10ml of 4%boric acid, 10ml sodium hydroxide and 50ml of distilled water mixed completely and distilled for 3 minutes. The distilled turn from dark blue to light blue. Distillate was titrated with 0.03N H_2SO_4 until it turned from light blue to transparent.

$$\text{Crude protein (\%)} = \frac{(14 \text{ X Normality}) \text{ X (Reading point} - \text{blank point x } 6.25)}{\text{dry matter X Sample weight}}$$

3.7.3 Determination of Ash

Weighing the crucible containing the dried sample and placing it in a muffle furnace at 550^0 c for three hours allowed us to determine the sample's ash content. The crucible containing the sample was reweighed and the difference between sample weights indicate the ash content.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{ x } 100$$

3.7.4 Determination of Fat

A dry sample weighing 2 g was placed in filter paper. The thimble was filled with filter paper and a sample, and the thimble was retained in the Soxhlet flask.

150ml of petroleum benzene ether was poured into the flask (half of the flask) and the flask was fixed in the heating system. The solution was heated at 160^0 c for 60 minutes and cooled. The extract was removed and left overnight. The beaker was placed in an oven for 2 hours and allowed to cool in a desiccator, and then the final weight was noted.

$$\text{Crude fat (\%)} = \frac{(\text{Flask + Fat weight}) - \text{Flask weight}}{\text{Sample weight X Dry matter}} \text{ x } 100$$

3.7.5 Determination of Crude Fibre

Crude fibre content was calculated by acid base- digestion using 1.25% H2SO4 (W/V) and 1.25% NaOH(W/V) solution.

3.8 Statistical analysis

All the results expressed are the mean of three measurements. The mean and standard deviation of the data were displayed. Statistics were performed using the Microsoft Office Excel program. One-way ANOVA was used to examine the variations across species. Differences were evaluated by ANOVA using Statistical Package of Social Science (IBM SPSS Statistics 28.0). Levels of statistical significance were $p < 0.05$.

4. RESULTS

4.1 Proximate composition

The proximate composition of small indigenous fish species of Budhi Rapti River is shown in tables 3 and 4 (Appendix). The proximate composition of different fish species shows there was a significant difference between the different nutrient levels among small indigenous fish species ($p < 0.05$) (Table 6-10, Appendix).

4.1.1 Moisture content

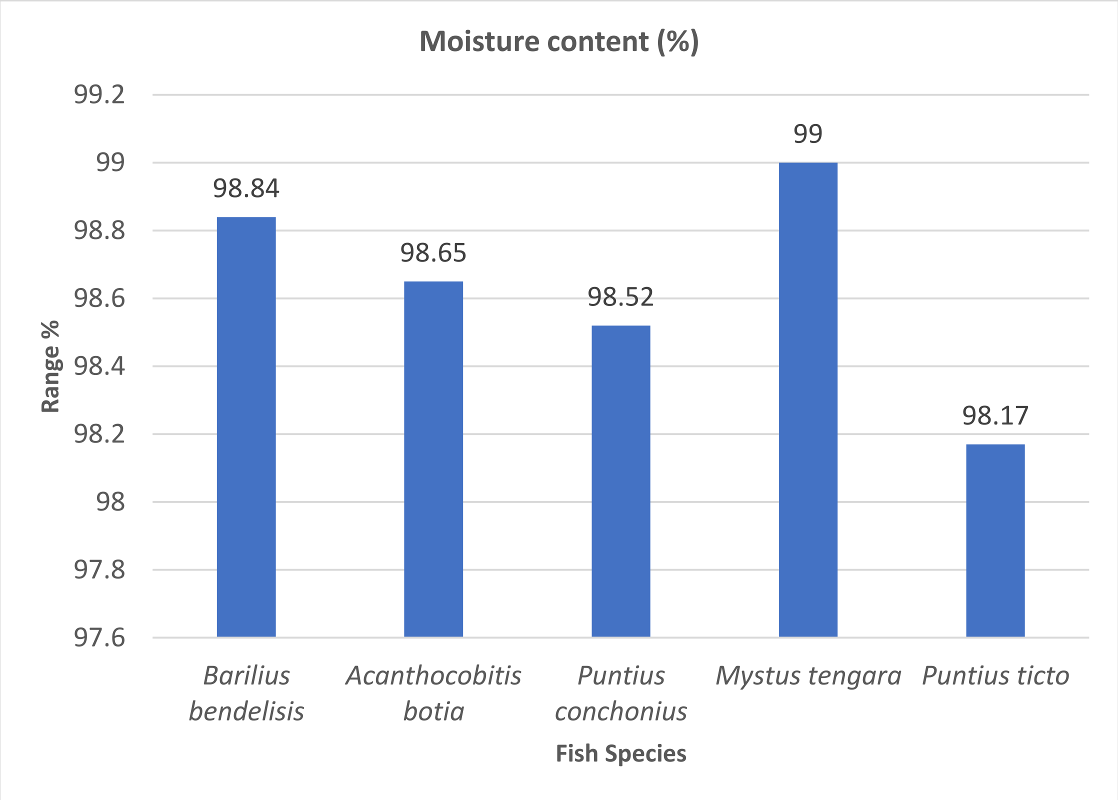


Figure 4: Moisture content (%) among the fish species

The estimated moisture content (%) for *Barilius bendelisis*, *Acanthocobitis botia*, *Puntius chonchonius*, *Mystus tengara and Puntius ticto* is 98.84, 98.65, 98.52, 99, 98.17, respectively.

The moisture content in the present study ranged between (98.17 - 99) % with the highest in *Mystus tengra* (99%) and lowest in *Puntius ticto* (98.17%) (Fig. 4). The moisture content in *Acanthocobitis botia* and *puntius chonchonius* were similar. There was no significant difference in moisture content among small indigenous fish species ($p > 0.05$) (Table 6, Appendix).

4.1.2 Total Ash content

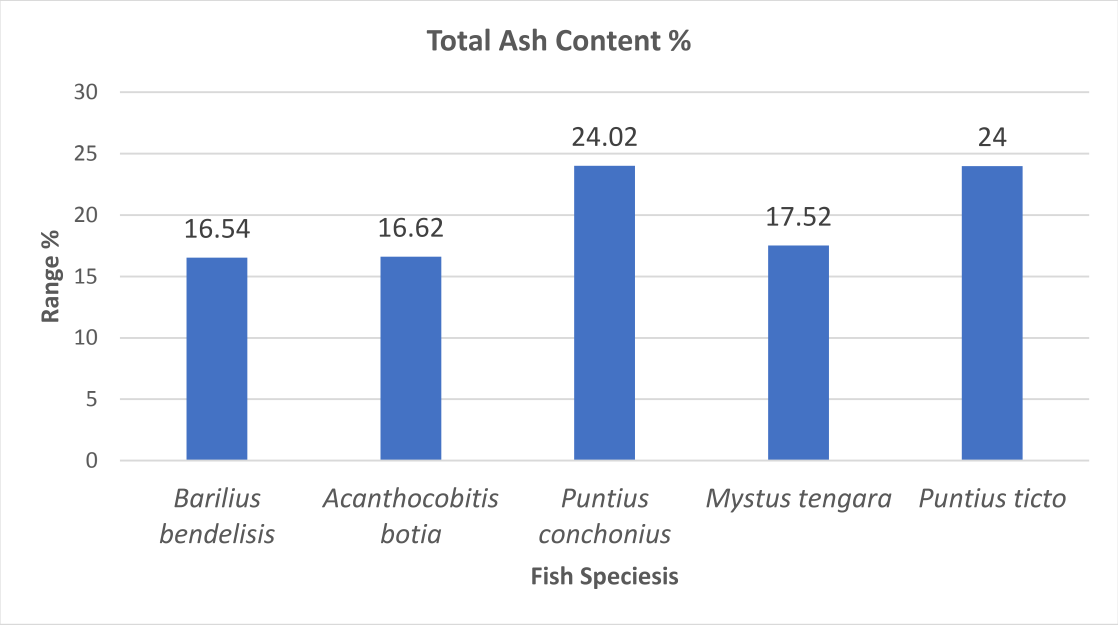


Figure 5: Ash content (%) among the fish species

The ash content of SIS in the present study ranged between 16.54 -24.02% with the highest in *Puntius conchonius* (24.02%) and lowest in *Barilius bendelisis* (16.54%) (Fig. 5). *Barilius bendilisis, Acanthocobitis botia* and *Mystus tengara* with less or similar ash content (16.62%). There was a significant difference in Total ash content among small indigenous fish species ($p < 0.05$) (Table 7, Appendix).

4.1.3 Crude Protein content

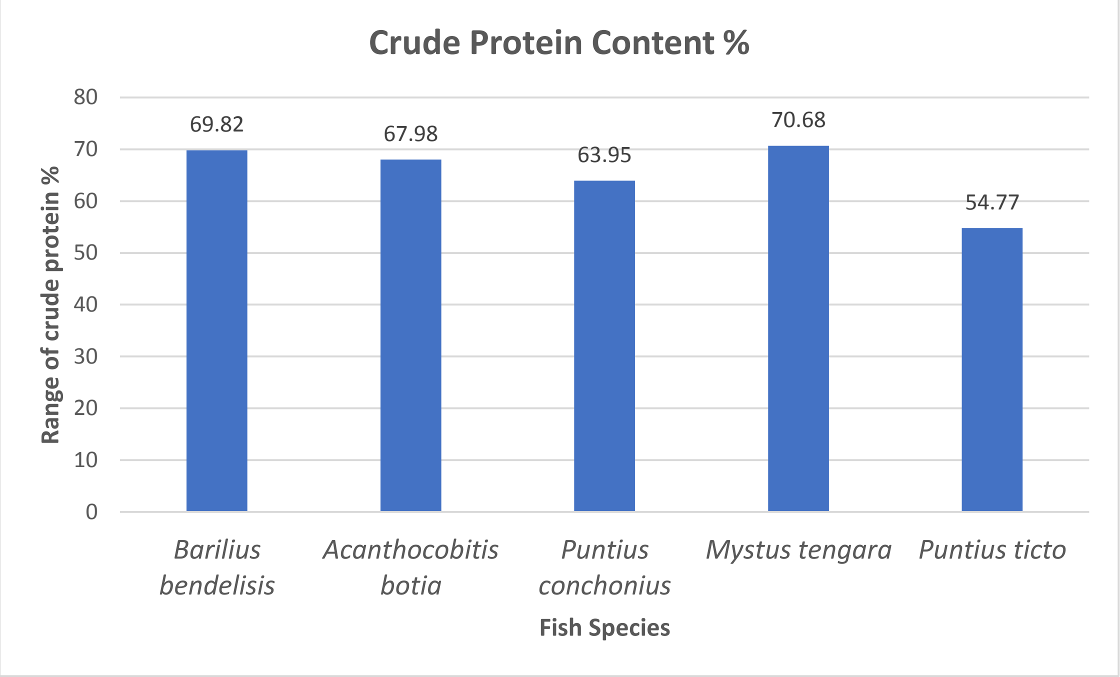


Figure 6: Variation of Crude protein content (%) among the fish species

The protein content of SIS in the present study varied between 54.77- 70.68% with the highest in *Mystus tengara* (70.68%) and lowest in *Puntius ticto* (54.77%) (Fig. 6). The protein content in *Barilius bendelisis* and *Mystus tengara* were less or more similar (69.82). There was a significant difference in Crude protein content among small indigenous fish species ($p < 0.05$) (Table 8, Appendix).

4.1.4 Crude Fiber content

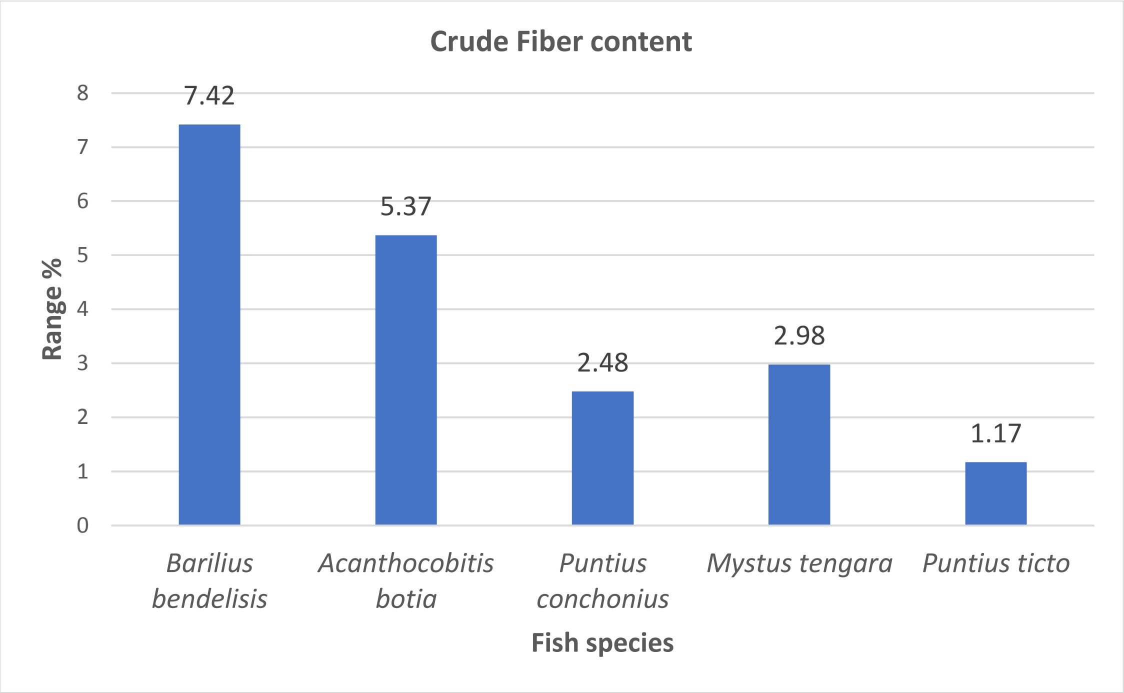


Figure 7: Variation of Crude fiber content (%) among the fish species

The crude Fibre content of small indigenous fish in the present study varied between (1.17-7.42) with the highest in *Barilius bendelisis* (7.42%) and lowest in *Puntius ticto* (1.17%) (Fig. 7). The fibre content in *Puntius chonchonius* and *Mystus tengara* were similar. There was a significant difference in Crude fiber content among small indigenous fish species ($p < 0.05$) (Table 9, Appendix).

4.1.5 Lipid content.

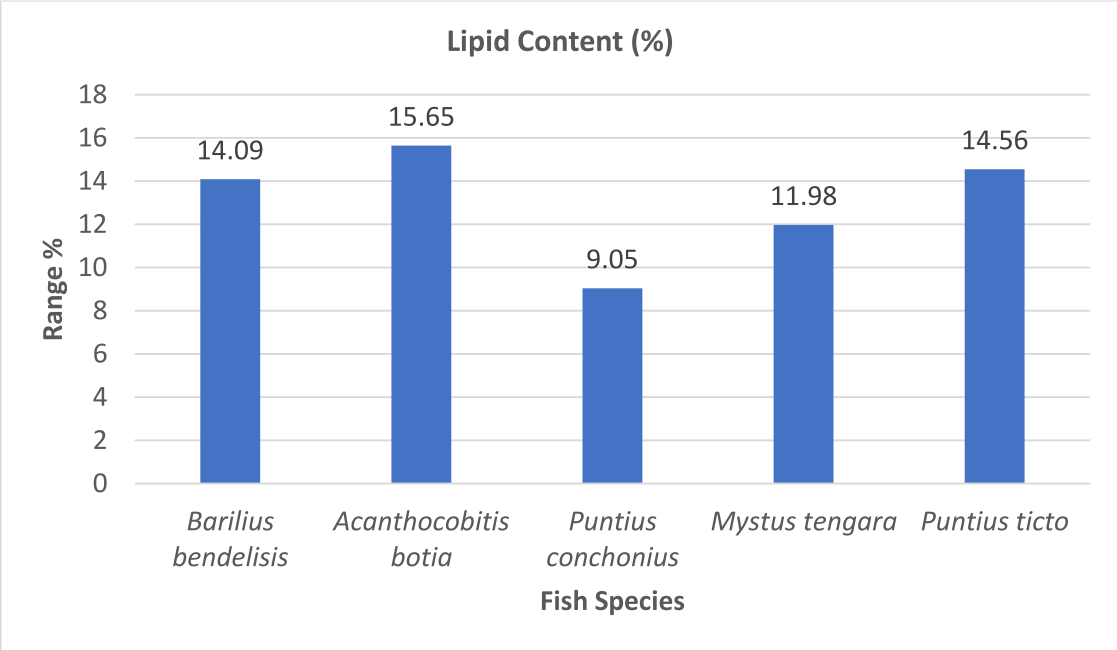


Figure 8: Variation of Lipid content (%) among the fish species

The Lipid content of SIS in the present study varied between 9.05- 15.65% with the highest in *Acanthocobitis botia* (15.65%) and lowest in *Puntius chonchonius* (9.05%) (Fig. 8). *Puntius ticto*, *Barilius bendelisis* and *Acanthocobitis botia* with less or similar lipid content (14.56%). There was a significant difference in Lipid content among small indigenous fish species ($p < 0.05$) (Table 10, Appendix).

5. DISCUSSION

The current investigation showed the proximate composition of some of the local small indigenous fish species of Budhi Rapti River Chitwan. The morphometric data (average length and weight) and proximate composition of five small indigenous fish species are displayed in Tables 2 and 3.

Moisture

Moisture is the major component in the fish muscle tissue. The value of moisture for most fish species typically fell between 60% and 80% (Aberoumand 2014; Love 1970). Mazumder et al. (2008) discovered that *Puntius chola, A. mola, P. atherinoides, and G. chapra* had moisture contents of 74.43%, 76.38%, 75.06%, and 73.32%, respectively, which were SIS and are not comparable to the current study.

(Ogundiran et al. 2014) suggested the high moisture content can cause the degradation of polyunsaturated fatty acids, increase the fish's vulnerability to spoilage by microorganisms, and consequently reducing fish quality for longer periods of preservation. The findings of our study show the fish species are highly vulnerable to spoilage with a high moisture range (98.17-99%).

According to (Rafikul Islam et al. 2020) the moisture content (%) of *C. fasciatus, A. mola, Puntius ticto, Puntius sarana, M. aculeatus, Mystus tengara, A. testudineus, H. fossilis, C. punctatus, and C. batrachus* were found to be 78.32, 77.99, 77.3, 75.27, 75.31, 79.12, 77.61, 79.89, 81.05, and 75.28 respectively which were SIS and are not like our present study.

According to this investigation, the range of the moisture contents of the various small indigenous fish species was between 98.17 to 99%. The outcome demonstrates that the main component of edible fish parts is moisture. The high moisture level can lead to the breakdown of polyunsaturated fatty acids, increase the susceptibility of the fish to microbial deterioration, and ultimately decrease the quality of the fish over extended durations of preservation.

Total ash

Ash serves as an indicator for a mineral level of food. After the biological stuff has been burned out, there is a substance left behind that is inorganic (Adewumi et al. 2014).

According to the range of ash found in this study (16.54 - 24 %). These fish species are likely rich sources of minerals including calcium, potassium, zinc, iron.

(Jana et al. 2018) and (Mohanty et al. 2019) illustrated the crude ash content ranging from 2 to 4 % in *Puntius sophore, Xenentodon cancilla, Mystus vittatus, Channa striatus and Heteropneustes fossilis* which seems to be lower than the present findings ranging from 16.54- 24 % with highest in *Puntius conchonius* (24.02%) and lowest in *B. bendelisis* (16.54%). The high value of ash in fish species is an indication of its high mineral content like magnesium, calcium, potassium, and zinc (Emmanuel et al. 2011). In the present study the fish species - *Puntius conchonius* contained high ash content (24.02%). Md. Akbal Husen et al. (2021) illustrated the total ash contents were varied from 12.64% to 19.65% with the lowest in the *Barilius barna* (12.64 ± 0.31) and highest in the *Puntius conchonius* (19.65 ± 1.54) in the studied native fish species of Begnas Lake which supports the present study.

Crude protein

The protein content of SIS in the present study varied between 54.77-70.68 % with the highest in *Mystus tengara* (70.68 %) and lowest in *Puntius ticto* (54.77%). According to (Ahmed et al. 2012) and (Mohanty et al. 2019), crude protein content in fish species- *Channa striatus, Puntius sophore, Mastacembelus armatus, Xenentodon cancilla, Mystus vittatus and Heteropneustes fossilis* ranged from 15 to 20 % which contrasted with our present findings (54.77 – 70.68%). The possible differences in protein level may be due to the availability of different fish food organisms in water (Jana et al. 2018). According to (Sultana et al. 2011) protein content ranged from 52.66 to 72.45% in seven dried fishes. These findings are like the present study.

M. S. A. Mazumder et al. studied the proximate biochemical composition of some small indigenous fish species from the study they found that variation of protein contents among the studied fish is not so high, and it ranged from 14.08% – 18.46%, which contrasted with present findings (54.77 – 70.68%).

Crude fiber

The proximate and mineral analysis of developed Brycinus *nurse* powder and tilapia fish powder were compared by (KASOZI et al. 2018). They discovered that crude fibre is just 0.1%, which appears to be lower than the available data, which ranges from 1.17

to 7.42 percent. Daniel and Imaobong E (2015) studied proximate analysis of three commercial fishes. They discovered that crude fiber is between 7.67% - 10.71% which appears like the available data, which ranges from 1.17 to 7.42 percent. Md. Mirazul Islam et al. (2020) studied evaluation of nutritional status of small indigenous fish species in Bangladesh. They found that Fibre content was in the range of 2.17 to 8.74% with the highest value obtained from Punti (*Puntius sophore*) and the lowest value from Mola (*Amblypharyngodon mola*). In the same way, the present experiment showed a similar percentage range (1.17 - 7.42%).

Lipid

The Lipid content of SIS in the present study varied between 9.05-15.65% with the highest in *Acanthocobitis botia* (15.65 %) and lowest in *Puntius chonchonius* (9.05%). (Prasanta Jana et al. 2018) discovered that the lipid content of six small indigenous fish species ranged between 1.18% and 5.21%. *B. Dario*, *Puntius sophore*, *and Puntius ticto* had the maximum amount of lipids, while *H. fossilis* had the lowest percentage, which does not match with the present findings. The possible difference in Lipid may be, lipid content is lower in smaller fish but rises with age and size according to (Sankar and Ramachandran 2001). According to (Hossain et al. 2015), the lipid content of Small indigenous fish species in Bangladesh typically ranged from 1.54 to 6.28%; however, this information is unrelated to our findings. According to (Hossain et al. 2015) The maximum total lipid percentage detected in *Amblypharyngodon mola* (Mola) by 6.28 percent and fewest by 1.54 in *Awaous grammepomus* (Bele) which were not like current findings.

6. CONCLUSION

6.1 Conclusion

The present study on proximate composition of small indigenous fish species from Budhi Rapti River, Chitwan indicated that, these fish species are excellent sources of moisture, protein, lipids, and ash. The present study concludes that *Mystus tengara* and *Barilius bendelisis* have high protein content and Lipid content is high in *Acanthocobitis botia* and *Barilius bendelisis*. Variation among the fish species was confirmed by the nutritional makeup of the investigated fish samples. Consuming these species is strongly advised because the current studies clarified more about the significance of small indigenous fishes. Low - income communities in Chitwan may have better nutritional security due to these small indigenous fish. Therefore, it is suggested that small indigenous fish species could be a good option for the vast majority of Chitwan impoverished people to satisfy their daily nutritional needs and improve their state of health. Present study will help people choose fish based on its nutritional worth rather than on its flavour, look, or other physical characteristics.

6.2 Recommendations

Based on this research, some important recommendations are given below:

- Only the five parameters of the proximate composition of small indigenous fish species is examined in the current study, So More research should be done on fish species with extra nutritional considerations. Specific detailed studies are needed to explore the amino acid profile, fatty acid profile and mineral quantities to establish a standard nutritional database of small indigenous fishes.
- Encouraging farmers and aqua culturists to consume small indigenous fishes as animal protein sources.
- Since the current result is inconsistent with earlier findings, lab work errors may be to blame. Therefore, I encourage additional research to reduce error for better outcomes.
- This is a preliminary investigation on a specific fish species. More research should be done on fish species with extra nutritional considerations.

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APPENDICES

Table 2: Length and weight of sample small indigenous fish species (Mean and ± SD)

S. N	*Mystus tengara*			*Barilius bendelisis*			*Puntius ticto*			*Puntius conchonius*			*Acanthocobitis botia*		
	Length (cm)	**Wt. (g) (before gut)**	**Wt.(g) (after gut)**	**Length (cm)**	**Wt. (g) (before gut)**	**Wt.(g) (after gut)**	**Length (cm)**	**Wt. (g) (before gut)**	**Wt.(g) (after gut)**	**Length (cm)**	**Wt. (g) (before gut)**	**Wt.(g) (after gut)**	**Length (cm)**	**Wt. (g) (before gut)**	**Wt.(g) (after gut)**
1.	10.5	10.1	9.5	9.5	8.9	8.6	4.1	3.6	3.4	6.7	5.1	5	5.5	1.9	1.8
2.	10.6	10.8	9.8	10.5	10.9	10.6	4.2	3.7	3.6	6.5	5.1	4.9	6.8	3.5	3.6
3.	9.1	6.3	6.2	10.5	11	10.8	4.6	4.5	4.4	6.5	4	3.9	6.3	2	2.1
4.	8.5	5.4	5	7.5	5.3	5	4.4	3.8	3.7	6.6	3.4	3.3	6.4	2.3	2.3
5.	8.5	5.4	5.3	8.3	5.5	5.2	4.6	4.2	4	6.4	4.3	4.1	6.3	2.2	2.3
6.	9.5	7.1	6.5	10	10.8	10.5	4.4	3.9	3.8	6.5	3.6	3.5	5	1.4	1.5
7.	7	3.4	3.1	8	5.7	5.4	4.7	4.2	4.1	6.5	3.2	3.1	6.5	2.7	2.8
8.	7	3.1	2.9	10.1	10.6	10.3	5.2	4.7	4.5	5.8	3	2.9	5.3	2	1.9
9.	7.1	3	2.9	11.8	17.7	17.1	4.5	4.2	4	6.3	4	3.8	5	1.8	1.7
10.	6.5	2.4	2.2	9.9	9	8.8	4.3	4	3.9	6.2	3.9	3.7	4.9	1.6	1.5
11.	9.2	6.8	6.4	10.2	12.5	12	4.4	4.2	4.1	6.4	4	3.9	5.4	2	1.9
12.	7.2	4.9	4.7	9.5	9	8.8	4.3	4	3.9	6.5	4.1	3.9	5.3	2	1.8
13.	7.2	4.8	4.7	9.1	9	8.7	4.6	4.3	4.1	6.6	4.2	4	5.1	1.9	1.8
14.	8.5	6.5	6.3	8.5	7	6.8	4.7	4.6	4.4	6.3	4	3.9	4.5	1.8	1.7
15.	9.4	6.8	6.6	7.9	6.8	6.6	4.5	4.3	4.2	6.4	4	3.8	5.9	1.8	1.9
16.	10.2	6.9	6.7	10.5	11	10.7	4.4	4.3	4.1	6.5	5.1	5	5.3	1.3	1.3
17.	8.7	6.2	6	8.4	8	7.8	4.7	4.5	4.4	6.4	5.1	4.9	4	1	1
18.	7.8	5.7	5.5	11.2	11.6	11.3	4.2	4.2	4.1	6.1	3.9	3.7	4.6	1.2	1.2
19.	10.1	7.1	6.9	10.3	11	10.8	5.1	4.5	4.3	6.3	5	4.9	4.7	1.3	1.3
20.	7.9	5.8	5.5	8.2	7.7	7.5	4.9	4.2	4	6.5	5.2	5	5	2	1.9
21.	9.5	6.7	6.4	7.9	7	6.8	4.4	4.2	4.1	5.9	4	3.9	6.1	2.7	2.6
22.	9.4	6.3	6.2	8.8	8.2	8	4.1	4	3.9	6.2	3.8	3.7	6.2	2.9	2.8
Mean	**8.61**	**5.98**	**5.70**	**9.39**	**9.28**	**9.00**	**4.51**	**4.19**	**4.05**	**6.37**	**4.18**	**4.04**	**5.46**	**1.97**	**1.94**
±SD	**1.25**	**2.02**	**1.88**	**1.20**	**2.82**	**2.76**	**0.29**	**0.28**	**0.27**	**0.22**	**0.65**	**0.64**	**0.75**	**0.59**	**0.61**

Table 3: Fish species (22 samples from each species) with their average length and weight before and after gut.

S. N	Name of Species	Length (cm) mean ±s.d.	Weight (g) before gutting mean ±s.d.	Weight (g) After gutting mean ±s.d.
1.	*Puntius ticto*	4.51 ±0.29	4.19 ±0.28	4.05 ± 0.27
2.	*Puntius chonchonius*	6.37 ±0.22	4.18 ±0.65	4.04 ±0.64
3.	*Mystus tengara*	8.61 ±1.25	5.98 ±2.02	5.7 ±1.88
4.	*Barilius bendelisis*	9.39 ±1.2	9.28 ±2.82	9.0 ±2.76
5.	*Acanthocobitis botia*	5.46 ±0.75	1.97 ± 0.59	1.94 ±0.61

Table 4: Proximate composition of small indigenous fish species (Mean and ± SD)

S. N	Name of Species	Moisture	Total Ash	Crude Protein	CF Crude Fiber	Lipid
1.	*Barilius bendelisis*	98.84 ±1.12	16.54 ±1.03	69.82 ±1.64	7.42 ±1.00	14.09 ±0.48
2.	*Acanthocobitis botia*	98.65 ±0.53	16.62 ±0.96	67.98 ±1.05	5.37 ±0.96	15.65 ±0.47
3.	*Puntius chonchonius*	98.52 ±0.70	24.02 ±0.99	63.95 ±0.44	2.48 ±0.43	9.05 ±1.41
4.	*Mystus tengara*	99 ±0.45	17.52 ±1.06	70.68 ±1.38	2.98 ±0.05	11.98 ±0.58
5.	*Puntius ticto*	98.17 ±0.80	24 ±0.40	54.77 ±0.80	1.17 ±0.03	14.56 ±0.60

Table 5: Fish species with their Total weight, Gutted weight, Dried weight, Homogenized weight, and Moisture

S. N	**Name of Fish Species**	**Number of Sample**	**Total Weight (g)**	**Total Gutted Weight**	**Total Dried Weight**	**Total Homogenized Weight**	**% Of moisture**
1	*Mystus tengara*	40	221	205.6	39.1	37.3	80.98
2	*Barilius bendelisis*	95	950.6	888.2	134.6	134	84.84
3	*Puntius ticto*	65	243.3	199.9	52.6	52.4	73.68
4	*Puntius conchonius*	55	190	168.8	43.8	43.4	74.05
5	*Acanthocobitis botia*	60	250	224.3	47.8	47.4	78.7

Table 6: One way ANOVA test by using SPSS software of Moisture between selected Fish species

ANOVA Test Moisture					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.212	4	0.303	0.521	0.723
Within Groups	5.812	10	0.581		
Total	7.024	14			

Table 7: One way ANOVA test by using SPSS software of Total Ash between selected Fish species

ANOVA Test Total Ash					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	184.192	4	46.048	53.719	<.001
Within Groups	8.572	10	.857		
Total	192.764	14			

Table 8: One way ANOVA test by using SPSS software of Crude Protein between selected Fish species.

ANOVA Test Crude Protein					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	507.332	4	126.833	96.259	<.001
Within Groups	13.176	10	1.318		
Total	520.508	14			

Table 9: One way ANOVA test by using SPSS software of Crude Fiber between selected Fish species.

ANOVA Test Crude Fiber					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	74.498	4	18.625	43.579	<.001
Within Groups	4.274	10	.427		
Total	78.772	14			

Table 10: One way ANOVA test by using SPSS software of Lipid between selected Fish species.

ANOVA Test Lipid					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	81.867	4	20.467	32.431	<.001
Within Groups	6.311	10	.631		
Total	88.178	14			

PHOTO PLATE

A. Pictures of field work

B. Pictures of lab work