# PROXIMATE COMPOSITION OF COMMERCIALLY IMPORTANT FISHES FROM THE FISH MARKET AROUND SAPTAKOSHI BARRAGE, EASTERN NEPAL



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**<u>Submitted to:</u>**

Central Department of Zoology

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September 2023

# DECLARATION

I hereby declare that the work presented in the thesis entitled "**PROXIMATE COMPOSITION OF COMMERCIALLY IMPORTANT FISHES FROM THE FISH MARKET AROUND SAPTAKOSHI BARRAGE, EASTERN NEPAL**" has been done by myself and has not been 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 COMMERCIALLY IMPORTANT FISHES FROM THE FISH MARKET AROUND SAPTAKOSHI BARRAGE, EASTERN NEPAL**" has been carried out by Mr. Bishal Pokhrel for the partial fulfilment of Master of Science in Zoology with special paper **Fish Biology and Aquaculture**. This is his 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 Mr. Bishal Pokhrel entitled "**PROXIMATE COMPOSITION OF COMMERCIALLY IMPORTANT FISHES FROM THE FISH MARKET AROUND SAPTAKOSHI BARRAGE, EASTERN 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 Mr. Bishal Pokhrel entitled "**PROXIMATE COMPOSITION OF COMMERCIALLY IMPORTANT FISHES FROM THE FISH MARKET AROUND SAPTAKOSHI BARRAGE, EASTERN 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.

## EVALUATION COMMITTEE

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## LIST OF ABBREVIATIONS

| **Abbreviated form** | **Details of abbreviations** |
|---|---|
| AA | Arachidonic Acid |
| ANOVA | Analysis of Variance |
| AOAC | Association of Official Analytical Chemists CF |
| | Crude Fiber |
| CP | Crude Protein |
| DHA | Docosahexaenoic acid |
| DM | Dry Matter |
| EE | Ether Extract |
| EPA | Eicosapentaenoic acid |
| FAO | Food and Agriculture Organization |
| IMCs | Indian Major Carps |
| NARC | National Agricultural Research Centre PUFAs |
| | Polyunsaturated Fatty Acids |
| T Ash | Total Ash |
| UNICEF | United Nations International Children's Emergency Fund |
| WHO | World Health Organization |

# ABSTRACT

Fish is one of the main food constituents in our diet as it includes essential fatty acids, amino acids, and some of the principal vitamins in sufficient amounts for healthy living. Commercially important freshwater fishes such as *Clupisoma garua, Labeo bata, Aspidoparia morar,* and *Wallago attu* might be a good alternative for the majority of the population in both rural and urban areas to meet their daily nutritional requirement in improving their health status. The main goal of this experiment was to find out the nutrient composition of the *Clupisoma garua, Labeo bata, Aspidoparia morar,* and *Wallago attu* from the fish market around the Saptakoshi Barrage. About 1.5 kilograms of each selected species were collected in November 2022 from the fish market near the Saptakoshi Barrage. The samples were cleaned, degutted, and oven-dried in an electric oven at between 70 – 80°C until the samples had constant weight 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 the National Agricultural Research Centre (NARC). Analyses of Proximate compositions such as Dry Matter (DM), Total Ash (T Ash), Crude Protein (CP), Ether Extract (EE), and Crude Fiber (CF) contents were carried out in triplicates according to the Association of Official Analytical Chemists (AOAC) methods. The Crude Protein content ranged from 72.83% to 90.62%, and *Wallago attu* was conspicuous in possessing a high content of Crude Protein (90.62%). The lowest Crude Protein content was found in *Labeo bata* (72.83%). The Crude Fiber content ranged from 0.74% to 3.12%. In *Clupisoma grua*, the Crude Fiber content was high (3.12%). The lowest was found in *Aspidosparia morar* (0.88%). The Ether Extract content varied from 12.67% to 36.12%. In *Aspidosparia morar*, the Ether Extract was very high (36.12%) when compared to other species. Among the four species, the lowest Ether Extract was observed in *Clupisoma garua* (12.67%). The Total Ash in the present study varied between 3.01% to 16.14% with the highest in *Clupisoma garua* (16.14%) and the lowest in *Aspidoparia morar* (3.01%). The Dry Mattercontent varied between 92.77% to 98.22% with the highest in *Aspidoparia morar* (98.22%) and the lowest in *Clupisoma graua* (92.77%). At the end of the study, a significant difference was observed between the different nutrient levels among commercial fish species ($p < 0.05$). This study shows that the investigated fish species are good sources of many major nutrients and essential elements.

# लेखसार

स्वस्थ जीवनको लागि पर्याप्त मात्रामा आवश्यक फ्याटी एसिड, एमिनो एसिड र केही प्रमुख भिटामिनहरू समावेश भएकाले माछा हाम्रो आहारको मुख्य खाद्य घटकहरू मध्ये एक हो। व्यावसायिक रूपमा महत्त्वपूर्ण ताजा पानीका माछाहरू जस्तै *Clupisoma garua, Labeo bata, Aspidosparia morar* र *Wallago attu* ग्रामीण र शहरी क्षेत्रका अधिकांश जनसंख्याको लागि दैनिक पोषण आवश्यकताहरू पूरा गर्न राम्रो विकल्प हुन सक्छ। यस अनुसन्धानको मुख्य लक्ष्य सप्तकोशी ब्यारेज वरीपरी रहेका माछा बजारमा पाइने *Clupisoma garua, Labeo bata, Aspidosparia morar* र *Wallago attu* को पौष्टिक संरचना पत्ता लगाउनु थियो। सप्तकोशी ब्यारेज नजिकको माछा बजारबाट २०२२ नोभेम्बरमा माछा सङ्कलन गरिएको थियो । रासायनिक विश्लेषणको लागि एकसङ्गित पाउडर प्राप्त गर्न नमूनाहरूलाई सफा गरी ७०- ८० डिग्री सेल्सियसको बीचमा इलेक्ट्रिक ओभनमा स्थिर तौल नभएसम्म सुकाइयो। राष्ट्रिय कृषि अनुसन्धान केन्द्र( NARC) को प्रयोगशालामा रासायनिक विश्लेषण गरिएको थियो। AOAC प्रोटोकल अनुसार सुक्खा पदार्थ , कुल खरानी, कच्चा प्रोटीन ,ईथर एक्स्ट्र्याक्ट,र कच्चा फाइबर  सामग्री जस्ता प्रोक्सिमेट रचनाहरूको परिक्षण ट्रिपलिकेटमा गरिएको थियो। पौष्टिक संरचना प्रजातिहरू बीचमा भिन्नता पाइयो। चार प्रजातीहरु बिचमा कच्चा प्रोटिन ७२.८३% - ९०.६२% सम्म पाइयो । *Wallago attu* मा कच्चा प्रोटिन (९०.६२%) उच्च रहेको र सबैभन्दा कम कच्चा प्रोटिन *Labeo bata* (७२.८३%) मा रहेको पाइयो। कच्चा फाइबर सामग्री ०.७४% देखि ३.१२% सम्म पाइयो । कच्चा फाइबर सामग्री सबै भन्दा धैरै *Clupisoma garua* (३.१२%) भने सबैभन्दा कम *Aspidosparia morar* (०.८८%) मा फेला पर्यो। ईथर एक्स्ट्र्याक्ट १२.६७% देखि ३६.१२% सम्म भिन्न थियो। *Aspidosparia morar* मा, ईथर एक्स्ट्र्याक्ट अन्य प्रजातिहरूको तुलनामा धैरै उच्च (३६.१२%) थियो। चार प्रजातिहरू मध्ये, सबैभन्दा कम ईथर एक्स्ट्र्याक्ट *Clupisoma garua* (१२.६७%) मा

देखियो। हालको अध्ययनमा कुल खरानी ३.०१% देखि १६.१४% को बीचमा भिन्न देखियो। जसमा *Clupisoma garua* (१६.१४%) मा उच्चतम र *Aspidosparia morar* (३.०१%) मा सबैभन्दा कम थियो। त्यसैगरी, सुक्खा पदार्थको सामग्री ९२.७७% देखि ९८.२२% को बीचमा भिन्न थियो। जसमा *Aspidosparia morar* (९८.२२%) मा उच्चतम र *Clupisoma garua* (९२.७७%) मा सबैभन्दा कम थियो। अध्ययनको अन्त्यमा, व्यावसायिक महत्वका माछा प्रजातिहरू ($p < 0.05$) बीचको विभिन्न पोषण तत्व बीच महत्त्वपूर्ण भिन्नता देखियो। यस अध्ययन अनुसार अनुसन्धान गरिएका माछा प्रजातिहरू प्रमुख पोषक तत्वहरूको राम्रो स्रोत हुन्।

# 1. INTRODUCTION

## 1.1. Background

According to WHO, “Nutrition is the intake of food, considered in relation to the body’s dietary needs”. Nutrition and health are related to each other as good nutrition is the cornerstone of good health. Fish in this context, is a healthy food and is a major player in human nutrition ensuring about 20% of protein intake to a third of the world’s population which is more evident in developing countries (Bene et al. 2007). Fishes are known for their high nutritional value. They are one of the most important sources of animal protein and have been widely accepted as a source of protein and other nutrients for human health (Andrew 2001). 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). The consumption of fish provides essential nutrients to a large number of people worldwide and plays a key role in nutrition. The proximate composition of fish includes the determination of moisture, protein, fat, and ash contents, which constitute about 96%−98% of the total constituents of the fish body (Imtiaz et al. 2022).

There are seven major classes of nutrients; they are carbohydrates, fats, fiber, minerals, proteins, vitamins, and water. These nutrient classes are categorized as either macronutrients or micronutrients. Macro and micronutrients available in seafood make it better compared to other protein sources. Moisture, protein, lipids, ash, and minerals are the most important macro and micronutrient components that act as sources of nutritive value of fish meat (Steffens 2006). Minerals are essential for the transmission of nerve impulses and muscle contraction, have a vital role in the acid-base equilibrium of the body, and thus regulate the pH of the blood and other body fluids. Furthermore, they serve as essential components of many enzymes, vitamins, hormones, and respiratory pigments, or as cofactors in metabolism, catalysts and enzyme activators (Albert 1987). The 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). Moreover, fish is a very important source of vitamins, and unsaturated essential fatty acids (PUFAS) particularly omega-3 (Raymond et al. 2020). The amount or percentage of each within a fish’s body is termed proximate composition. It is often determined in studies of fish pathology, growth, and nutrition (Copeland et al. 1999).

The freshwater habitat covers 5.3 % of the country's total area. Nepal is rich in aquatic biodiversity. 236 species of fish are reported in Nepal (Economic survey 2079/80). In this study, we have selected four commercially important freshwater fish species from the fish market around the Saptakoshi Barrage. The selected fish represented typical habitats, food niches, and market prices. Fish species, in general, are considered a good source of essential minerals required by the human body for the maintenance of certain physiological processes which are essential to life. Despite having such marvelous nutritive value, nutritionists are lacking to experiment with the nutritional value of Nepalese fish. Nowadays consumer wants to know and ensure the nutritional value of the products that they are eating. Also, the determined nutritional value of experimented fishes is expected to contribute to small-scale fisheries to breed and propagate those fishes which can help rural peoples generate income sources and hence alleviate poverty. In view of these facts, the present study was therefore initiated to compare the proximate of four different commercially important fishes in the fish market around the Saptakoshi Barrage.

## 1.2 Objectives of the study

### 1.2.1 General objective

The main objective of this study is to investigate the proximate composition viz. Dry Matter (DM), Total Ash (T Ash), Crude Protein (CP), Ether Extract (EE), and Crude Fiber (CF) of four commercially important fishes - *Clupisoma garua, Labeo bata*, *Aspidoparia morar* and *Wallago attu* from the fish market in Koshi Barrage.

### 1.2.2 Specific objectives

- To estimate the Dry Matter, Total Ash, Crude Protein, Ether Extract, and Crude Fiber of four fish species.
- To compare the difference in proximate composition of macro-nutrients in selected fish species from the fish market around Saptakoshi barrage.

## 1.3 Rationale of the study

Scanty works were carried out in the proximate composition of fishes in Nepal. The nutraceutical feature of fish is of great importance for healthy living. It may be therefore imperative to determine the proximate composition and mineral content of fish which will be helpful to dieticians, nutritionists, researchers, fish farmers, and related stakeholders to promote fish as a healthy food in human nutrition. 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. Furthermore, the nutrition status of the species will definitely motivate for conservation and propagation of their culture.

# 2. LITERATURE REVIEW

## 2.1 The nutrient composition in fishes

Fish is high in vitamins and minerals, making it ideal for both young and old consumers (Edem 2009). The nutritional value of high-quality fish and fishery products is increasing year after year, owing to the fact that they include a variety of helpful healthful elements (FAO, 1986). Proteins with high nutraceutical value, minerals, group B vitamins, vitamins A and D, and unsaturated essential fatty acids (PUFAS), particularly arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Zmijewski et al. 2006, Memon et al. 2010, Raymond et al. 2020). Despite having such exceptional nutraceutical properties, Nepalese fish nutrition has received little attention in comparison to other fauna. Biochemical research into the proximate and mineral content of fresh varieties of fish is relatively a new approach in Nepal.

According to Begum et al. (2012), moisture, protein, fat, and ash makeup 96% - 98% of the body composition of fish. Fish contains a substantial amount of protein (13-20% by fresh weight) and all of the essential amino acids. Fish fat content ranges from 0.2 to 15% and fluctuates based on the species and season in general, with fish having less fat than red meats (Calder 2004). Fish can be classified into four groups based on their fat level, according to Ackman (1989): lean fish (less than 2% fat), low fat (2-4% fat), and medium fat (4-8% fat). Normally high-lipid fishes have less water and more Protein than low-lipid fishes. Moreover, there is an inverse relation between the size and moisture content of the fish. In general, the protein contents show an inverse relationship with moisture contents, and the reduction in protein level is connected with the denaturation of fish protein that is associated with freezing. Similarly, the lipid content is in inverse relation to the freezing time (Siddiqui et al. 2010, Hossain et al. 2015, Jim et al. 2017).

Minerals are a class of micronutrients that are required for fish. Twenty-nine of the 90 naturally occurring inorganic elements are thought to be needed for all farmed animals, including fish (Antony 2015). The necessary mineral elements are typically divided into two groups based on their concentration in the animal body: macro elements and microelements. All fish species are considered good suppliers of minerals in general, with levels ranging from 1.2-1.5% (Love 1970, Fawole et al. 2007). Feeding habits, ecological demands, metabolism, age, size, and length of the fish, as well as their environments, all have a significant impact on mineral concentrations in different species (Yilmaz 2005). Concentrations inside an organism can vary depending on tissue, weight, and age (Erkan and Ozden, 2007, Paul et al. 2016). Furthermore, the difference could be due to the metal detection method utilized (Mahanaty et al. 2014). The mineral content of habitat fish is influenced by the concentration of minerals in harvest streams. In general, all fish species are considered good suppliers of minerals.

Fish makes up more than 50% of the animal protein consumed in many Asian nations, whereas in Africa, the percentage is only 17.50% (Willmann et al. 1998). The combined amount of fish produced in Nepal in 2013/2014 from aquaculture and capture fisheries was expected to be roughly 64,900 metric tons (Durbar 2014). In Nepal, fishing is practiced by local fishing communities such as Tharu, Chaudhary, and Jalari. Small indigenous species are their main food source, which they catch from nearby rivers, lakes, marshy plains, reservoirs, and small 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 (Mohanty 2015). Forty-five percent of mortality in children under five is attributable to undernutrition; nutritional deficiencies are responsible for 50% of years lived with disability in children aged four and under (Golden et al. 2016). Forty- one percent of Nepalese children are thought to be stunted or chronically malnourished (Faruk et al. 2012). Nepal is one of the least developed countries when it comes to food and nutrition. According to Faruk et al. (2012), 41% of Nepalese children are believed to be stunted or chronically malnourished.

## 2.2 Nutritional contribution of commercial fish species

The proximate composition of two clupeid species (*Pellonula afzeliusi* and *Sierrathrisa leonensis* in both fresh and sundried forms) revealed that crude protein and ash were higher in sundried samples of both fishes, whereas moisture content was higher in fresh samples (Balogun 1988). According to Memon et al. (2010), the crude protein, crude fat, and ash content of *Clupisoma garua* (CG) is higher than that of *Wallago attu* (WA), however, the moisture content of CG is lower than that of WA. Among the six freshwater fish studied, *Wallago attu* has the highest crude protein and n-3 fatty acid content (Pradhan et al. 2020). Similarly, Sankar and Ramachandran (2001) examined the crude protein levels of three freshwater fishes, including *Labeo rohita*, and discovered that the crude protein of all three fishes was higher in small fishes. The difference in the proximate composition of fishes may arise as a result of species, sex, size, zoogeography, seasonal variation or eating habits of the fish, organs, and fasting condition of the fish. The spawning period of the fish also has a negative impact on protein content. Furthermore, these characteristics are linked to low levels of pollution and high oxygen concentration in harvest streams. According to Mahanty et al., (2014) *Puntius sophore* has a higher crude fat and total ash content than the indian major carps (IMCs) *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala*. Raymond et al. (2020) studied the proximate composition of wild tilapia (*Oreochromis niloticus*) and wild african catfish (*Clarias gariepinus*) and discovered that crude fat, crude protein, and ash content increases with size in both fishes, while moisture content decreases with size in both fishes. The lower moisture content in wild fish could be attributed to increased physical efforts required by wild fish species to collect food items in their natural habitat, as opposed to farmed fish, which have an ample food supply in the ponds (Jim et al. 2017).

Paul et al. (2016) investigated the mineral content of different weight groups of Indian main carps (*Labeo rohita*, *Catla catla,* and *Cirrhinus mrigala*) and discovered that calcium concentration was greater ($P<0.05$) in *Labeo rohita* in all weight groups. Similarly, the sodium, potassium, and iron content of *Labeo rohita* were greater ($P<0.05$) in >2000g when compared to others, and the zinc content of *Labeo rohita* 51-500 g group was higher ($P<0.05$) when compared to others. Phosphorus and manganese concentrations were similar across all groups, regardless of weight ranges or species. Mahanty et al. (2014) discovered that the mineral profile of *Puntius sophore* is richer in microminerals and calcium than the mineral profile of Indian big carp. Sharma et al. (2015) discovered that the mineral composition of *Tor putitora* in three different geo-climatic situations and three different body weights is high in calcium and potassium, but the mean concentration of sodium is low. Furthermore, the calcium and potassium content dropped as body weight increased.

Death rates are greater in affluent and even developing nations due to nutritional inadequacies caused by a lack of proteins including key amino acids, vitamin A, iron, and other nutrients in their diets (Muler and Krawinkel 2005). Fish is an extremely nutritious meal in the human diet Fish offers nutritional stability and is one of the most influential foods in lowering mortality rates due to nutritional inadequacies. Fish include a variety of critical elements, including minerals and polyunsaturated fatty acids (PUFA), particularly Eicosapentaenoic acid (EPA) and Docosahexaenoic Acids (DHA) (Golden et al. 2016). PUFA has a vital function in lowering the risk factors associated with a variety of cardiovascular illnesses (Gammone et al. 2019).

# 3. MATERIALS AND METHODS

## 3.1 Study area

The Saptakoshi River is Nepal's largest and drains eastern Nepal, mainly the area east of Gosaikunda (north of Kathmandu) and west of Kanchenjunga. It has three major affluents: The Sunkoshi in the west, the Arun in the center, and the Tamor in the east. As a pride of the Eastern area, the Koshi Bridge, one of Nepal's longest and most intricate bridges, is situated between the borders of the Saptari and Sunsari districts. The fish samples for the present study were collected from the fish market around Koshi Barrage.

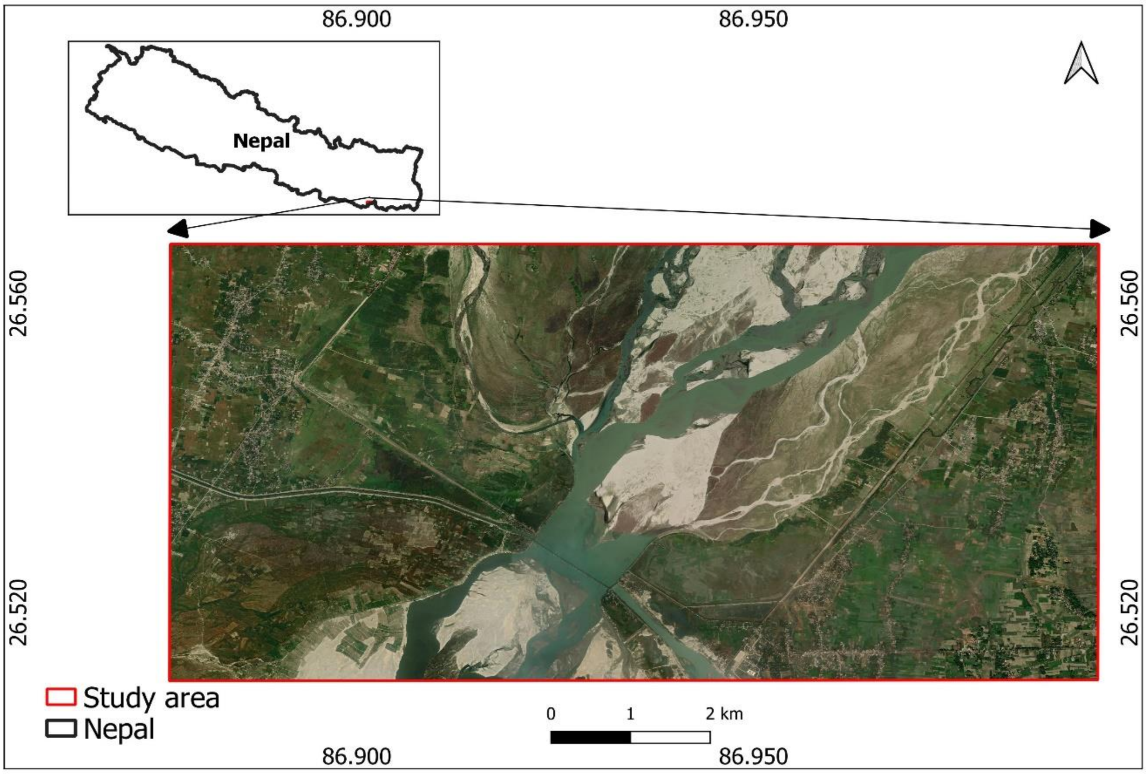


**Figure 1**: Study area map

## 3.2 Collection and identification of fishes

### 3.2.1 Collection

Samples of four commercially important fish species were bought from the fish market around the Saptakoshi barrage. The keys provided in the book, Ichthyology of Nepal, (Shrestha 2008) were used to identify the various kinds of fish that were bought. The fish samples were transported to the Nilgiri College, Itahari, Sunsari in an icebox for preparation of sample in powdered form.

### 3.2.2 Identification

**Table 1:** Local name, Scientific name, Order, and Family of fish species bought from Fish market nearby koshi barrage.

| **S.N** | **Local Name** | **Scientific Name** | **Order** | **Family** |
|---|---|---|---|---|
| 1. | Jalkapoor | *Clupisoma garua* | Siluriformes | Schilbeidae |
| 2. | Rohu | *Labeo bata* | Cypriniformes | Cyprinidae |
| 3. | Karangi | *Aspidoparia morar* | Cypriniformes | Cyprinidae |
| 4. | Bohari | *Wallago attu* | Siluriformes | Siluridae |

***Clupisoma garua***

**Labeo bata**

***Aspidosparia morar***


***Wallago attu***

**Figure 2:** Selected commercial fishes for analysis of proximate composition.

### a. *Clupisoma garua* (Hamilton, 1822)

It has elongate and compressed body devoid of scales. Color is silvery with dark back and silvery sides. Caudal fin is deeply forked and the lower lobe is longer. Barbels 4 pairs. Eye with board adipose lids. Wide gill opening. Dorsal spine slender and comparatively weak than pectoral. It is commonly found in freshwater bodies. Niche is bottom layer of water body. Bottom dweller fish species and feeds on molluscs, insects, small fishes and decaying matter.

### b. *Labeo bata* (Hamilton, 1822)

Body is elongated. Dorsal profile is more convex than the ventral. Color is predominantly silvery but dark along the back appear yellow on dorsal half of flanks, silvery on lower half of flanks and belly; an irregular faint black blotch is present on anterior scale of lateral line. Snout is bluntly pointed. Snout slightly projecting beyond mouth of ten studded with pores. A pair of small maxillary barbells is hidden inside the labial fold. Darkish on upper half, silvery below is light orange. *L. bata* is predominantly a bottom feeder. It is an herbivorous fish and feeds mainly on algae, plant parts, decayed organic matter, insects, mud and debris.

### c. *Aspidoparia morar* (Hamilton, 1822)

Body is elongated and laterally compressed. Mouth small and inferior, upper jaw longer than lower. No Barbels. Dorsal fin originated behind pelvic base. Lateral line present, curved and runs through the lower half of body. Body color is black brownish, belly yellowish-silvery, fins dark yellow. *A. morar* is omnivorous hence feed on both phytoplankton and zooplankton (Hossain et al. 2015).

### d. *Wallago attu* (Bloch & Schneider, 1801)

It has elongate, strongly compressed body. Head is large and fairly depressed. Mouth very deeply cleft, its corner reaching far behind eyes. Teeth in jaws set in wide bands; vomerine teeth in two small patches. Barbels two pairs; maxillary barbels extending to anterior margin posterior of anal fin, mandibulary barbels to angle of mouth. Eyes small, with a free orbital margin. Dorsal fin small, anal fin very long. This is a migratory catfish and can live in running and stagnant water. A predatory fish with voracious feeding capacity by the virtue of a large mouth best with sharp teeth.

### 3.3 Preparation of samples for analysis

Physical cleaning, descaling, and degutting were performed on the samples. They were also oven dried for 24 hours at 70 – 80°C. Finally, the homogenization procedure was carried out using a homogenizer and a grinder mixture, and the homogenized weight was recorded. After homogenization, the final minced powdered samples were placed in sealed plastic airtight containers and cooled for a short period of time before testing. The dried samples were used for proximate analysis.


**Figure 3**: Flow chart of sample preparation of four commercially important fish species for proximate analysis.

## 3.4 Proximate analysis

Following the protocol of the Association of Official Analytical Chemists (AOAC 1990), the proximate composition of sample fishes was determined. The proximate analysis was determined at the National Agricultural Research Centre (NARC) in Khumaltar, Lalitpur, Nepal. Dry Matter (Oven Drying Method), Crude Protein (Micro-Kjeldahl Method), Ether Extract (Soxhlet Method), Crude Fiber (Weende method) and Total Ash was determined using AOAC (2000) standard techniques.

### 3.4.1 Calculation of Dry Matter

Firstly, crucible was oven dried at 100℃ for 24 hours and desiccated for 30-40 minutes. The crucible was then weighed. 1gram of sample was poured in crucible and weighed. Again, the sample with crucible was oven dried at 100℃ for 24 hours and desiccated for 30-40 minutes. The crucible with sample was then weighed. After that Ashing was carried out at furnace of 500℃ -600℃ for 2 hours. The sample was finally allowed to cool at about 100℃ and placed in desiccator for 30-40 minutes. The sample was then weighed.

$$\text{DM \%} = \frac{\text{Dry sample weight}}{\text{Sample weight}} \text{ X } 100$$

### 3.4.2 Calculation of Total Ash

We determined the ash content of the sample by weighing the crucible containing the dried sample and heating it in a muffle furnace at 5500°C for three hours. The sample crucible was reweighed, and the difference in sample weights indicated the ash content.

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

### 3.4.3 Calculation of Crude Protein

To determine Crude Protein, three distinct steps were used: digestion, distillation, and titration. The 0.5-1gram sample was weighed and placed in a digestive tube. And 5 gm of the digestion combination ($Na_2SO_4$ and $CuSO_4$ in a 4:1 ratio) was carefully poured into the digestion tube, followed by 20 ml of concentrated Sulphuric acid. The tube was then placed in a digesting chamber and heated at $400^o$-$500^o$C for two hours before being cooled. After heating, the solution turns green. In a volumetric flask, 100 ml of volume (Sample + distilled water) was prepared, and the color of the solution changed to blue with the addition of distill water and left overnight. For the distillation process, 10ml of solution was placed in a tube and placed in a distillation chamber. The sample is thoroughly mixed with 10ml of 4%boric acid, 10ml of sodium hydroxide, and 50 ml of distilled water before being distilled for 3 minutes. The distilled color changes from dark to pale blue. The distillate was titrated with 0.03N $H_2$ $SO_4$ until it became clear.

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

### 3.4.4 Calculation of Ether Extract

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.

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

### 3.4.5 Calculation of Crude Fiber

One gram of the sample was placed in a crucible and was digested with 50 ml of 1.25% $H_2SO_4$ for half an hour at 400-500°C. Then, the sample was washed with hot distilled water. Furthermore, Acetone was also used 3-4 times for washing the sample. The sample was then digested with 50 ml of 1.25% NaOH solution for half an hour. Again, the sample was washed with hot distilled water and Acetone was separately used 3-4 times for washing the sample. Now, the sample was oven-dried for 24 hours at 65°C and desiccated for 10-15 minutes. The crucible was then weighed. After weighing the crucible, Ashing was carried out at 500 to 550°C for 3.5 hours and then the crucible was weighed after putting it in desiccated for 10-15 minutes.

$$\text{Ether Extract (\%)} = \frac{\text{Dry sample weight} - \text{Ash weight X 100}}{\text{Fresh sample weight X DM}}$$

### 3.5 Statistical Analysis

The results reported for each species were in the form of means ± standard deviation (SD) obtained from a triplicate determination of four fish species. The investigated parameters (proximate composition) were subjected to one-way analysis of variance (ANOVA) in R Studio to compare variances across the means (or average) of different groups, with statistically significant differences reported at ($P < 0.05$). Furthermore, the Tukey test was used to assess the mean difference between each pair of groups. The results were presented in box plot formats.

# 4. RESULTS

## 4.1 Proximate composition

The proximate composition of commercial fish species in the fish market at Koshi Barrage is shown in Table 7 (Appendix). The proximate composition of different fish species shows there was a significant difference between the different nutrient levels among commercial fish species ($p < 0.05$).

### 4.1.1 Dry Matter

The DM content ranged from 92.77% to 98.22%. *Aspidoparia morar* was conspicuous in possessing a high content of DM (98.22%). The lowest content of DM was found in *Clupisoma garua* (92.77%). The DM content in *Labeo bata* and *Wallago attu was* recorded at 94.20% and 94.76%, respectively. ANOVA test in response to DM (Table 2, Appendix) indicates that there was a statistically significant difference in sample means of several groups of data for selected fish species. Tukey's (Figure 9, Appendix) identified significant differences between *Clupisoma garua* and *Aspidosparia morar* only among the selected fishes for DM.

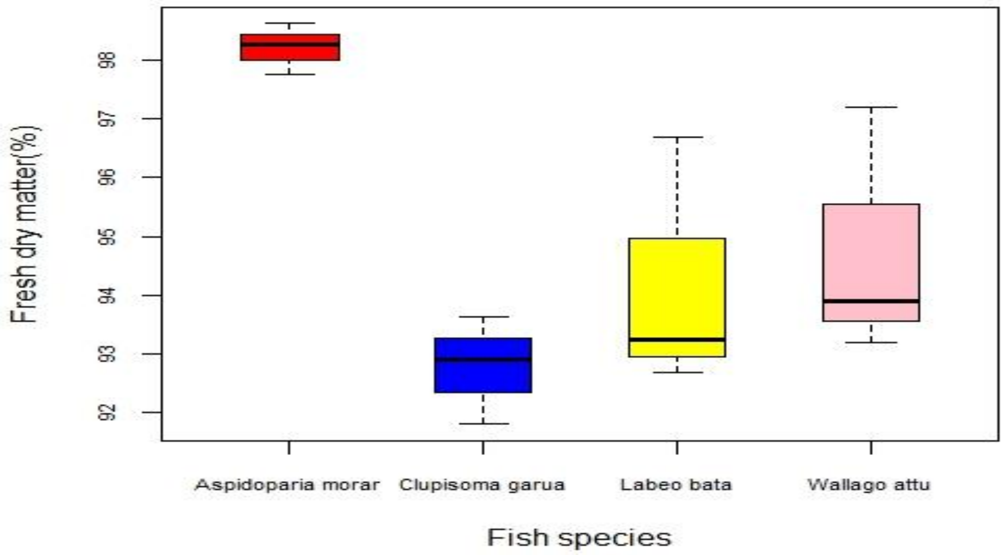


**Figure: 4** Box plots of dry matter (%) among selected fish species.

### 4.1.2 Total Ash

The Total Ash content ranged from 3.01% to 16.14%. *Clupisoma garua* (16.14%) was conspicuous in possessing high content of Total Ash. The lowest content of DM was found in *Aspidoparia morar* (3.01%). The Total Ash content of *Labeo bata* and *wallago attu* was recorded at 14.68% and 3.25%, respectively. ANOVA test in response to T Ash (Table 3, Appendix) indicates that there was a statistically significant difference in sample means of several groups of data for selected fish species. Tukey's (Figure 10, Appendix) identified significant differences between *Clupisoma garua* and *Aspidosparia morar*, *Labeo bata* and *Aspidosparia morar, Wallago attu* and *Clupisoma garua* & *Wallago attu* and *Labeo bata* among the selected fishes for T Ash.

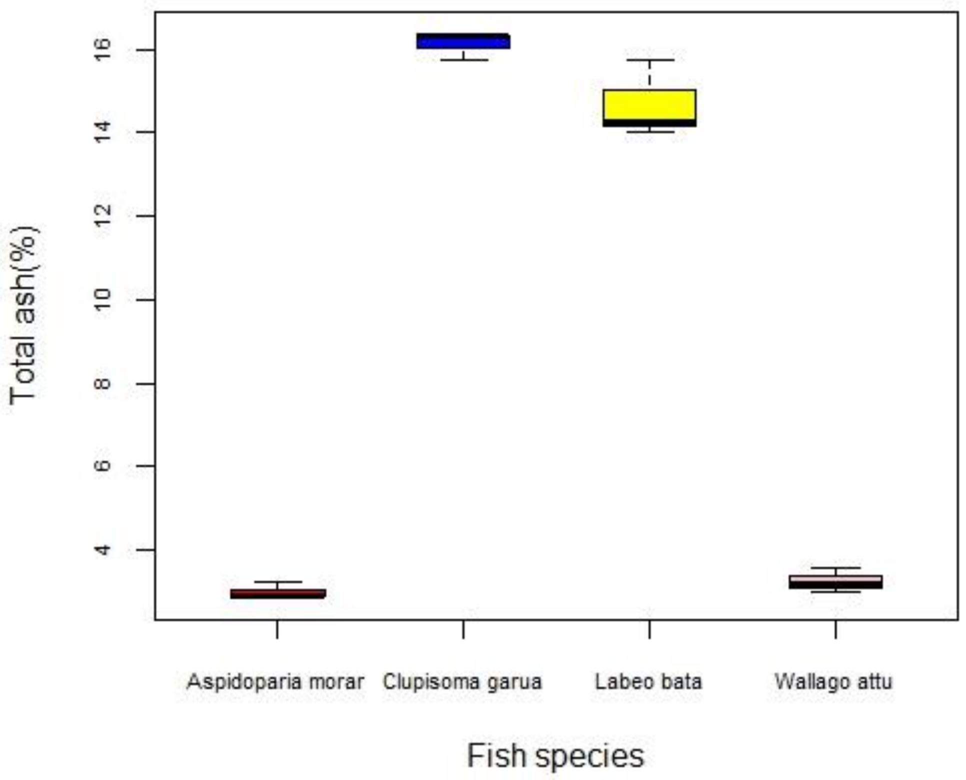


**Figure: 5** Box plots of total ash (%) among selected fish species.

### 4.1.3 Crude Protein

The crude protein content ranged from 72.83% to 90.62% and *Wallago attu* was conspicuous in possessing a high content of crude protein (90.62%). The lowest crude protein content was found in *Labeo bata* (72.83%). *Clupisoma garua* and *Aspidosparia morar* were recorded with 80.64% and 82.36% crude protein, respectively. ANOVA test in response to CP (Table 4, Appendix) indicates that there was a statistically significant difference in sample means of several groups of data for selected fish species. Tukey's (Figure 11, Appendix) identified significant differences between *Labeo bata* and *Aspidosparia morar, Wallago attu* and *Aspidosparia morar, Wallago attu* and *Clupisoma garua & Wallago attu* and *Labeo bata* among the selected fishes for CP.

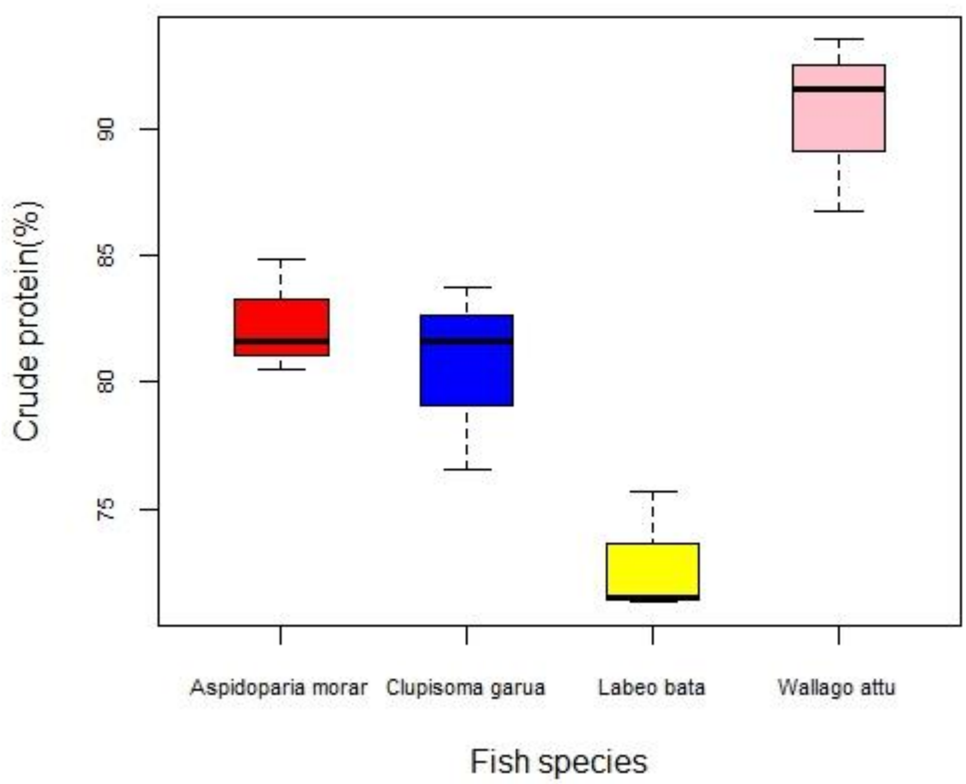


**Figure: 6** Box plots of crude protein (%) among selected fish species.

### 4.1.4 Ether Extract

The EE content varied from 12.67% to 36.12%. In *Aspidosparia morar*, the EE was very high (36.12%) when compared to other species. Among the four species, the lowest EE was observed in *Clupisoma garua* (12.67%). *Labeo bata* and *Wallago attu* were recorded with 27.02% and 12.91% EE, respectively. ANOVA test in response to EE (Table 5, Appendix) indicates that there was a statistically significant difference in sample means of several groups of data for selected fish species. Tukey's (Figure 12, Appendix) identified significant differences between *Clupisoma garua and Aspidosparia morar*, *Wallago attu and Aspidosparia morar*, *Labeo bata and Clupisoma garua & Wallago attu* and *Labeo bata* among the selected fishes for EE.

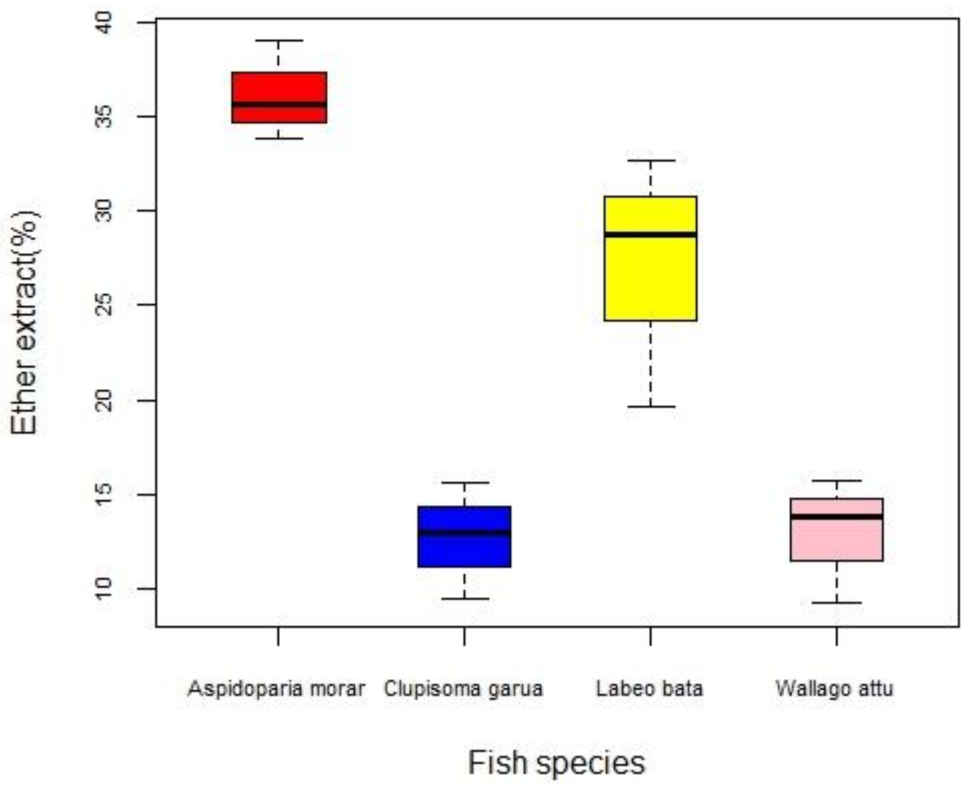


**Figure: 7** Box plots of ether extract (%) among selected fish species.

### 4.1.5 Crude Fiber

The CF content ranged from 0.74% to 3.12%. However, in *Clupisoma garua*, the CF content was high (3.12%). The lowest was found in *Labeo bata* (0.74%). *Aspidosparia morar* and *Wallago attu* were recorded with 0.88% and 1.58% CF, respectively. ANOVA test in response to CF (Table 6, Appendix) indicates that there was a statistically significant difference in sample means of several groups of data for selected fish species. Tukey's (Figure 13, Appendix) identified significant differences between *Clupisoma garua* and *Aspidosparia morar & Labeo bata* and *Clupisoma garua* among the selected fishes for CF.

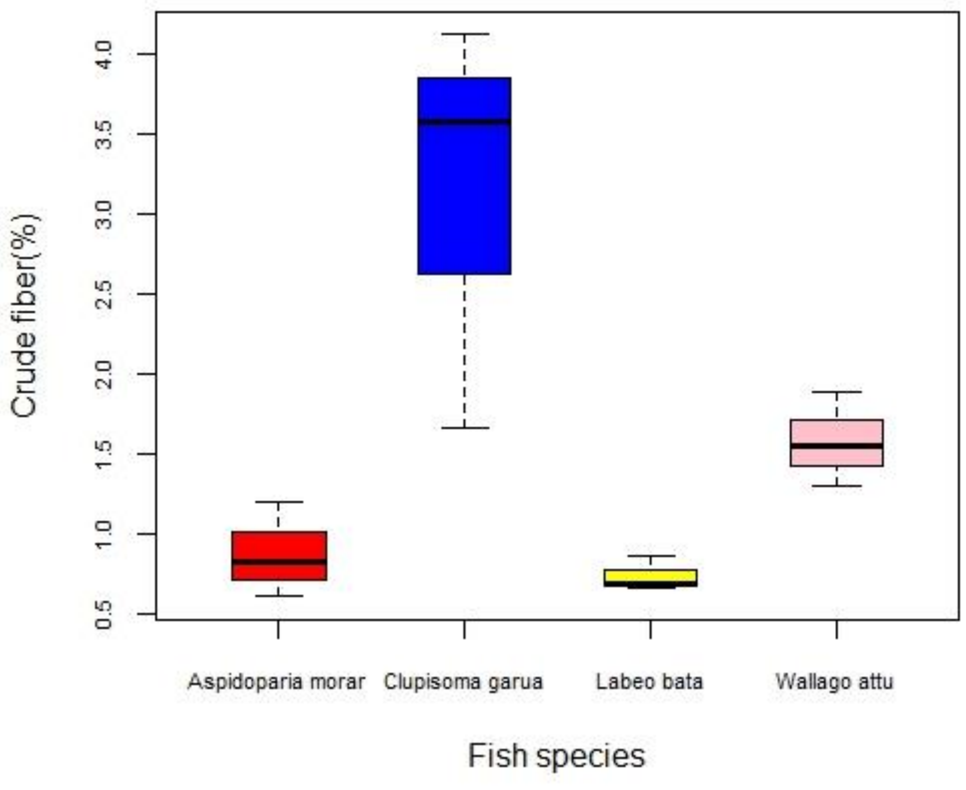


**Figure: 8** Box plots of crude fiber (%) among selected fish species.

# 5. DISCUSSION

## Dry Matter

Generally, moisture is an important component of a fish's body composition. A sample's moisture content is a measure of how much water it possesses. Dry matter is calculated by reducing the moisture content of the dried sample. The dry matter in the present study content varied between 92.77% to 98.22% with the highest in *Aspidoparia morar* (98.22%) and the lowest in *clupisoma graua* (92.77%). The proximate composition in the body tissue of four selected fish species by (Fawole et al. 2007) ranged from 92.10 % to 94.20%. Similarly, Musad and Haldar (2017) calculated the dry matter of four selected fish species ranging from 92.58% to 94.31% including *Aspidosparia morar* of 92.58% which satisfies our findings. Fish have soft skin, which provides for easy water permeability at the skin-water contact, allowing them to easily absorb more water. Organisms benefit from high moisture content because it allows for smooth enzymatic reactions. High moisture content in fish, on the other hand, might be a negative since it makes the fish more susceptible to microbial spoilage, accelerates oxidative destruction of polyunsaturated fatty acids, and lowers the quality of the fish, reducing its preservation time (Omolara and Omotayo, 2009).

## Total Ash

Ash serves as an indicator of the mineral level of food. After the biological stuff is burned out, there is a substance left behind called inorganic (Adewumi et al. 2014). The high value of ash in this study (3.01% to 16.14%) is an indication of its high mineral content like magnesium, calcium, potassium, and zinc (Emmanuel et al. 2011). Jahan et al. (2018) illustrated the total ash contents varied from 4.34% to 18.43% with the lowest in the *Mastacembelus pancalus* (4.34±0.23) and the highest in the *Mystus cavasius* (18.43±0.77) including *Wallago attu* (4.62±0.65) in the studies fish species of Chalan beel area, Bangladesh which satisfies the data obtained in our study. Similarly, Memon et al. (2010) reported the total ash content of *Wallago attu* and *Clupisoma garua* 2.90 ± 0.14 and 4.95 ± 0.54 respectively on a wet basis (%). This value differed from our findings could be attributed to the kind of sample taken for analysis. Furthermore, the ash content of fish wild fish is significantly higher contents as compared to the farmed fish (Raymond et al. 2020). Mahafuj et al. (2012) discovered that the crude ash content of *Labeo bata* was 16.63±0.20%

which satisfies our findings. Furthermore, Sharma et al. (2019) illustrated the total ash content in *Aspidoparia morar* at 2.36% which seems to be similar to our finding (3.01%).

## Crude Protein

Proteins are considered the most versatile biomolecules which are made up of amino acids held together by a peptide bond. In the current investigation, the crude protein content of four selected fish species ranged from 72.83% to 90.62% and *Wallago attu* was conspicuous in possessing a high content of crude protein (90.62%). The lowest crude protein content was found in *Labeo bata* (72.83%). Calder (2004) reported that on a fresh weight basis, fish contains protein of about 13-20% and all the essential amino acids. Similarly, Love (1970) stated that fishes constitute 16%−21% protein. The proximate composition (crude protein and crude fat) of oven-dried fish is generally higher as compared to raw fish due to loss of moisture content (Jahan et al. 2018). This could be attributed to the extent of drying which lowered moisture and concentrated proteins. Memon et al. (2010), reported that the crude protein content in *Clupisoma garua* (CG) is higher than that of *Wallago attu* (WA) whereas the content of moisture is lower in CG compared to WA. This finding remains just the reverse of our findings. Jahan et al. (2018) calculated the average protein content of five oven-dried fish ranged from 56.71±0.75% to 77.12±0.36 % including WA 72.40±0.52% which contrasted with our present findings. The deviation may have occurred in the proximate composition of fishes due to species, sex, size, zoogeography, seasonal variation or feeding habits of the fish, organs, and fasting condition of the fish (Jim et al. 2017). According to Mahafuj et al. (2012) on a dry, the protein content of *Labeo bata* was obtained to 66.75±0.39 %. Similarly, Masud and Haldar (2017) evaluated 60.79 % of protein content in *Aspidosparia morar* on a dry weight basis. These findings seem to be agreed with our findings.

## Ether Extract

The ether extract content varied from 12.67% to 36.12%. In *Aspidosparia morar*, the ether extract was very high (36.12%) when compared to other species. Among the four species, the lowest ether extract was observed in *Clupisoma garua* (12.67%). Mahafuj et al. (2012) discovered that the lipid content of *Labeo bata* on a Dry Matter was 13.44±0.08 %, which does not match our findings. According to Masud and Haldar (2017), the fat content of four selected fish species ranged from 15.56 to 29.76% including *Aspidosparia morar* at

24.53%. The percentage means of proximate composition in the body tissue of four selected fish species ranged from 3.50% to 8.25% (Fawole et al. 2007). However, this information is unrelated to our findings. The possible difference in Lipids may be, age variation and sex maturity in the same species (Memon et al. 2010). Furthermore, research work carried out by Kingsley and Patience (2021) states that fat content generally varies much more widely than other proximate components of fish, as species variation, age, lifecycle, and size have been shown to influence lipid content in fishes. Jahan et al. (2018) reported the lipid content of oven-dried *Wallago attu* is 11.32%. This fact supports our findings. Similarly, research carried out by Memon et al. (2010) found a lipid content of selected eight species ranging from 0.85% to 18.32% on a wet basis including *Clupisoma garua* (3.75%) and *Wallago attu* (0.85%) and the results seem to contrast with the present findings. This deviation may be because drying methods have effects on the proximate compositions (Chukwu and Shaba 2009, Abraha et al. 2018).

## Crude Fiber

The crude fiber content ranged from 0.74% to 3.12%. In *Clupisoma garua*, the crude fiber content was high (3.12%). The lowest was found in *Labeo bata* (0.74%). Chukwu and Shaba (2009) illustrated the mean crude fiber content of electric dried catfish 1.96±0.03% which supports our findings for four fishes (0.74%-3.12%). Daniel and Imaobong (2015) studied the proximate analysis of three commercial fishes. They discovered that crude fiber is between 7.67% - 10.71% which shows disparities with our findings. This finding might be attributed to the difference in season, size, sex, organ, and fasting condition of the fish.

# 6. CONCLUSION

The current investigation describes the proximate composition of four selected commercial fish species in the fish markets around the Saptakoshi Barrage. The fish contains a comparatively high amount of protein. The current study concludes that *Wallago attu* has high Protein content while *Aspidosparia morar* has high ether extract content. Except for moisture, all other nutrients were higher dried samples. The nutritional composition of the analyzed fish samples supported the variation among the fish species. Consuming these species is strongly encouraged because recent research has shown more about the importance of selected commercial fishes. It is expected that low-income populations in Saptakoshi Barrage fishermen may have higher nutritional security by consuming these commercial fish. Furthermore, increasing the production of the species can reduce the animal Protein requirements of customers.

# 7. RECOMMENDATION

There is limited information on the nutritive value difference of fish species in Nepal. This study only looked at the species' major nutritional content; further research is needed to determine the species' mineral, vitamin, and important fatty acid constituents. Because fish contain a significant amount of Protein, aqua-farming and proper management of the species' natural habitat should be implemented to boost production. The supply of fry culture can also be increased by artificial breeding of the species.

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# APPENDICES

**Table 2:** Dry Matter in response to the fish species.

| | Df | Sum Sq | Mean Sq | F value | Pr (>F) |
|---|---|---|---|---|---|
| Species | 3 | 48.11 | 16.04 | 6.167 | 0.0178* |
| Residuals | 8 | 20.80 | 2.60 | | |

**Table 3:** Total Ash in response to the fish species

| | Df | Sum Sq | Mean Sq | F value | Pr (>F) |
|---|---|---|---|---|---|
| Species | 3 | 455.7 | 151.89 | 545.9 | **1.37e-09 *** ** |
| Residuals | 8 | 2.2 | 0.28 | | |

**Table 4:** Crude Protein in response to the fish species.

| | Df | Sum Sq | Mean Sq | F value | Pr (>F) |
|---|---|---|---|---|---|
| Species | 3 | 479.3 | 159.77 | 17.16 | **0.000761 *** ** |
| Residuals | 8 | 74.5 | 9.31 | | |

**Table 5:** Ether Extract in response to the fish species.

| | Df | Sum Sq | Mean Sq | F value | Pr (>F) |
|---|---|---|---|---|---|
| Species | 3 | 1182 | 394.1 | 21.74 | **0.000335 *** ** |
| Residuals | 8 | 145 | 18.1 | | |

**Table 6:** Crude Fiber in response to the fish species.

| | Df | Sum Sq | Mean Sq | F value | Pr (>F) |
|---|---|---|---|---|---|
| Species | 3 | 10.70 | 3.567 | 7.755 | **0.00941 **** |
| Residuals | 8 | 3.68 | 0.460 | | |

**Table 7**: Proximate composition of commercial fish species (Mean ± SD)

| S.N. | Name of the Species | Dry Matter | Total Ash | Crude Protein | Ether Extract | Crude Fiber |
|---|---|---|---|---|---|---|
| 1. | *Clupisoma garua* | 92.77 ± 0.925 | 16.14 ± 0.338 | 80.64 ± 3.694 | 12.67 ± 3.096 | 3.12 ± 1.285 |
| 2. | *Labeo bata* | 94.20 ± 2.183 | 14.68 ± 0.936 | 72.83 ± 2.487 | 27.02 ± 6.690 | 0.74± 0.104 |
| 3. | *Aspidoparia morar* | 98.22± 0.437 | 3.01± 0.190 | 82.36± 2.260 | 36.12± 2.625 | 0.88± 0.298 |
| 4. | *Wallago attu* | 94.76± 2.141 | 3.25± 0.292 | 90.62± 3.507 | 12.91± 3.356 | 1.58± 0.296 |

**Table 8:** Proximate composition of commercial fish species in triplicates.

| **Fish sample** | **FDM** | | | **TAsh** | | | **CP** | | | **EE** | | | **CF** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1st read** | **2nd read** | **3rd read** | **1st read** | **2nd read** | **3rd read** | **1st read** | **2nd read** | **3rd read** | **1st read** | **2nd read** | **3rd read** | **1st read** | **2nd read** | **3rd read** |
| *Clupisoma garua* | 93.63 | 92.89 | 91.79 | 15.75 | 16.32 | 16.35 | 81.6 | 83.76 | 76.56 | 15.6 | 12.98 | 9.43 | 4.12 | 3.57 | 1.67 |
| Labeo bata | 92.67 | 93.23 | 96.7 | 14.01 | 14.28 | 15.75 | 71.5 | 75.7 | 71.29 | 32.69 | 28.73 | 19.64 | 0.86 | 0.69 | 0.67 |
| *Aspidosparia morar* | 97.76 | 98.63 | 98.27 | 3.23 | 2.89 | 2.91 | 81.65 | 84.89 | 80.54 | 38.97 | 35.59 | 33.8 | 1.2 | 0.83 | 0.61 |
| *Wallago attu* | 93.19 | 93.89 | 97.2 | 3.56 | 3.21 | 2.98 | 86.73 | 93.54 | 91.59 | 13.76 | 15.76 | 9.21 | 1.55 | 1.89 | 1.3 |

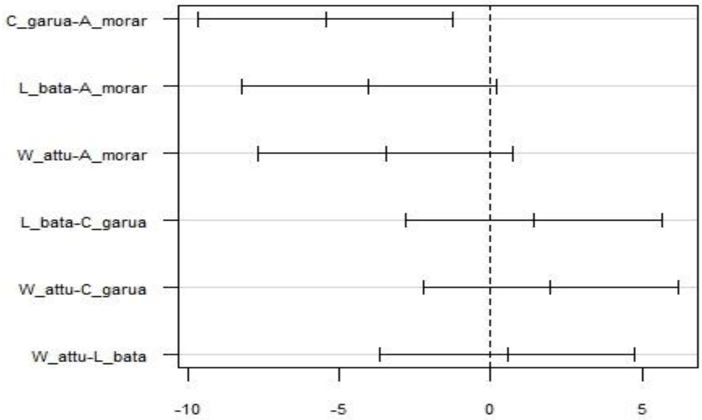


**Figure 9**: Tukey test comparing all possible pairs of means between fish species in response to dry matter.

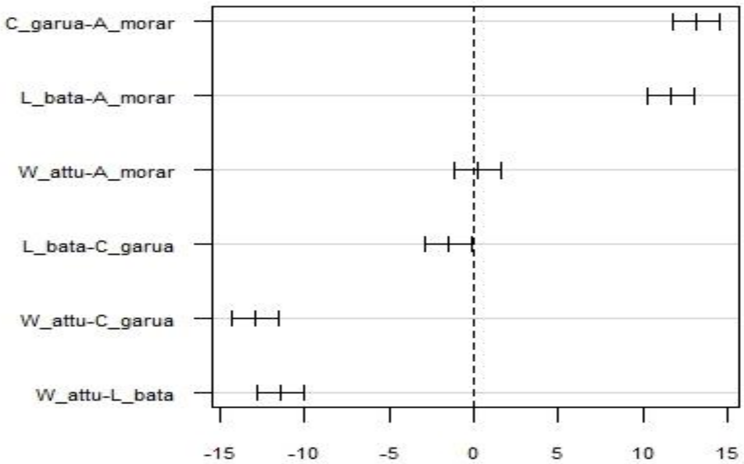


**Figure 10:** Tukey test comparing all possible pairs of means between fish species in response to total ash.

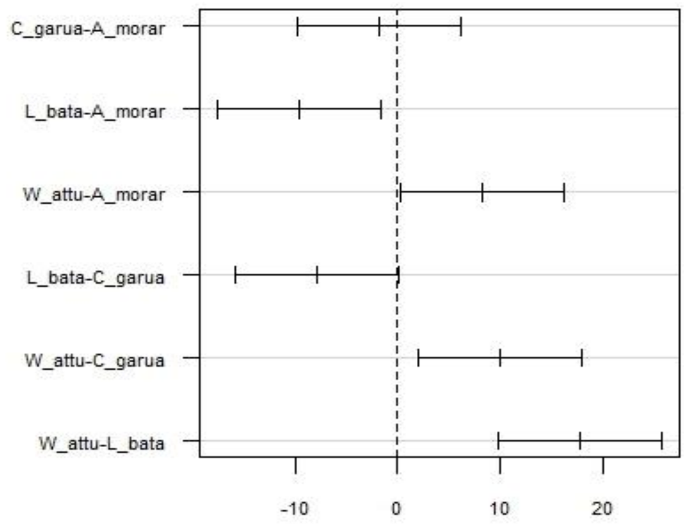


**Figure: 11.** Tukey test comparing all possible pairs of means between fish species in response to crude protein.

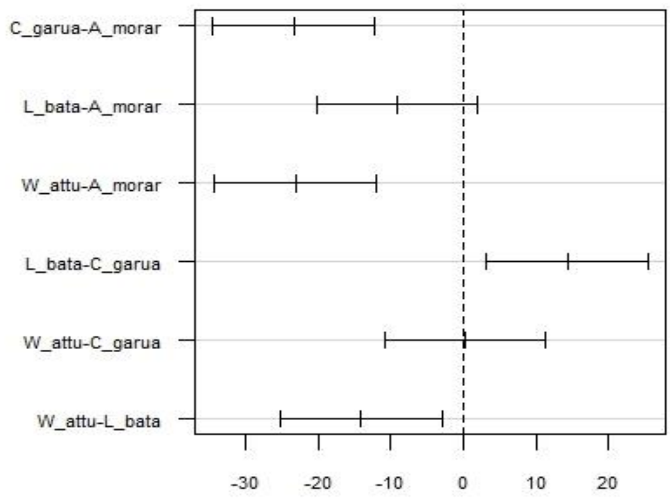


**Figure: 12.** Tukey test comparing all possible pairs of means between fish species in response to ether extract.

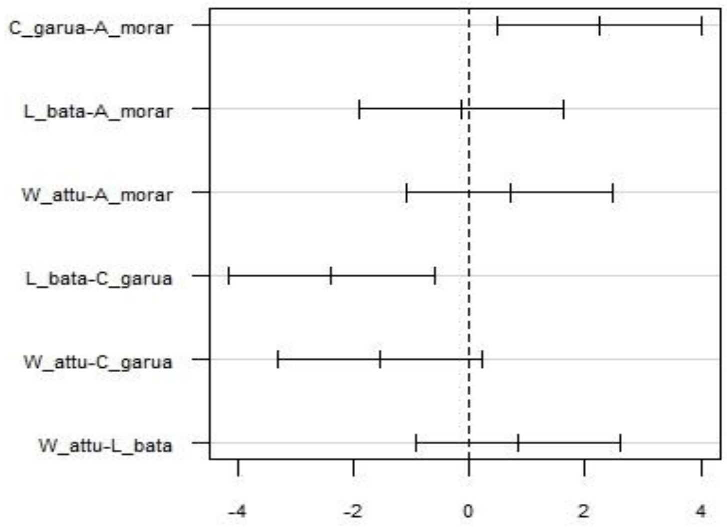


**Figure: 13**. Tukey test comparing all possible pairs of means between fish species in response to crude fiber.

## PHOTO PLATE