NUTRIENT COMPOSITION IN SMALL INDIGENOUS FISH SPECIES OF BEGNAS LAKE, POKHARA, NEPAL

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Submitted to

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Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

April, 2021

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author or institution.

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RECOMMENDATION

This is to recommend that the thesis entitled **"Nutrient Composition in Small Indigenous Fish Species of Begnas Lake, Pokhara, Nepal"** has been carried out by Sweety Nakarmi for the partial fulfilment of Master's Degree of Science in Zoology with a 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 institution.

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LETTER OF APPROVAL

On the recommendation of supervisor "Associate Professor Dr Archana Prasad" this thesis submitted by Sweety Nakarmi entitled, **"Nutrient Composition in Small Indigenous Fish Species of Begnas Lake, Pokhara, Nepal"** is approved for the examination in a partial fulfilment for the requirements of Master's Degree of Science in Zoology with a special paper Fish Biology and Aquaculture.

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CERTIFICATE OF ACCEPTANCE

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Sweety Nakarmi

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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
PUFA	Polyunsaturated Fatty Acids
RAE	Retinol Activity Equivalents
SIFFS	Small Indigenous Freshwater Fish Species
WHO	World Health Organization

ABSTRACT

Small indigenous fishes are very tasty and costly compared to other food fishes. In this present study, an attempt has been made to find out the nutrient composition in small indigenous fish species of Begnas Lake, Pokhara, Nepal. Sample collection was done from February to July 2020. Fish individuals of different species were collected from Begnas Lake, nearby outlets, irrigation canal as well as Fish Collection Centre. The proximate and minerals analysis were done by methods: determination of moisture content (hot air oven method), crude protein (Micro-Kjeldahl), crude fat (ether extraction method), total ash and minerals (calcium, phosphorous and iron) were done based on AOAC (2000) standard methods. The results shows the highest moisture content in *Puntius sophore* (8.39%) and lowest in *Mystus vittatus* (6%). The highest amount of fat (13.88%) was found in *Danio devario* and lowest in *Mastacembelus armatus* (6.62%). Similarly, the highest percentage of protein (68.19%) was found in *Mastacembelus armatus* and lowest in *Puntius sophore* (43.46%). The highest percentage of ash was found in *Puntius conchonius* (19.5%) and lowest in *Mastacembelus armatus* (12.64%). Regarding the calcium content, *Puntius conchonius* (6282.45 mg/100 gm) was found to be highest and lowest in *Xenentodon cancilla* (2376.60 mg/100 gm). *Channa striatus* shows highest phosphorus content (4510.80 mg/100 g) and lowest was observed in *Puntius sophore* (917.02 mg/100 gm). Iron content was found highest in *Xenentodon cancilla* (14.53 mg/100 gm) and lowest in *Danio devario* (1.65 mg/100 gm). From the nutritional point of view, it shows that the small indigenous fish species are good sources of protein and minerals especially calcium and phosphorus.

1. INTRODUCTION

1.1 Background

Nutrition is the intake of food, considered in relation to the body's dietary needs (WHO 2014). Nutrition and health are related to each other as good nutrition is the cornerstone of good health. Reduced immunity, higher susceptibility to disease, impaired physical and mental development, and decreased productivity can all be consequences of poor nutrition (Organization 2018). Human nutrition is concerned with the supply of key nutrients in foods that are required for human survival and wellness. 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 (Béné et al. 2007). Furthermore, fish is high in health-promoting oils such as omega-3 polyunsaturated fatty acids (PUFAs), eicosepantaenoic acid (EPA), and docosahexaenoic acid (DHA), and small indigenous fishes (SIFs) are micronutrient-dense, which could help eradicate micronutrient deficiency diseases that are common in developing countries (Mohanty et al. 2016). 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 healthy source of protein and other nutrients (Bolawa et al. 2011). Consumption of fish provides essential nutrients to a large number of people worldwide and plays a key role in nutrition.

1.2 Fish as a source of nutrient

Nutrients are the substances that nourish the body, promote growth, maintain and repair body parts (Srivastava et al. 2008). Nutrients can be divided into micro and macro nutrients that are vital for good health. Macronutrients such as proteins, lipids, ash and carbohydrates are present in the fishes (Lilly et al. 2017). Micronutrients such as vitamins (fat-soluble vitamins A, D, E and K and water-soluble vitamins B complex, vitamin C) and minerals (calcium, sodium, potassium, magnesium, iron, copper, selenium) are essential dietary elements that are essential in very small quantities i.e. they must be supplied from outside sources to the body (Mohanty 2015). Fish consumption on a regular basis can also help to prevent heart disease (Chrysohoou et al. 2007). In this context, fish is a major contributor owing to its richness in essential nutrients necessary to provide a balanced nutrition.

Fish is a high-quality animal protein source with a higher satiety effect than other animal protein sources such as beef and chicken (Uhe et al. 1992, Mahanty et al. 2014). In comparison to the other dietary animal proteins sources, consumers have a vast choice for fish as far as affordability is concerned as there are many varieties of fish species available in tropical countries. Fish protein is easily digestible. Additionally, it is an important source of both essential and non-essential amino acid (Astawan 2004). Its amino acid content has a high quantity of cysteine than a large amount of other protein sources. Protein from fish contributes to the overall protein intake significantly as the digestibility of protein from fish is approximately 5–15% higher than that from plants (Joint 1973).

As proteins, deficiency of the essential fatty acids can lead to decreased growth of infants and children, higher susceptibility to infection as well as poor wound healing (Jeppesen et al. 1998). In addition to this, fat is an integral component of the human body that acts as a source of energy during excessive need of energy. Beneficial polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been reported to be present in adequate quantities in the tissues of fish (Njinkoué et al. 2002, Rasoarahona et al. 2005). These polyunsaturated fatty acids have been reported to have the ability to both prevent and also cure some diseases of man including cancers, heart diseases, rheumatoid arthritis and inflammation (Clandinin et al. 1997, Raatz et al. 2013). The lipid composition of fish is unique, having PUFA in the form of arachidonic acid (20:4n-6), EPA (20:5n-3) and DHA (22:6n-3), with many potential beneficial effects for adult health (Wang et al. 2006) and child development (Koletzko et al. 2007).

Fish is also rich in micronutrients which tend to be more easily available than those from plant foods (Lilly et al. 2017). Hidden hunger, the chronic lack of minerals and vitamins, affects one in three people globally and keeping this in view, at the United Nation Millennium Summit (Summit 2000), micronutrient supplementations programs were incorporated as an essential component of Millennium Development Goals and their vision is through micronutrient initiatives to build a world free of hidden hunger.

1.3 Small Indigenous Fishes

Small indigenous fishes are considered as an important source of macro and micronutrients that can play a vital role in the elimination of malnutrition in the country (Roos et al. 2007). They are the freshwater species that reach a mature or adult size of about 25- 30 cm (Mohanty et al. 2019). Small indigenous fishes (SIS) comprise a major part of captured and cultured inland fisheries and contribute notably to the nutritional as well as livelihood security of the rural people. In Nepal, 200 fish species are available of which around 190 are indigenous species and the remaining are exotic species (Sharma 2008). As small fish are more perishable, so processing technology can be used to preserve these species for future consumption and spoilage free. One kind of a short time- handy drier having capable of removing dirt and chemicals has been invented for small fish drying without changing the nutritious value in Mali (Sen 2008).

At present, more than two billion people worldwide, in particular in developing countries, are estimated to be deficient in essential vitamins and minerals, especially in vitamin A, Fe and Zn (Organization 2001, Initiative 2009). Regarding species consumed by the poor in rural areas in Asia, a variety of small indigenous species account for 50–80 % of the total amount of fish consumed (Hels 2002, Islam 2007). This higher dependence on smaller fish is explained by the fact that poor people can afford only comparatively cheaper fish species, whereas the better-off households purchase larger, medium-sized fish species, which they prefer because they have fewer bones, more flesh and taste better (Chamnan et al. 2009).

Small Indigenous Freshwater Fishes (SIFFS) are not only a rich source of animal protein but also a vital source of micro-nutrients such as calcium, zinc, iron and fatty acids to the rural poor (Sarkar and Lakra 2010). Vitamin A, iron and zinc level in *Esomus danricus* and *Esomus longimanus* from Cambodia is high (Roos et al. 2007). Like the Indian major carps (IMCs), large catfishes and other food fishes, the small indigenous fishes contribute high-quality animal protein for human nutrition, proportional to their muscle biomass. Small indigenous fishes, like large fishes, contribute to food and nutritional security and thus serve as a health food; however, large fishes contribute more to protein and fish oil requirements, particularly PUFAs, whereas small indigenous fishes are significant for their micronutrient richness.

1.4 Objectives

General objective of this study was to find the nutrient composition in small indigenous fish species of Begnas Lake, Pokhara, Nepal.

Specific objectives were:

- To estimate the moisture, crude fat, crude protein, crude ash and mineral contents (calcium, phosphorus, iron) of small indigenous fish species.
- To compare the different nutrient levels among small indigenous fish species.

1.5 Rationale of the study

This study gives the knowledge on nutrient composition of small indigenous fish species. Hence, the result is useful to the consumers in selecting the fish depending on their nutritional value. In terms of nutrition, the macro and micronutrients found in fish are suitable for human consumption. (Lilly et al. 2017). It is observed that partial harvesting of SIFFs from polyculture of Indian major carps yields better productivity for both SIFFs and IMC. Knowledge of the chemical composition of fishery products is one of the basic aspects to assess their nutritional value, as well as the benefits associated with their consumption. This study is helpful to gain knowledge of the risk and benefits associated with the indiscriminate consumption of freshwater fishes.

1.6 Limitations

- Sample collection was difficult during the study period because of the pandemic.
- Sample collection from additional sites was not possible.
- Analysis could not be done at the Fishery Research Station, Begnas due to the lack of resources.

2. LITERATURE REVIEW

2.1 Biochemical composition of fish

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). 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). Protein and fat are the most important nutrient present in fish and their composition help to describe the nutritional status of a particular organism (Aberoumad and Pourshafi 2010). Minerals are essential nutrients that are important in enzymes metabolism and contribute to the growth of the fish (Glover and Hogstrand 2002). The amount or percentage of each within a fish's body is termed proximate composition. The study of the chemical composition of fish is important since it influences keeping quality and technological characteristics of the fish (Kumar et al. 2016). Measurement of proximate profiles such as protein, lipids, and moisture content is often necessary to ensure that they meet the requirements of food regulations and commercial demands (Waterman 1980). They also influence postharvest processing and the shelf-life of the fish (Clement and Lovell 1994). Different nutrients present in fishes have a unique and very important function in human health.

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). It is often determined in studies of fish pathology, growth and nutrition (Copeland et al. 1999). Data concerning the chemical composition of fish is essential for providing information on nutritious healthy foods of low fat and high protein content (Mozaffarian et al. 2003, Foran et al. 2005). Fish contains 15-20% protein of its overall live body weight. The fish protein contains the essential amino acids which improve the overall nutritional quality of a mixed diet (Sujatha et al. 2013, Mohanty 2015).

2.2 Nutrient intakes from fish

Fish is a food with excellent nutritive value, which provides high-quality protein and a large variety of vitamins and minerals including vitamin A & D, magnesium and phosphorus (Balami et al. 2019). Fishery resources are an important source of both macro and micro-nutrients for humans. Fish provides essential nutrients especially proteins of high biological values and fats, so it is often referred to as 'rich food for poor people' and the micro and macronutrients present in the fish make it better than other animal protein sources (Sujatha et al. 2013). Fish species had received tremendous attention from researchers due to the excellence in their nutritional aspects. Apart from being a food source, fish also functions to prevent human beings from a variety of diseases in the world (Abraha et al. 2018). A portion of 140 gm of fish can provide about 50-60% of the daily protein required by an adult human (FAO 2012). As compared to land-living animals, fishes are a rich source of protein and have a high content of omega-3 long-chain fatty acids. Intake of animal source foods (ASF) in young children has been associated with improved dietary quality and growth outcomes (Murphy and Allen 2003, Neumann et al. 2003).

Fish can be beneficial to be consumed whole as it shows a high protein-fat ratio as compared to the meat of goat, lambs, buffalo and chicken (Steffens 2006). Fisherman communities such as the Tharu, Chaudhary, and Jalari involved in fishing activities in Nepal. Their main food is small indigenous species (SIS) captured from nearby rivers, lakes, marshy lands, reservoirs and small water bodies. This provides a major protein diet to the people (Sonawane 2013, Mohanty 2015). However, as national statistics on fish production and consumption fail to capture data on these small fish species, their importance in diets is neglected (Roos et al. 2007). Very few consumption surveys have reported on fish intake at the species level.

Nepal underlies itself as one of the food and nutrition-deficient countries. It has been estimated that 41 % of Nepalese children suffer from stunting or chronic malnutrition having a potential negative impact on the human population and economic development (Faruk et al. 2012, Gurung 2016). The Government of Nepal has recommended at least 30 g per day fish or animal protein diet to each man, women, adolescent girls and boys (Gurung 2016). While average per capita fish consumption may be low, even small quantities of fish can have a significant positive nutritional impact by providing essential amino acids, fats and micronutrients that are scarce in vegetable-based diets. Poor nutrition is cited as the major factor in more than half of all child deaths in Nepal - a significantly higher proportion than those claimed by other infectious diseases (Faruk et al. 2012). In the early 1990s, the Nepalese standard food intake had a very small amount of fish in their diet, but recently the amount of fish protein in common people diets is increasing (Nestel et al. 2015). This suggests that the fish production, availability, affordability, purchasing capacity and awareness might have led the increased consumption, implying that the role of fisheries in food and nutritional security is becoming indispensable and increasing. Coinciding with these facts, the Agriculture Development Strategy (2015–2030) has included fish as one of the prioritized commodities (Fox et al. 2014).

Fish foods are beneficial in the development of neuron in infants and fat glycemic control (Mozaffarian et al. 2015). Moreover, fish has a good contribution towards essential amino acids, specifically lysine which is low in cereals, providing nutritional balance in the quality of mixed diet (Ababouch et al. 2005). Presently people are more sensitive to healthy eating than in the past (Obemeata et al. 2011). People prefer white meat like fish over red meat due to its high nutritional contents (Mahboob et al. 1996, Ayisi et al. 2017).

2.3 Nutritional contribution of small indigenous fish species

Small Indigenous fish species are not only a rich source of animal protein but also a vital source of micro-nutrients such as calcium, zinc, iron and fatty acids to the rural poor (Sarkar and Lakra 2010). Studies have shown that the bioavailability of calcium from SIFFS is on par with milk (Roos et al. 2007) and indigenous knowledge about the health benefits of SIFFS is significant among the rural communities. The research report of Farid et al. (2014) on the fish species *Channa striatus* and *Channa punctatus*, revealed that studied fresh fish species contained high moisture and low protein contents.

It has been reported that some species such as *Amblypharyngodon mola*, *Esomus danricus* and *Channa soborna* contain high amount of vitamin A and other micronutrients and minerals (Thilsted and Roos 1999). *Esomus danricus* as well as *Esomus longimanus* from Cambodia have a high iron and zinc content (Roos et al. 2007). Some SIS fish like *Puntius sp.* contains double the amount of iron compared to many cultured carps like Silver carp (*Hypopthalmichthys molitrix*) and Rohu (*Labeo rohita*); and Mola (*Amblypharyngodon mola*) contains three times more calcium and 50 times more vitamin- A than that of Silver carp and Rohu (Villif and Jorgensen 1993). These small fishes are an important item in the daily diet of people of all categories. The majority of the people of Bangladesh has no choice but to eat different species of small fishes with their staple food rice since they cannot afford to purchase high priced large carp species (Hossain et al. 1997).

Bogard et al. (2015) worked on the nutritional composition of important fish species in Bangladesh and their potential contribution to recommended nutrient intakes. The research work concluded significant contributions of small indigenous fishes containing rich nutrient profiles, to the pregnant as well as lactating mothers. A comparative study about the body composition of different small indigenous species, Shoal fish and Hilsa were performed by Begum and Minar (2012). They observed that the small fishes contained good nutritional value and not less than the larger fish helping to decrease the nutrient deficiency of the people.

In Bangladesh, two species, *Amblypharyngodon mola* and *Parambassis baculis* were identified as having a vitamin A content as high as 2500 and 1500 mg retinol activity equivalents (RAE) /100 g raw edible parts, respectively (Roos 2001). In Cambodia, the small indigenous species *Parachela sianensis* and *Rasbora tornieri* were reported to contain > 1500 mg RAE/100 g raw edible parts (Roos et al. 2007). Debnath et al. (2014) worked on protein and mineral composition of some local fishes of Tripura, India. The selected fish species *Amblypharyngodon mola*, *Esomus danricus*, *Puntius sophore*, *Channa fasciata*, *Labeo bata*, *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* were analyzed and the reports concluded that all the fish species were nutritionally competitive even in their dried state. The study revealed that the small indigenous fishes were highly nutritive and these species can assure the nutritional security of the poor classes owing to their low cost and tremendous availability.

Mahanty et. al (2014) with his team studied on proximate composition, amino acid, fatty acid and micronutrient profiles of small indigenous fish *Puntius sophore*. Their work recorded that the studied fish species was rich in proteins and minerals. The essential amino acids, Histidine was most prominent in that species. Moreover, the fish *Puntius sophore* was rich in unsaturated fatty acid, especially oleic acid. According to the research report of Bijayalakshmi and group (2014), the small indigenous fish species namely *Channa striata*, *Trichogaster fasciatus* and *Puntius sophore* contained higher lipid content than the other fish species. In Kishoreganj, Bangladesh, field data show that daily consumption of small fish contributes 40% of the total daily requirement of vitamin A at the household level (Roos et al. 2007). The small indigenous fish species *Amblypharyngodon mola* (555.0 µg/100g), *Anabas testudineus* (89.8 µg/100g), *Puntius sophore* (70.9 µg/100g), marine fish *Epinephelus spp.* (379.3 µg/100g), *Sardinella longiceps* (346.4 µg/100g), and migratory fish *Tenualosa ilisha* (260.7µg/100g) are rich in vitamin A (Mahanty et al. 2014). The majority of the people of Bangladesh has no choice but to eat different species of small fishes with their staple food rice since they cannot afford to purchase high priced large carp species (Hossain et al. 1997).

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in "Fishery Research Station" situated in Begnas, Pokhara from February to July 2020 (Fig. 1).

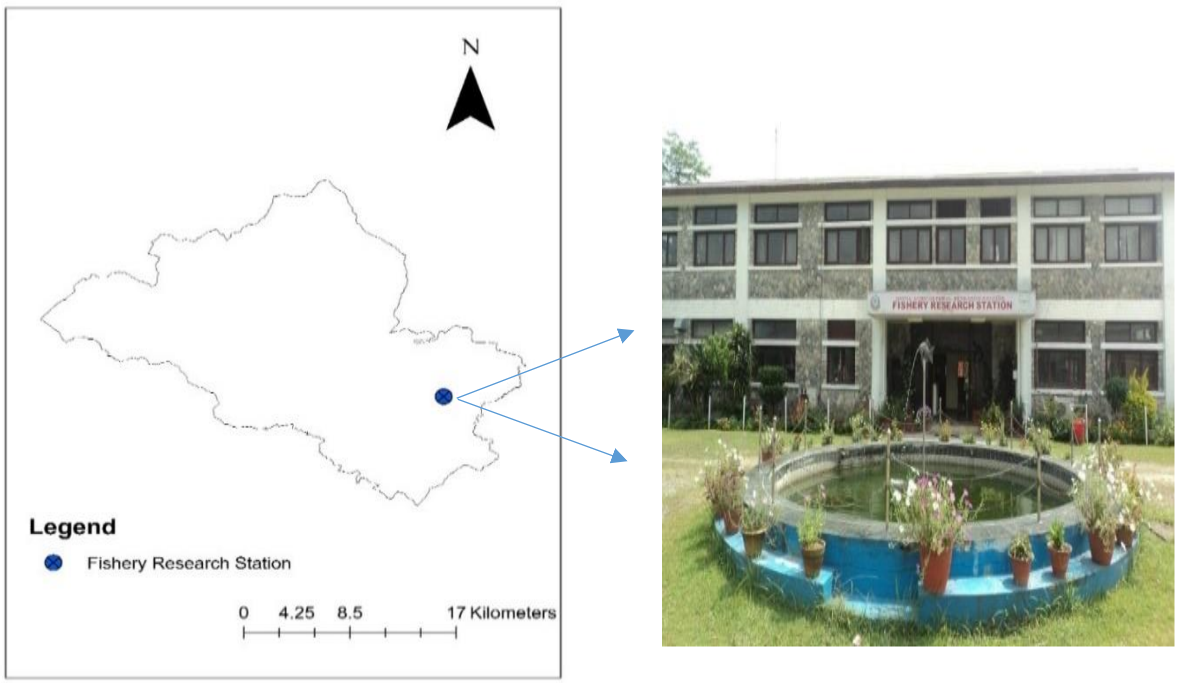

Fig 1. Fishery Research Station, Begnas, Pokhara

3.2 Materials

Weighing balance, gloves, scissors, dissecting box, scale, aluminium foil, tissue papers, trays, drying oven, homogenizer, blender mixture, tongs, zipper bags, refrigerator, cast net, gill net etc.

3.3. Methods

3.3.1 Collection of fish species

Fish individuals (small indigenous fishes) of different species were collected from Begnas Lake, nearby outlets, irrigation canal as well as Fish Collection Centre by using cast net and gill nets (Photo 1). Sample collection was done from February to July 2020.

3.3.2 Identification of small indigenous fishes

From the above mentioned sites under study fish samples were collected. The identification of fishes was done as per the key given by Shrestha (2008) (Table 1, Appendix I) which is as follows:-

a. *Puntius sophore* (Hamilton, 1822)

It has a distinct orange golden spot below the eye as well as opercles are golden red and distinct black blotch on the caudal peduncle and dorsal fin base whereas the tip of the fin is reddish. The head of this fish is small and the eye is comparatively large. There is a faint black band on the lateral line. Barbels of this fish are absent and the lateral line system is complete.

b. *Puntius conchonius* (Hamilton, 1822)

It is a deep-bodied silvery fish with a darkish back and silvery belly. It is common to fish found in shallow water stream and lakes. It has scales with dark bases and a black blotch is present at the caudal region. Rosy colour is noticeable when fish is in breeding condition. The lateral line system is incomplete. The paired fins have dark tips and caudal fin is transparent.

c. *Danio devario* (Hamilton, 1822)

It is common ornamental fish with silvery greenish body with yellowish pectoral, dorsal, pelvic anal and caudal fins. A dark band runs from the middle of the caudal above the middle of anal fin. Head is small, snout obtusely pointed. Scales cycloid and barbels absent. Mouth oblique and directs upward.

d. *Barilius barna* (Hamilton, 1822)

The body of this fish is usually crossed by fine dark vertical bands. Barbels are absent, open pores are present on both jaws and snout in the adults. The body of the fish is silvery with 9-11 bluish vertical bands, originating from dorsal sides and crossing the lateral line. Generally, the dorsal and caudal fins are tinged with black.

e. *Mastacembelus armatus* (Hamilton, 1822)

Long, slender and elongated body having anal and dorsal fin confluent with caudal. Snout pointed. General body colour is brownish becoming lighter on the belly. There is a row of distinct rounded black spots along the base of the dorsal fin. The body of fish is covered over on small scale.

f. *Xenentodon cancilla* (Hamilton, 1822)

The body is elongated with beak-like jaws. Body is greenish above and whitish below. A series of four or five blotches on sides of body between pectoral and anal fins present in adult. Dorsal and anal fins dark- edged. The lower jaw is slightly longer than upper. Sharp opposing teeth are present on both jaws. Eyes are large golden coloured.

g. *Channa striatus* (Hamilton, 1822)

A moderate-sized dark brown fish having a blackish and yellowish belly. Dark vertical bands descend from lateral sides to the abdomen on yellow ground from middle of the body. The ventral and anal fins are greyish. Generally, the scales are large, irregular on dorsum of the head. Mostly caught in hook line.

h. *Mystus vitttus* (Hamilton, 1822)

A small sized silvery fish with a brief median longitudinal groove on its head but the groove does not reach the base of the occipital process. The general body colour yellowish with a distinct bluish spot shoulder. Four longitudinal bands on either lateral side. Barbel 4 pairs.

i. *Heteropneustes fossilis* (Hamilton, 1822)

A dirty brown purplish catfish with four faint bands along the body. The juvenile fishes are reddish-brown. This fish has got long exploring 4 pairs of barbels. It has a short dorsal fin and pectoral fins with spine which inflicts a wound. The anal fin is separated from caudal by a deep notch. The caudal fin is rounded. This fish can breathe air through its accessory respiratory organs.

3.3.3 Preparation of samples

After the identification of the fish species, the individual length (cm) and weight (gm) of fishes were recorded (Table 2, Appendix I). Samples were manually cleaned and degutted and their gutted weight was measured. Further, they were dried in an oven for 24 hours at 50℃ and their dried weight was recorded. Finally, the homogenization process was carried out by the use of a homogenizer and a grinder mixture respectively and the homogenized weight was recorded respectively (Table 3, Appendix II). The homogenized samples which are the final minced powdered ones were then placed in zipper bags, sealed and refrigerated for a certain period before analysis.

3.3.4 Proximate analysis

The proximate composition of the minced samples for their nutrient analysis was performed in Food Research Division and Animal Nutrition Division, Khumaltar, Nepal. The analysis included Proximate Analysis i.e. Crude Protein, Crude Fat, Crude Ash, Moisture, Minerals-Calcium, Iron and Phosphorus. The proximate and minerals analysis were done by methods: determination of moisture content (hot air oven method), crude protein (Micro-Kjeldahl), crude fat (ether extraction method), total ash and minerals (iron, phosphorous, and calcium) were done based on AOAC (2000) standard methods (Table 4, Appendix III). All analyses were conducted in triplicates as the data obtained for analysis were presented on a dry weight basis.

3.3.5 Statistical analysis

Microsoft Excel 2013 and one way ANOVA was employed to understand the variation in the quantity of nutrients with respect to different fish species.

4. RESULTS

4.1 Proximate and mineral composition

The proximate and mineral composition of small indigenous fish species of Begnas Lake is shown in tables 5 and 6 (Appendix IV). 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 7, Appendix IV).

a) Moisture content

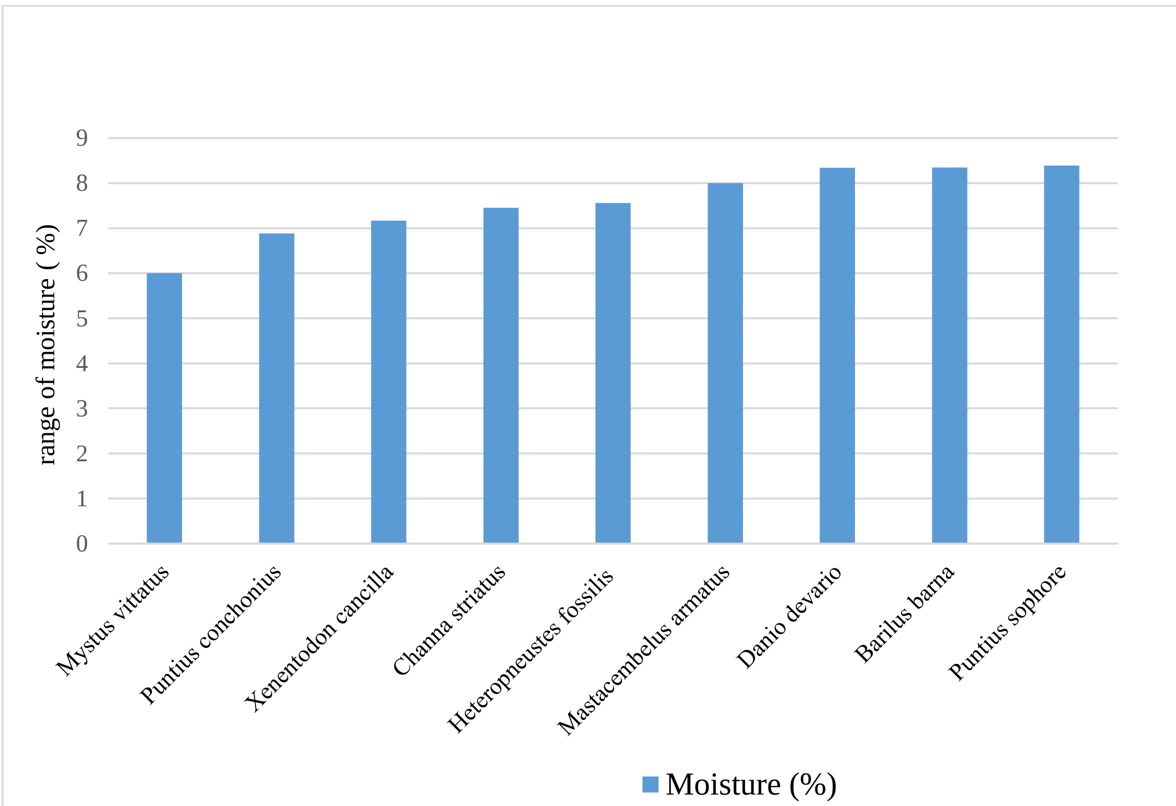

Fig 2. Variation of moisture content in different fish species

The moisture content in the present study ranged between 6-8.39 % with the highest in *Puntius sophore* (8.39%) and lowest in *Mystus vittatus* (6%) (Fig. 2). The moisture content in *Danio devario*, *Barilius barna* and *Mastacembelus armatus* were less or more similar (8.23 ± 0.199 %). *Puntius conchonius*, *Xenentodon cancilla*, *Channa striatus* and *Heteropneustes fossilis* with less or more similar (7.265 ± 0.3046 %) moisture content.

b) Crude Fat

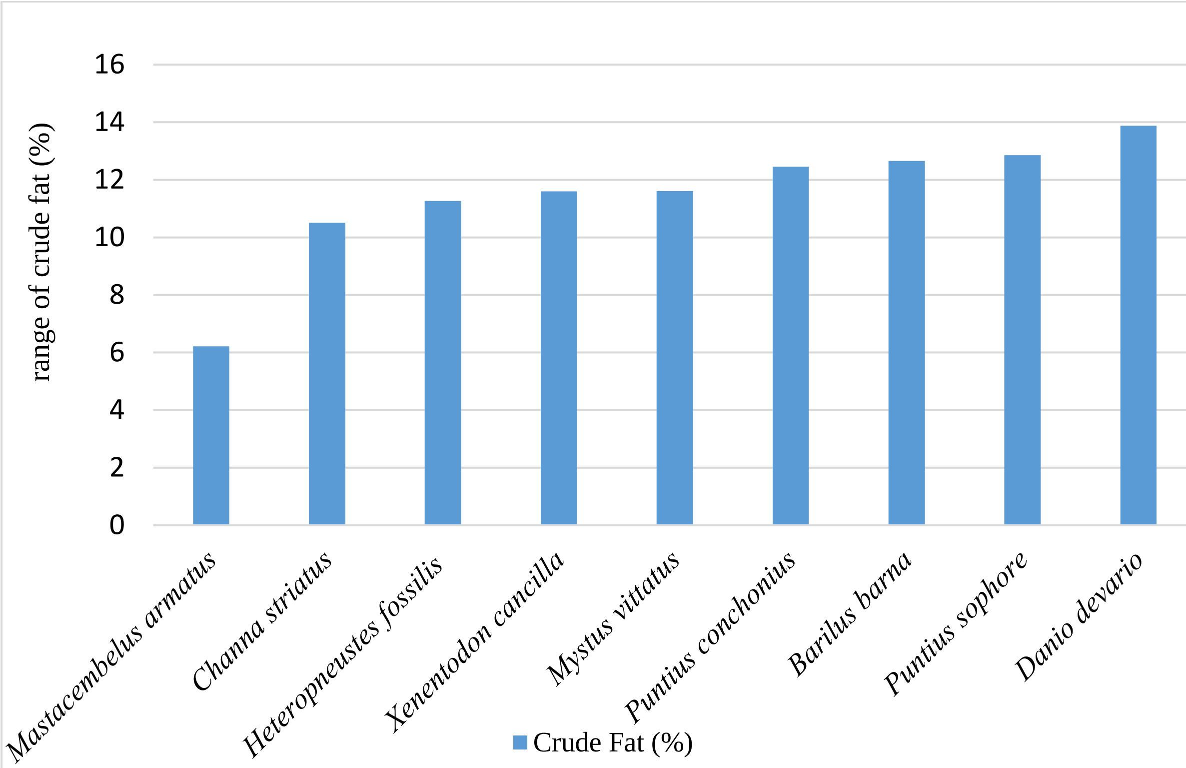

Fig 3. Variation of fat content in different fish species

The fat content of SIS in the present study varied between 6.22- 13.88% with the highest in *Danio devario* (13.88%) and lowest in *Mastacembelus armatus* (6.22%) (Fig. 3). *Puntius conchonius*, *Barilius barna* and *Puntius sophore* with less or similar fat content (12.65 ± 0.200%). *Channa striatus*, *Heteropneustes fossilis*, *Xenentodon cancilla* and *Mystus vittatus* with less or similar fat content (11.2475 ± 0.516 %).

c) Crude Protein

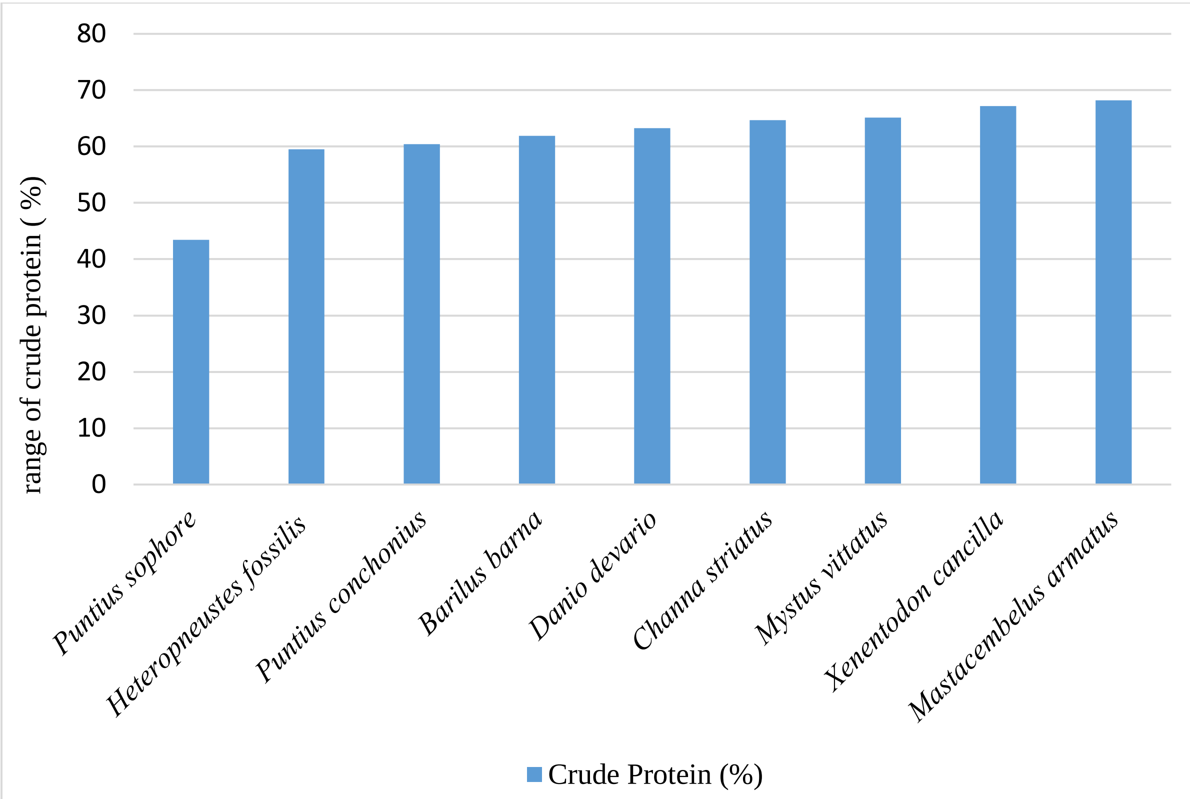

Fig 4. Variation of protein content in different fish species

The protein content of SIS in the present study varied between 43.46- 68.19% with the highest in *Mastacembelus armatus* (68.19%) and lowest in *Puntius sophore* (43.46%) (Fig. 4). The protein content in *Heteropneustes fossilis*, *Puntius conchonius* and *Barilius barna* were less or more similar (60.61 ± 1.184 %). *Danio devario* and *Channa striatus* with less or similar protein content (63.98 ± 0.989949 %). *Mystus vittatus* and *Xenentodon cancilla* with less or similar protein content (66.15 ± 1.428%).

d) Crude Ash

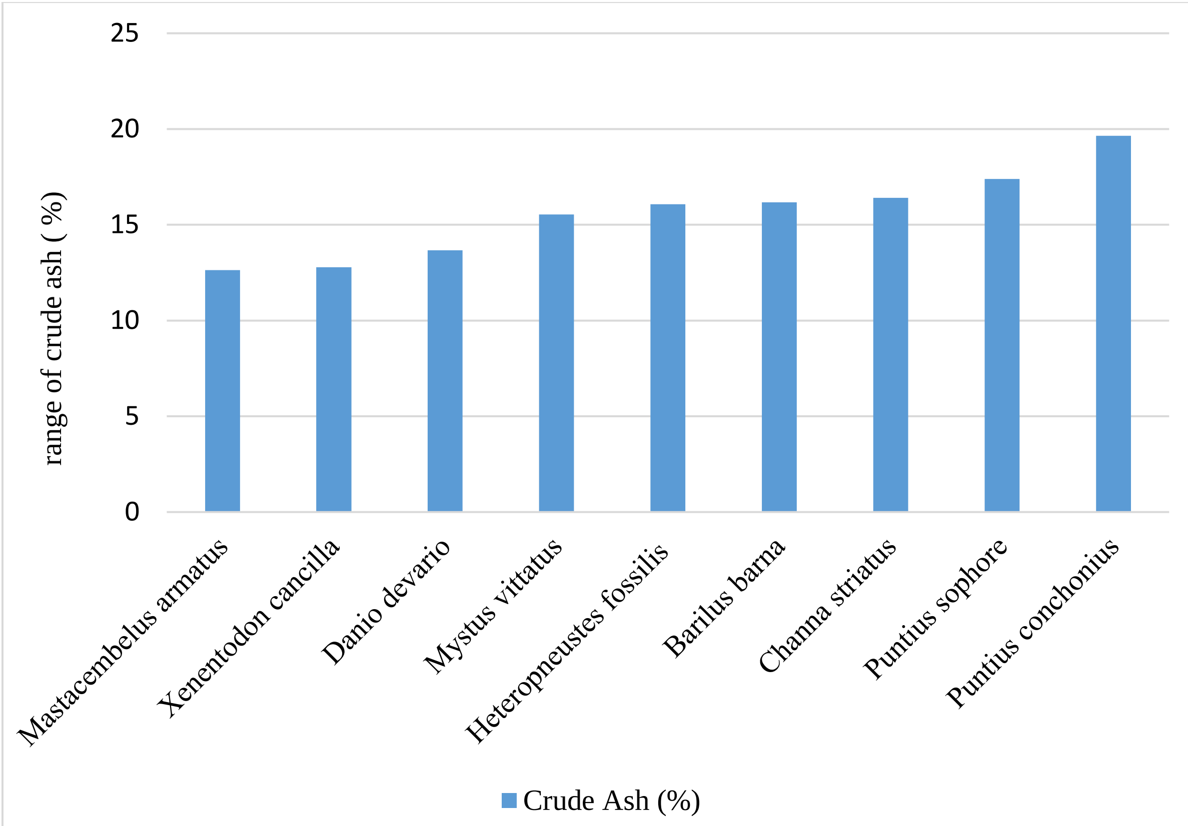

Fig 5. Variation of ash in different fish species

The ash content of SIS in the present study ranged between 12.64 -19.65 % with the highest in *Puntius conchonius* (19.65%) and lowest in *Mastacembelus armatus* (12.64%) (Fig. 5). *Xenentodon cancilla* and *Danio devario* with less or similar ash content (13.225 ± 0.6151%). *Mystus vittatus*, *Heteropneustes fossilis*, *Barilius barna*, *Channa striatus* and *Puntius sophore* with less or similar ash content (16.316 ± 0.68053%).

e) Calcium content

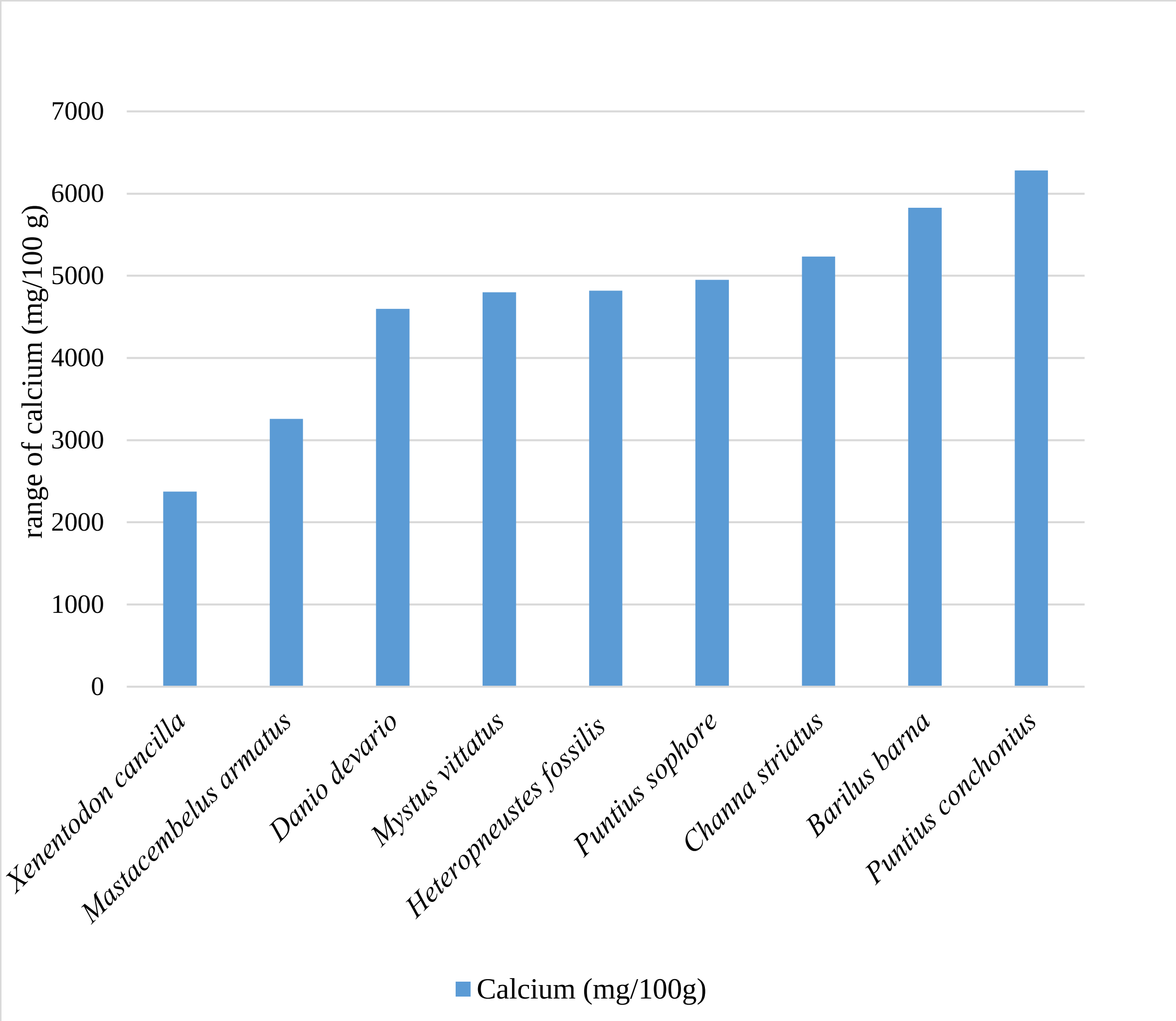

Fig 6. Variation of calcium content in different fish species

In the present study, the calcium content ranged between 2375.6 to 6282.45 mg/100 g with the highest in *Puntius conchonius* (6282.45 mg/100 g) and lowest in *Xenentodon cancilla* (2375.6 mg/100 g) (Fig. 6). *Danio devario*, *Mystus vittatus* and *Heteropneustes fossilis* with less or similar calcium content (4737.56 ± 122.33 mg/100 g). *Mastacembelus armatus* with 3259.63 mg/100 g calcium content. *Puntius sophore*, *Channa striatus* and *Barilius barna* with less or similar calcium content (5337.08 ± 447.3932 mg/100 g).

f) Phosphorus content

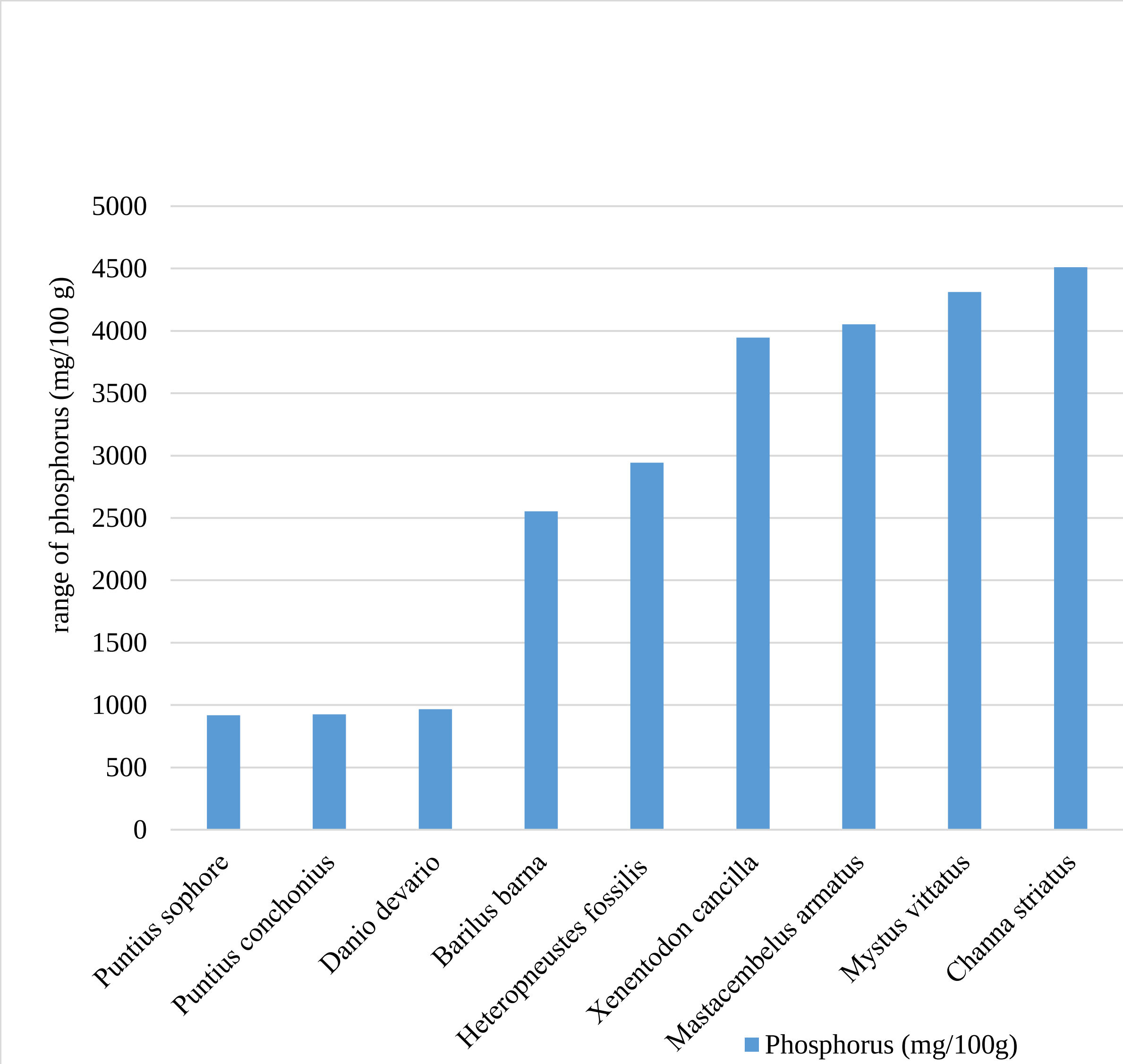

Fig 7. Variation of phosphorus content in different fish species

The phosphorus content in the present study ranged between 917.02 to 4510.8 mg/100 g with the highest in *Channa striatus* (4510.8 mg/100 g) and lowest in *Puntius sophore* (917.02 mg/100 g) (Fig. 7). *Puntius conchonius* and *Danio devario* with less or similar phosphorus content (945.98 ± 30.561 mg/100 g). *Barilius barna* and *Heteropneustes fossilis* with less or similar phosphorus content (2747.78 ± 276.1818 mg/100g). *Xenentodon cancilla*, *Mastacembelus armatus* and *Mystus vittatus* with less or similar phosphorus content (4104.043 ± 188.2993 mg/100 g).

g) Iron content

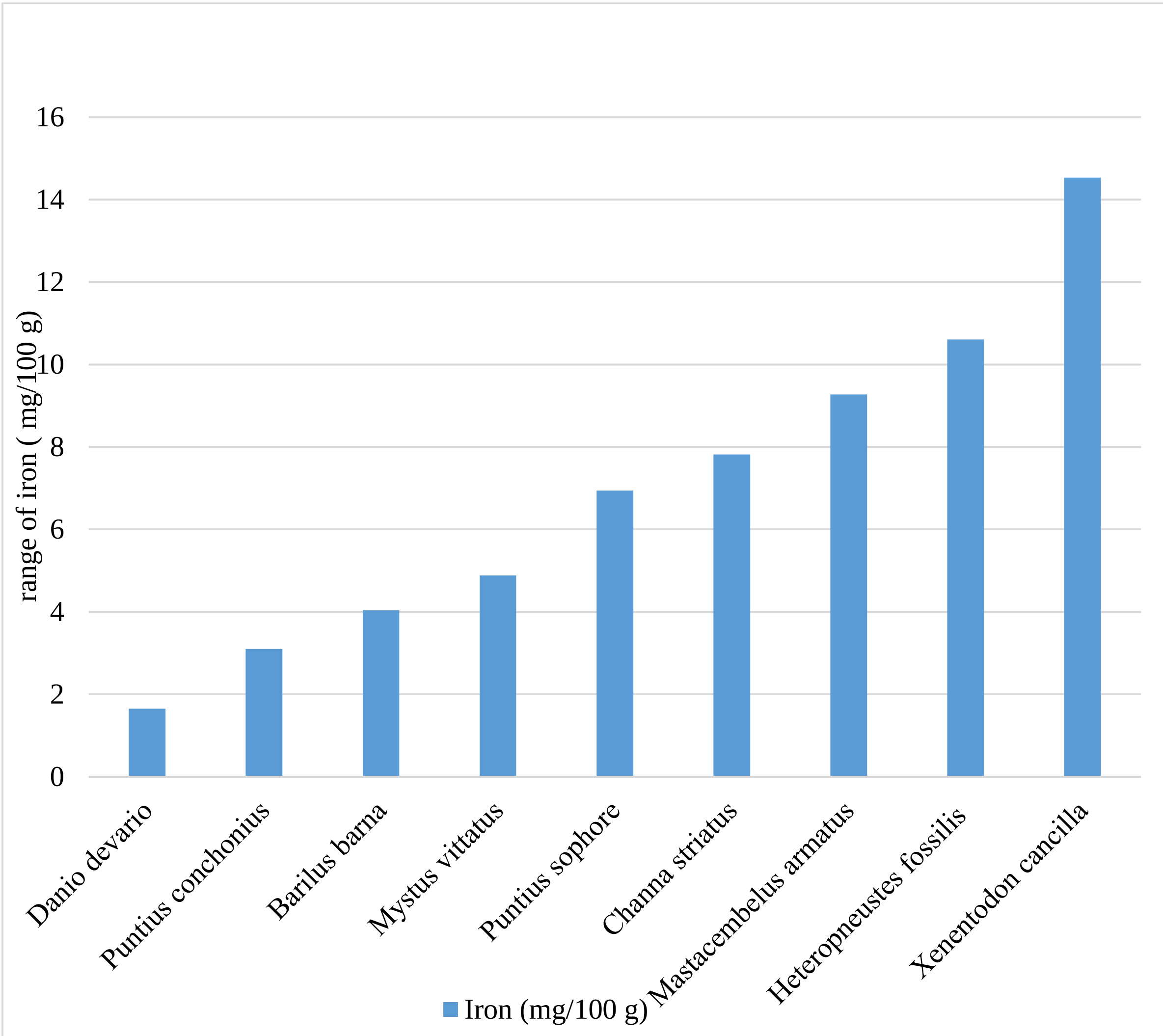

Fig 8. Variation of iron content in different fish species

The iron content in the present study ranged between 3.1 to 14.53 mg/100 g with the highest in *Xenentodon cancilla* (14.53 mg/100 g) and lowest in *Danio devario* (3.1 mg/100 g) (Fig. 8). *Puntius conchonius*, *Barilius barna* and *Mystus vittatus* with less or similar iron content (4.006 ± 0.8904 mg/100 g). *Puntius sophore* and *Channa striatus* with less or similar iron content (7.38 ± 0.622 mg/100 g). *Mastacembelus armatus* and *Heteropneustes fossilis* with less or similar iron content (9.94 ± 0.9475 mg/100 g).

5. DISCUSSIONS

The present study focuses on each nutrient components as some fish species are rich sources of macronutrients and some in micronutrients. Imported sun-dried fish and smoked indigenous fish are the common dried fish products available in Nepal. Sun-dried fish normally contain 10-20% moisture, 60-80% crude protein (Mitra et al. 2004) , 0.20- 25% fat (Love 1970). The experiments are performed on a dry basis and findings show the variation of seven different nutrient contents in nine fish species.

The moisture content of different fish species shows wide variation among each other in the present study and the values are within the range of 6 - 8.39 % with the highest in *Puntius sophore* (8.39%) and lowest in *Mystus vittatus* (6%). Ogundiran et al. (2014) suggested the high moisture content can cause the degradation of polyunsaturated fatty acids, increase the fishes' vulnerability to spoilage by microorganisms, and consequently reducing fish quality for longer periods of preservation. The findings of our study shows the fish species are less vulnerable to spoilage with a low moisture range (6-8.39%). (Ahmed et al. 2012, Jana et al. 2018) illustrated the moisture content in *Mastacembelus armatus*, *Puntius sophore*, *Heteropneustes fossilis*, *Mystus vittatus* and *Channa striatus* ranging from 72 to 83 % on a wet basis and the results seem to contrast with the present findings (6 - 8.39 %). Similar studies was done by Hazarika et al. (2016) showing lower moisture content of the dried fish as 2.8% in Seleng (*Barilius tileo*) to 8.9% in Mukanga (*Amblypharyngodon mola*) that matched with the present findings (6 - 8.39%) performed on dry basis.

The protein content of SIS in the present study varied between 43.46- 68.19% with the highest in *Mastacembelus armatus* (68.19 %) and lowest in *Puntius sophore* (43.46%). 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 % on the wet basis which were in contrast to our present findings (43.5 – 68.19%). The possible differences in protein level may be due to the availability of different fish food organisms in water (Jana et al. 2018). The results of the dried fish species- *Chanda ranga*, *Mystus vittatus, Channa striatus*, *Puntius sophore* and *Channa* *punctatus* shows the protein content ranging from 42 to 55% (Azam et al. 2003). In the same way, present experiment showed a similar protein range (43.46- 68.19%).

Variation in fat content could be influenced by the variation of species, diet, temperature, salinity, selective mobilization and distribution (Lovern 1950). The fat content in the present study varied among the fish species that are reported within the range of 6.22- 13.88% with the highest in *Danio devario* (13.88%) and lowest in *Mastacembelus armatus* (6.22%). Similarly, Ahmed et al. (2012) and Jana et al. (2018) performed experiments on small indigenous fish species- *Channa striatus*, *Puntius sophore*, *Mastacembelus armatus*, *Xenentodon cancilla* and *Heteropneustes fossilis* and results shows variation in crude fat ranging from 1.5 to 5% which were similar to the low fat content fish *Mastacembelus armatus* (6.22%) in present experiment. Rahnan et al. (1995) reported variation in fat content (1.17–34.00%) among 20 species of freshwater Malaysian fish on wet basis which are in contrast with the present findings ranging from (6.22- 13.88%) on dry basis.

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 12.64-19.65% with highest in *Puntius conchonius* (19.65%) and lowest in *Mastacembelus armatus* (12.64%). 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). The fish species- *Puntius conchonius* contained high ash content (19.65%) and also rich in calcium content (6282.45 mg/100 g) which supports the study.

The calcium content ranged between 2375.6 to 6282.45 mg/100 g with the highest in *Puntius conchonius* (6282.45 mg/100 g) and lowest in *Xenentodon cancilla* (2375.6 mg/100 g) in the present study as the calcium content was found 944.6 ± 55.4 mg/ 100 g , 5310 ± 23.5 mg/100 g and 164.4 ± 21.5 mg/100 g in *Puntius sophore*, *Xenentodon cancilla* and *Heteropneustes fossilis* (Mohanty et al. 2019). The experiments were performed on a wet weight basis so there seems variation compared to the present study ranging from (2375.6 to 6282.45 mg/100 g). According to Roos (2001), small indigenous fish species as *Mastacembelus armatus*, *Mystus vittatus* and *Puntius sophore* shows the different calcium contents as 198 mg/100 g, 481 mg/100 g and 698 mg/100 g (per 100 g, raw edible parts) which shows the vast differences compared to our study (2375.6 – 6282.45 mg/100 g). In the same way, the phosphorus content in the present study ranged between 917.02 to 4510.8 mg/100 g with the highest in *Channa striatus* (4510.8 mg/100 g) and lowest in *Puntius sophore* (917.02 mg/100 g). The iron content in the present study ranged between 3.1 to 14.53 mg/100 g with the highest in *Xenentodon cancilla* (14.53 mg/100 g) and lowest in *Danio devario* (1.65 mg/100 g). *Mastacembelus armatus*, *Mystus vittatus* and *Puntius sophore* shows the iron content ranging from 1.5 to 4 mg/100g as illustrated by (Roos (2001))on wet basis which can be related to the present findings (1.65 to 14.53 mg/100 g). Thus iron content seems to be neutral based on results of either wet ones or dried ones as it is not related to sample dried or wet weight.

The research report of Fishery Research Station, Begnas (FRS 2018) on fish species- Rewa (*Cirrhinus reba*), Sahar (*Tor putitora*) and Rohu (*Labeo rohita*) showed that iron and calcium contents is higher in small native fish Rewa i.e. iron content- 12.36 mg/100 g and calcium content- 5476.94 mg/100 g and protein level is higher in Rohu i.e. 66.1% which seems to be similar to our present findings (iron -14.53 mg/100 g, calcium- 6282.45 mg/100 g and protein-68.19%) performed on dried basis.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

Present study was carried to find out the nutritional quality of nine small indigenous fish species of Begnas Lake. Results showed that these fish species are source of high quality moisture, protein, fat, ash and minerals. The nutritional composition of studied fish samples confirmed variation among the fish species. Since the present work elucidated more on the importance of small indigenous fishes so consumption of these species is highly recommended. In this study, we focused only on selected elements in fish, and their high levels indicated that they also would be rich in other nutrients which can be expected to be high in fish. Hence, an overall study has helped to generate and document comprehensive information on the nutritional component of several commonly consumed fish species.

6.2 Recommendations

There is limited information on the nutritive value difference of fish species in Nepal. Since the present study focuses on the nutritional value of small indigenous fish species so it is advised for further works regarding other larger food fishes. Comparison of small indigenous fish species with other larger ones on the basis of nutrient profile might be helpful in food nutrition and consumption. Since the study was based on the same habitat so further works need to be done inhabiting the different habitat. There needs an exposure of such small indigenous fish species to good market which minimizes the dependency on imported fish species in one hand and improves the livelihood of local people on the other hand.

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APPENDIX I

Table 1: List of local fish species collected from Begnas during sample collection

S.N.	Fish species	Order	Family	Local names
1	*Puntius sophore*	Cyprniformes	Cyprinidae	Vitte
2	*Puntius conchonius*			Rato Vitte
3	*Danio devario*			Serra Machha
4	*Barilius barna*			Phageta
5	*Mastacembelus armatus*	Synbranchiformes	Mastacembeloidei	Bam
6	*Xenentodon cancilla*	Beloniformes	Belonidae	Chuche Bam
7	*Mystus vittatus*	Siluriformes	Bagridae	Junge Machha
8	*Heteropneustes fossilis*		Heteropneustidae	Singe/ Chille
9	*Channa striatus*	Ophiocephaliformes	Channidae	Bhote

Table 2: Length weight of small indigenous fish species collected from Begnas Lake

S.N.	Fish species	Length (cm) mean ± s.d.	Weight (gm) ± s.d.
1	*Puntius sophore*	6.33125 ± 0.804	4.30875 ± 1.041
2	*Puntius conchonius*	6.41875 ± 0.718	4.768625 ± 1.580
3	*Danio devario*	5.633 ± 0.739	3.18666 ± 0.781
4	*Barilius barna*	9.171 ± 0.579	4.766 ± 0.3784
5	*Mastacembelus armatus*	17 ± 0.3915	13.525 ± 0.298
6	*Xenentodon cancilla*	17.9 ± 0.697	18.65 ± 4.09
7	*Mystus vittatus*	12.4166 ± 1.105	17.8 ± 1.586
8	*Channa striatus*	12.11 ± 1.089	13.381 ± 0.49 1
9	*Heteropneustes fossilis*	12.9 ± 0.406	7.25 ± 0.4332

APPENDIX II

Table3: Fish species with their different body weight, gutted weight, dried weight and homogenized weight (gm)

S.N.	Fish species	No. of individuals	Total body weight (gm)	Total gutted weight (gm)	Total dried weight (gm)	Total homogenized weight (gm)
1	*Puntius sophore*	118	428.66	365.64	102.06	96.84
2	*Puntius conchonius*	110	442.31	364.13	123.58	120.89
3	*Danio devario*	168	399.29	321.965	113.66	106.48
4	*Barilius barna*	95	498.485	409.51	94.34	86.79
5	*Mastacembelus armatus*	23	419.5	381.12	119.64	91.98
6	*Xenentodon cancilla*	22	407.15	359.45	89.15	82.42
7	*Mystus vittatus*	38	513.583	448.804	105.495	100.835
8	*Channa striatus*	48	463.17	333.91	150.69	144.09
9	*Heteropneustes fossilis*	43	297.395	276.49	102.29	94.17

APPENDIX III

Table 4: Protocols for analysis of nutrient composition of small indigenous fish species

S.N.	Proximate composition	Protocol
1.	Moisture composition (hot oven method)	A clean weighing bottle was taken. It was dried in a drying oven at 135^0C for two hours. Then it was cooled in a desiccator for 30 minutes. Constant weight (A) was measured. The process was repeated until the weight becomes constant (0.0001 g). 1.000 – 2.000 g sample was taken in a weighing bottle (B). The bottle should not be touched directly with a hand. Then the weighing bottle was dried in an electric air oven at 110^0C for 20-24 hours and was cooled in a desiccator for 30 minutes. The constant weight (C) was measured. The heating process was repeated for 1 hour and was cooled for 30 minutes until the weight of the bottle becomes constant.
2.	Ash content AOAC (2000) standard methods	A clean crucible was taken and was heated in a muffle furnace at 550^0C for two hours. Then it was cooled in desiccator for 30 minutes. The stopper of desiccator was opened after about 1 minute to release the hot air before vacuum. The constant weight (A) was measured and the process was repeated until weight becomes constant (0.0001 g). 1.000 – 2.000 g sample was taken in crucible (B). The crucible should not be touched by hand directly. The crucible was heated on electric heater until smoke does not come off. Then, the crucible was heated on muffle furnace at 550^0C for 4-6 hours. Finally, was cooled in desiccator for 30 minutes. Constant weight (C) was measured. The process was repeated for hour and was cooled for 30 minutes until the weight of crucible becomes constant.
3.	Crude fat (ether extraction method)	Extraction flask was placed in an oven at 110℃ for 1 hour and was cooled in desiccator for 30 minutes and was weighed for constant weight (A). It was re-heat for 30 minutes and the process was repeated until change weight within 0.000 g. For the dry sample, 1-2 g sample was weighed into a thimble filter tube and was covered with thin layer of absorbent cotton (B). The thimble was placed into the thimble filter tube in the extraction chamber and the pre-weighted extraction flask was connected in the bottom just over the water bath. About 100 ml of Ethyl ether was added into the extraction chamber and was extracted on water bath at 60 – 70℃ for 16 hours. The thimble filter tube was removed from the extraction chamber and the ether gets evaporated from the extraction flask. The extraction flask was dried in an oven at 110℃ for 1 hour and was cooled in desiccator for 30 minutes until the weight becomes constant (C).

4.	Crude Protein ((Micro-Kjeldahl method)	**a. Digestion** For Digestion, 0.5 – 1.0 gm of sample was weighed in nitrogen free weighing paper and the weighed sample was wrapped with weighing paper and was placed in a Kjeldahl flask. 2.0 – 3.0 gm of catalyst and 10 ml sulfuric acid was added. Flask was placed on the digestion rack and was heated gently for 30 minutes for 1 hour. Heating should be vigorously for 3 – 4 hours until solution becomes clear (bright green). Flask was cooled and 20- 30 ml distilled water should be added. Again flask was cooled and the solution from Kjeldahl flask was transferred to 100 ml volumetric flask. The Kjeldahl flask should be washed with distilled water until all solution becomes take off. The sample solution in 100 ml volumetric flask was brought up to the volume (by adding distilled water). **b. Distillation** The Kjeldahl distillation apparatus was kept in heating condition. 10 ml of 0.05 N sulfuric acid was taken and 2 drops of indicator was added in an Erlenmeyer flask and was placed under the delivery tube of condenser. 10 ml of sample solution was taken to put into the distillation flask from the funnel and should be washed with 1 ml of distilled water then, 20 ml of 30% NaOH solution was added to the distillation flask. The alkaline mixture in the distillation was steam distilled for about 10 minutes until the volume of solution raise up to 40 ml in flask. The Erlenmeyer flask was then lowered so that tip of condenser tube was above the surface of the solution and steam destination was continued for 1~2 minutes. **c. Titration** The Erlymeyer flask was taken out and solution was titrated with the standard 0.05 N sodium hydroxide solution.
5.	Minerals AOAC (2000) standard methods	In all mineral analyses, samples (1.000 g) were incinerated in porcelain crucibles at 450℃ overnight, and then treated with 5 ml of 6 M HCl, boiled to dryness on a hot plate, cooled and the residue re-dissolved with 10 ml of 0.1 m Nitric acid. The solutions were left standing for 2 hour and then, transferred to 50 ml volumetric flasks, topped with ultra-pure water and used for the determination of Ca, Mg, K, Na, Zn, Cu, Mn and Fe.

APPENDIX IV

Table 5: Proximate composition of small indigenous fish species (Dry Basis) (Mean ± S.D.)

S.N.	Fish species	Moisture (%)	Crude Fat (%)	Crude Protein (%)	Crude Ash (%)
1	*Puntius sophore*	8.39± 0.01	12.85 ± 0.0435	43.46 ± 0.0458	17.39 ± 0.0608
2	*Puntius conchonius*	6.88 ± 0.0173	12.45 ± 0.01	60.44 ± 0.026	19.65 ± 0.017
3	*Danio devario*	8.34 ± 0.1732	13.88 ± 0.02	63.28 ± 0.0360	13.66 ± 0.01732
4	*Barilius barna*	8.35 ± 0.01	12.66 ± 0.01	61.87 ± 0.0171	16.18 ± 0.01
5	*Mastacembelus armatus*	8 ± 0.026	6.22 ± 0.01	68.19 ± 0.01	12.64 ± 0.01
6	*Xenentodon cancilla*	7.17 ± 0.026	11.6 ± 0.0171	67.16 ± 0.01732	12.79 ± 0.01
7	*Mystus vittatus*	6 ± 0.0360	11.61 ± 0.01732	65.14 ± 0.02	15.53 ± 0.02
8	*Channa striatus*	7.45 ± 0.02	10.51 ± 0.01732	64.68 ± 0.01	16.4 ± 0.01
9	*Heteropneustes fossilis*	7.56 ± 0.01	11.27 ± 0.01	59.52 ± 0.01732	16.08 ± 0.01

Table 6: Mineral contents in small indigenous fish species (Dry Basis) (Mean ± S.D.)

S.N.	Fish species	Calcium (mg/100 g)	Phosphorus (mg/100 g)	Iron (mg/100 g)
1	*Puntius sophore*	4950.86 ± 0.7501	917.02 ± 0.0608	6.94 ± 0.017
2	*Puntius conchonius*	6282.45 ± 0.476	924.37 ± 0.1126	3.1 ± 0.0248
3	*Danio devario*	4596.8 ± 0.3119	967.59 ± 0.0360	1.65 ± 0.02645
4	*Barilius barna*	5827.32 ± 0.208	2552.49 ± 0.0781	4.04 ± 0.017
5	*Mastacembelus armatus*	3259.63 ± 1.171	4053.35 ± 0.1670	9.27 ± 0.01732

6	*Xenentodon cancilla*	2375.6 ± 0.3777	3946.28 ± 0.0529	14.53 ± 0.0305
7	*Mystus vittatus*	4797.77 ± 0.330	4312.5 ± 0.3132	4.88 ± 0.026
8	*Channa striatus*	5233.06 ± 0.0721	4510.8 ± 0.35	7.82 ± 0.01
9	*Heteropneustes fossilis*	4818.13 ± 1.0179	2943.07 ± 0.07211	10.61 ± 0.01732

Table 7: ANOVA

Test for equal means					
	Sum of sqrs	df	Mean square	F	p (95%)
Between groups:	1.94E+08	6	3.23E+07	59.8	1.47E-22
Within groups:	3.02E+07	56	539883	Permutation p (n=99999)	
Total:	2.24E+08	62	1.00E-05		

PLATE I

Different types of net used during sampling

Photo 1: Cast net

Photo 2: Gill net

PLATE II

Indigenous fish species of Begnas Lake

Photo A: *Puntius sophore* (upper)

Photo B: *Puntius conchonius* (lower)

Photo 1: *Puntius spp*.

Photo 2: *Danio devario*

Photo 3: *Barilius barna*

Photo 4: *Mystus vittatus*

PLATE II

Photo 5: *Xenentodon cancilla*

Photo 6: *Mastacembelus armatus*

Photo 7: *Channa striatus*

Photo 8: *Heteropneustes fossilis*

PLATE III

Lab work

Photo A **Photo B**

Photo 1: Photo A and B showing the sample preparation

Photo 2: Weighing of gutted samples

Photo 3: Gutted samples being ready for drying

Photo 4: Drying Oven

PLATE III

Photo 5: Dried samples

Photo 6: Homogenizer

Photo 7: Weighing of Homogenized sample

Photo 8: Homogenized samples