Seasonal variation in proximate, fatty acid and amino acid profile of freshwater mud eel, *Monopterus cuchia* (Hamilton, 1822)

A thesis submitted to the Department of Fisheries, University of Dhaka
in partial fulfillment of the requirements for the degree of
Master of Science (MS) in Fisheries

Submitted By

Examination Roll: Curzon-816

MS Session: 2015-2016

Registration No: 2011-312-767

Registration session: 2011-2012

DEPARTMENT OF FISHERIES

UNIVERSITY OF DHAKA

Bangladesh

February 2017

Declaration by Student

I hereby declare that the dissertation entitled "Seasonal variation in proximate, fatty acid and amino acid profile of freshwater mud eel, *Monopterus cuchia* (Hamilton, 1822)" submitted to the Department of Fisheries, University of Dhaka for the degree of Master of Science (MS) in Fisheries, is based on self-investigation, carried out under the supervision of **Md. Hasan Faruque** and**Dr Mohammad Shamsur Rahman**, Department of Fisheries, University of Dhaka, Dhaka-1000, Bangladesh.

I also declare that this or any part of this work has not been submitted for any other degree anywhere. All sources of knowledge used have been duly acknowledged.

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Certificate

We certify that the research work embodied in this thesis entitled **" Seasonal variation in proximate, fatty acid and amino acid profile of freshwater mud eel, *Monopterus cuchia* (Hamilton, 1822) "** submitted by **Sohel Rana, Examination Roll: Curzon-816, Session 2015-2016**, **Registration No: 2011-312-767,** has been carried out under our supervision. This is further to certify that, it is an original work and suitable for the partial fulfillment of the degree of Master of Science (MS) in Fisheries from the Department of Fisheries, University of Dhaka.

We wish every success in his life.

…………………………….	..……………………………….
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Acknowledgements

At first I want to express all of the praise to the most merciful and compassionate **Almighty Allah**who has enable me to finish the research work successfully for the degree of Master of Science (MS) in Fisheries.

I would like to express my heartfelt gratitude, deepest sense of appreciation and profound indebtedness to my reverend teacher and research supervisor, **Md. Hasan Faruque**, Assistant Professor, Department of Fisheries, University of Dhaka and Co-supervisor **Dr Mohammad Shamsur Rahman**, Associate Professor, Department of Fisheries, University of Dhaka for their day to day supervision, valuable suggestions, advice, fruitful criticisms, encouragement and inspirations throughout the entire period of research work.

I wish immeasurable pleasure to convey honest appreciation and gratefulness to all of my respected and beloved teachers of the Department of Fisheries, University of Dhaka, especially Chairperson of the Department **Professor Dr. Kaniz Fatema** and Chairman of the MS 2016 (2015-2016) examination committee **Professor Dr. Md. Ghulam Mustafa,** Department of Fisheries, University of Dhakafor their academic counsel and encouragement.

I convey profound respect and sincerest gratification Selina Khan, Principal Scientific Officer, IFST, BCSIR, Dhaka and Umma Fatema Sahahjadee, Senior Scientific Officer, IFST, BCSIR, Dhaka for their exemplary guidance, monitoring and constant encouragement about this research work.

I sincerely express my gratefulness to Government of the People's Republic of Bangladesh, Ministry of Science and Technology, Bangladesh Secretariat for their financial support to complete my thesis work

Cordial thanks to all of my beloved friends and well-wishers especially Md. Munjur Hossain, Md. Mostave Enan Eshik, Utpal Chandra Ray, Nusrat Jahan Punom and Md. Masud Rana (Orchid Trading Corporation, Dhaka) for their kind support throughout the study.

Finally, I am grateful to my beloved parents and family members for their moral supports, blessings and encouragement in executing thesis work and preparing this report.

February, 2017

Author

Abstract

Throughout the year, fish are subjected to considerable environmental changes and fluctuations in availability and compositions of feed that will affect their proximate, fatty acids and amino acids composition. The purpose of this study was to clarify the seasonal variation in proximate, amino acid and fatty acid profile of freshwater mud eel, *Monopterus cuchia*. The proximate compositions, total amino acid and fatty acid profile were determined by AOAC, HPLC and GC respectively. Results indicated that proximate (Moisture, Protein and Lipid), amino acid and fatty acid profile varied significantly ($p<0.05$) among these three seasons (pre-monsoon, monsoon and post-monsoon). However, ash content showed no significant variation among seasons. The amount of protein was higher in monsoon than in pre-monsoon and post-monsoon season. The fat and fatty acid as well as protein and amino acid contents of the investigated fish species showed a significant ($p<0.05$) seasonal dependency. Among the 14 recorded amino acids (Essential Amino Acid-6, Nonessential Amino Acid-8), the major amino acids in the total crude protein were lysine, glutamic acid, arginine, aspartic acid, leucine and methionine. The ratio of EAA/NEAA was higher in post-monsoon season than pre-monsoon and monsoon season. The findings of the present study also showed that unsaturated fatty acids represented 44.2142%, 57.2339%% and 66.0548% of the total fatty acids, whereas saturated fatty acids accounted for 55.7858%, 42.7661% and 33.9452% in pre-monsoon, monsoon and post-monsoon seasons. The major fatty acids in muscle of*M. cuchia* were oleic acid (18:1), palmitic acid (16:0), linoleic acid (18:0),palmitoleic acid (16:1) and lauric acid (C12:0). Fatty acid profile resulted that some fatty acid: tridecanoic acid (C13:0), pentadecenoic acid (C15:1), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosapentaenoic acid (C20:5) and docosapentaenoic acid (C22:5) were not detected in pre-monsoon experimental fish. Most nutritionally important Docosahexaenoic Acid (DHA, C22:6 n-3) was dominant fatty acid in monsoon than post-monsoon. Linoleic acid was the most abundant Poly

Unsaturated Fatty Acid (PUFA) in *M. cuchia.* Fatty acid composition and n-3/n-6 is higher in monsoon than pre-monsoon and post-monsoon season which indicates fishes caught in monsoon season arenutritionally rich, thus advantageous for human health. Therefore, a comprehensive study on *Monopterus cuchia* (Cuchia) is required for the further confirmation of the result of present study.

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List of Abbreviations and Symbols

DoF	Department of Fisheries
FAO	Food and Agriculture Organization
FRSS	Fisheries Resources Survey System
GDP	Gross Domestic Product
IUCN	International Union for Conservation of Nature
EPA	Eicosapentaenoic Acid
DHA	Docosahexaenoic Acid
EFA	Essential Fatty Acid
ATP	Adenosine Tri- Phosphate
PUFAs	Polyunsaturated Fatty Acids
COI	Cytochrome C Oxidase Subunit I
BCSIR	Bangladesh Council of Scientific and Industrial Research
AOAC	Association of Official Analytical Chemists
GPS	Global Positioning System
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
FAMEs	Fatty Acid Methyl Esters
SD	Standard Deviation
SEM	Standard Error of Mean
SPSS	Statistical Package for the Social Sciences
ANOVA	Analysis of Variance

+	Plus
-	Minus
%	Percentage
±	Plus-Minus
×	Multiplication
>	Greater than
<	Less than
=	Equal to
kg	Kilogram
p	Level of Significance
Ca	Calcium
Mg	Magnesium
K	Potassium
Na	Sodium
P	Phosphorus
S	Sulphur
Cl	Chlorine
Cu	Copper
Mn	Manganese
Ba	Barium
Ni	Nickel
Mo	Molybdenum
Sr	Strontium
I	Iodine
Br	Bromine
Cu	Copper
Mn	Manganese
Hg	Mercury
Cd	Cadmium
Co	Cobalt
Al	Aluminium
Zn	Zinc
HCl	Hydrochloric acid

H_2SO_4	Sulphuric acid
NaOH	Sodium hydroxide
Na_2SO_4	Sodium sulfate
NH_4	Ammonium
H_2BO_3	Dihydrogen borate
H_3BO_3	Boric acid

1. Introduction

1.1 Background

Bangladesh is considered as the most suitable regionfor aquaculture and fisheries in the world. It is not only one of the resourceful countries with its wide range of aquatic bio-diversities but also one of the world's leading inland fisheries producer countries.In 2014-2015, Inland fisheries contribute about 83.72% of countries total fish production (inland capture: 27.79% and inland culture: 55.93%) respectively. Last 10 years average growth rate of fisheries is 5.4% where aquaculture shows the growth performance of 8.2% (DoF, 2016).Bangladesh is ranked 4th in inland capture fisheries production and 5th in aquaculture production all over the world (FAO, 2015).

Fisheries is now themost valuable agricultural activities in Bangladesh, playing a substantial role in nutrition, food security, employment and income generation, trade, source of foreign exchange earnings and above all poverty reduction in the economy (Sarker et al., 2014). This sector plays a very important role in the national economy having a share 3.69% in national GDP, almost one-fourth (23.12%) in agricultural GDP and 2.01% to the country's export earnings (2nd largest) next to Ready-Made Garments. It also creates employment facilities not only for 17.80 million people (approximately 11% of total production) but also employment for women about 1.40 million (approximately 8.5% of fisheries sector employment). This sub sector also provides about 60% of the animal protein intake (FRSS, 2016).

Fish is the second most valuable agricultural crop in Bangladesh with its irreplaceable animal protein sourcewhich is consumed at 18.1 kg per person per year (Belton et al., 2014). The culture and consumption of fish has important implications for national income and food security. Bangladeshi people are popularly referred to as "Mache Bhate Bengali" or "Fish and rice makes a Bengali". Freshwater fishes are an extremely diverse group that have evolved to occupy a wide range of habitat types, some which are extremely harsh environments for the aquatic organisms.

The freshwater habitat of Bangladesh is very suitable for living the swamp mud eel, *Monopterus cuchia* and the population of this fish is very high. The Gangetic Mud eel, *Monopterus cuchia* is a freshwater air breathing mud eel is locally known as "Cuchia" belongs to the family Synbranchidae of the order Synbranchiformes (Rosen and Greemwood, 1976). In Bangladesh, it is commonly found throughout the country, plenty in mud-holes, boro-paddy, floodplains, swamps, canals, haors, particularly in Sylhet, Mymensingh and Tangail district. Due to present of highly modified accessory respiratory organs, this fish can survive 90 to 132 days without food and water. This fish is very tasty, nutritionally rich with medicinal value and highly priced in foreign markets (Miah et al., 2015). Although, this fish has a quite large economic demand, the population of the freshwater mud eel are declining at an alarming rate from the natural water bodies due to several reasons especially for overexploitation and loss of habitats. Nowadays, this fish species is recorded as not only “rare species” but also “red-listed species” (IUCN, 2000).

1.1.1 Fish nutrients

Fish contain essential fatty acids, amino acids and some of the principal vitamins and minerals in sufficient amounts for healthy living. Fish oil has been utilized by humans for hundreds of years to help relieve medical problems with its high nutritional value (Hunter and Roberts, 2000). Fish oil is one of the most crucial natural resources of polyunsaturated fatty acids inclusive of Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) are received from fish that have been validated to have useful consequences on human fitness (Rafflenbeul, 200; Saoud et al., 2008).

In addition, fish oil is a rich source of nutrients inclusive of diet A, D, E, and K which might be soluble in oil and should be taken on a normal foundation due to their key roles in human health and metabolism(Kinsella, 1987).Dietary responses to quick term diets of fish and the fine effects on protein calorie malnutrition, bronchial asthma, arthritis, vehicle-immunity, coronary heart illnesses and arteriosclerosis had been independently and unanimously stated (Cobiac et al., 1991; Gerhard et al., 1991).Everyday consumption of vital fatty acid (EFA: EPA+DHA) can also play an important role inside the prevention of cardiovascular sicknesses and neural disorders (Lauritzen et al., 2001; Kris-Etherton et al., 2002).

Arnett (2000) suggested that in being pregnant and lactation an adequate intake of DHA and EPA is in particular crucial. At some stage in this time DHA and EPA must be furnished with meals because human body cannot synthesize these important fatty acids. The composition of nutrients in fish varies significantly from species to species and also from character to individual relying on age, sex, environment and season (Huss, 1995).

1.1.2 The major components of fish muscle

1.1.2.1 Moisture

Moisture content is expressed as the amount of water as a percentage (%) and the remaining portion is the dry matter. It is the main constituent of fish flesh which usually accounts for about 80 percent of the weight of a fresh fish fillet while the average water content of the flesh of fatty fish is ready 70 per cent. Generally the range moisture content varies between 60-80% in many fish(Murra and Burt, 2001).

The water in fresh fish muscle is tightly bound to the proteins within the structure in any such manner that it cannot effectively be expelled even underneath excessive stress. After extended chilled or frozen storage the proteins are less capable of maintain all the water, and some of it, containing dissolved materials, is misplaced as drip. As a consequence moisture content is a critical measure of fish satisfactory, because the flesh obviously has excessive water content material.

1.1.2.2 Protein

Due to the amino acid composition and degree of digestibility, fish is the excellent source of protein. Protein consists of amino acids. The amount of protein in fish muscle is between 15 to 20 percent (Murra and Burt, 2001).

The proteins in fish muscle tissue can be divided into three groups: i) Structural proteins (actin, myosin, tropomyosin and actomyosin) which are soluble in neutral salt solutions of fairly high ionic strength (0.5 M), constituting 70-80 percent of the total protein content (Nowsad, 2007); ii) Sarcoplasmic proteins (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic strength (<0.15 M). This fraction constitutes 25-30 percent of the protein (Nowsad, 2007) and iii) Connective tissue proteins (collagen), which constitute approximately 3 percent (Nowsad, 2007) of the protein in teleosts and about 10 percent in elasmobranch (compared with 17 percent in mammals).

1.1.2.3 Lipid

Fish lipid has been recognized as being beneficial for human health. Lipids are widely classified as fat, phospholipids, sphingomyelins, waxes and sterols. Lipids are the widespread names assigned to a group of fats soluble compounds observed in the tissues of animals.

1.1.2.4 Fat

Fat deposition is normally below the pores and skin, across the stomach wall and after alongside lateral line. Fat are made of different styles of fatty acids. Those fatty acids range in the quantity and association of the carbon and hydrogen atoms. Fat are the fatty acid esters of glycerol and are the number one energy depots of animals. Those are used for long-term electricity requirements in the course of intervals of massive exercising or throughout periods of inadequate food and power intake. Fats content material of fish varies with the species (Exler et.al., 1975). Variation is also caused by feeding, size, age, season, physiological status and geographical location (Stansby, 1954).

1.1.2.5 Fatty acids

Fatty acids are crucial resources of gasoline due to the fact, while metabolized, they yield massive quantities of ATP. It's far a carboxylic acid with an extended aliphatic chain, which is either saturated or unsaturated. Maximum clearly going on fatty acids have a chain of a good variety of carbon atoms, from fatty acids are usually derived from triglycerides or phospholipids. When they are no longer connected to different molecules, they are called "free" fatty acids.

1.1.2.6 Amino acids

Amino acids are the building blocks of proteins and serve as body builders. Fish protein is an excellent source of these amino-acids especially lysine and methionine are generally found in high concentrations in fish proteins, in contrast to cereal and meat proteins. All proteins, are chains of chemical units (called amino acids) linked together to make one long molecule. There are about twenty types of amino acids. Some of them are essential and some are non-essential. Human body cannot synthesize essential amino acids and it must take from the diet for the maintenance of good health. Essential amino acids are isoleucine, lysine, tryptophan, valine, leucine, threonine, phenylalanine, methionine (Chandrashekar and Deosthale, 1993).

1.1.3 The minor components of fish muscle

1.1.3.1 Carbohydrate

The quantity of carbohydrate in fish muscle is typically too small. In white fish the amount is typically less than 1 percent, however in the dark muscle of some fatty species it may once in a while be up to 2%. A few mollusks contain up to 5% of the carbohydrate glycogen (Love, 1970).

1.1.3.2 Vitamins

Vitamins can be divided into two categories, those that are soluble in fats, inclusive of nutrients A, D, E and K, and those which might be soluble in water, inclusive of nutrients B and C. All of the nutrients are essential for true fitness. It calls for no longer only to sell true health however also to maintain existence itself. Fish does provide a well-balanced deliver of minerals in a conveniently usable form.

Water-soluble nutrients in fish especially present inside the skin. However in the liver and the intestine, they are more uniformly allotted and the flesh commonly contains extra than half of the full quantity present within the fish. The roe is also a very good source of those vitamins. The mineral and diet content of fish isn't markedly stricken by cautious processing or through preservation, furnished storage isn't always very prolonged.

1.1.3.3 Minerals

Minerals especially focused in fish bone. A few factors together with boron, fluorine, bromine, lithium, strontium are present in more awareness in each marine and clean water fish. FAO Corporate Document Repository has mentioned that typically fish muscle rarely exceeds 1-2% minerals.

The principal minerals are Calcium(Ca), Magnesium(Mg), Potassium(K), Sodium (Na), Phosphorus(P), Iron (Fe), Sulphur (S), Chlorine(Cl), Copper (Cu), Manganese (Mn), Iodine (I), Bromine (Br). Some trace elements also present in small quantity. Such trace elements may be Strontium(Sr), Mercury (Hg), Cadmium (Cd), Molybdenum (Mo), Cobalt (Co), Nickel (Ni), Aluminum(Al), Barium (Ba), Zinc(Zn) etc.

Either endogenous or exogenous factors affect fish proximate composition. The endogenous factors are genetically controlled and are associated with the life cycle of fish. The proximate and fatty acid compositions are also affected by exogenous factors (temperature and salinity). Based on species, sexual cycle, age, feed, stage of maturity, environment, season, organs and muscle location, the composition of fish muscle also varies greatly. It does appear that seasonal changes with its resultant effect on the activities of fishes may cause variations in their nutrient quality.

1.2 Rationale

Muscle is the main part of fish consumed by human which contains protein, lipid, carbohydrate, moisture, ash, minerals etc. The measurement of these proximate profiles is often necessary to ensure that they meet the dietary requirements and commercial specifications (Onyia et al., 2010).A few quantitative studies have also indicated that body constituents and energy resources vary with seasonal life cycles (Puwastien et al., 1999). However, the flesh protein contents much less influenced with the aid of outside feeding for the reason that it is particularly depending on intrinsic elements which includes the fish species, subculture environment and size (Shearer, 1994). It is far extensively believed that the wild fish acceptability is higher than that of farmed fish.

Many studies had been performed at the metabolism and characteristic of polyunsaturated fatty acids (PUFAs) in popular and on the levels and ratios of n-3 and n-6 fatty acids specifically. Humans cannot synthesize long chain polyunsaturated fatty acids (PUFAs) like linoleic acid, arachidonic acids, α-linolenic acid, Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) that are known as essential fatty acid which are gained from diets (Hunter and Roberts, 2000; Sushchik et al., 2007).

Today it is known that the balanced ratio of n–3/n-6 fatty acids in the diet, are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, diabetes, hypertension and cancer. It is thus important, for human health, to increase the consumption of fish or fish, which are rich in polyunsaturated fatty acids of the n-3 family and poor in polyunsaturated fatty acids of the n-6 family.

M. cuchia has played to fulfil tremendous demand by contributing in employment opportunities, income augmentation, poverty alleviation, foreign exchange earnings, providing food and nutritional security to the castes as well as ethnic tribal group. Direct consumption of fresh blood of the fish is reported to cure weakness, anemia and asthma (Jamir and Lal, 2005). Consumption of gall bladder of the fish either fresh or sun dried is believed to have anti- asthmatic and anti-rhinitic properties (Kakati et al., 2006). No culture system of *Monopterus cuchia* is developed in Bangladesh. Monopterus cuchia business completely depends on natural capture only. The determination of fatty acid and amino acid profile provide the necessary information concerning the nutrient value for both consumerand researcher.

1.3 Research gap

Diverse fish species do not offer the same nutrient profile relying on the seasons and cultured surroundings. An excellent number of works has already been achieved on proximate composition of various freshwater and marine fishes (Stansby, 1954; Mazumder et al., 2008; Hossain et al., 2015).Alemu et al. (2013) worked on effect of endogenous factors on proximate composition of Nile tilapia (*O. niloticus*) filet from Lake Zeal. Seasonal variations of proximate and total fatty acid composition of wild Brown Trout in Munzur River, Tunceli Turkey were done by Ates et al., 2013. Harlioglu et al. (2016), studied on a comparison of fatty acid, Cholesterol and vitamin composition in sea bass (*D. labrax*) and sea bream (*S. aurata*) from three cage farm areas.

Fatty acid and amino acid composition of marine Eels, *C. talabanoides* and *T. macrura* from Parangipettai coastal water were conducted by Peninal et al., 2012. Rao et al.(2014) worked on seasonal changes of biochemical constituents in the four fish species represented in the trawl net by cletchs off Visalihapatnam, east coast of India.Wang et al. (2012) acted on comparison of proximate composition, amino acid and fatty acid profiles in wild, pond and cage cultured Longsnout catfish (*Leiocassis longirostris*). Wang et al. (2014) also worked on comparative study of proximate composition and amino acid in farmed and wild *Pseudobagrus ussuriensis* muscles. Suschik et al. (2007) studied on seasonal dynamics of fatty acid content of a common food fish from the Yenisei River, Siberian grayling, *Thymallusarcticus*.Seasonal variations in proximate and fatty acid composition of golder grey mullet, *Liza aurata* from the Tunisian coast were performed by Khitouni et al., 2014.

Moreover, Onyia et al. (2013) made a thorough study on variation on proximate composition depending on cultured environments. Commercial feed trials and its effect on proximate composition were done by several researchers (Brahney et al., 1981; Aursand et al., 1994; Badiani et al., 1996). In addition, Yeganeh et al. (2012), Hassan et al. (2010), Sigurgisladottir and Palmadottir (1993) worked on fatty acid and amino acid profiles and their distribution of different habitat fish. Hussain et al. (2011) studied on comparative analysis of proximate composition of head from wild and farmed *C. catla.* Proximate composition of some small indigenous fish species in Bangladesh were done by Mazumder et al., 2008. Moreover, Shamim et al., 2011) carried out a research works to know the proximate composition of different portion of Hilsa (*T. ilisha*) from two regions of the Bay of Bengal in Bangladesh and Nargis (2006) worked on seasonal variation in the chemical composition of body flesh of koi fish *Anabas testudineus* (Block) (Anabantidae, Perciformes). Seasonal variation of the proximate composition of freshwater Gobi, *Glossogobius giuris* (Hamilton) from the River Padma was done by Islam and Joadder, 2005). Yeganeh et al. (2012) acted on comparison of farmed and wild common carp, *C. carpio*: Seasonal variation in chemical composition and Fatty Acid profile.

Studied on *M. cuchia* focusing the molecular identification and sexual differentiation, reproductive biology and induced breeding status, rearing and production performance, health condition, catching techniques from wild habitat and nutritional composition were done by several researcher (Narejo et al., 2003; Ahmed et al. 2009; Barman et al. 2013; Dutta, 2013; Miah et al., 2013; Miah et al. 2015a; Miah et al. 2015b;). Moreover, Devi et al. (2014) worked on comparative mitochondrial DNA sequences and amino acid analysis of the Cytochrome C Oxidase Subunit I (COI) from two Eel species *M. cuchia* and *M. albus*.

But there is no combined work which compares seasonal variation of proximate composition, fatty acid and amino acid profile of freshwater mud eel, *Monopterus cuchia* in Bangladesh or elsewhere. Seasonal variation in the biochemical and nutritional composition of the freshwater mud eel were not recorded strongly. In view of these facts, the aim of this study is to determine seasonal variations of the proximate, fatty acid composition and n-3/n- 6 ratios and amino acid profile of *Monopterus cuchia*.

1.4 Objectives

The overall objective of the thesis work was to know the seasonal variation in proximate, fatty acid and amino acid profile of freshwater mud eel, *Monopterus cuchia*.

To fulfill the main objective specific objectives were-

i. To analyze proximate composition of *Monopterus cuchia*;
ii. To determine total fatty acid composition of *Monopterus cuchia*;
iii. To estimate amino acid profile of *Monopterus cuchia* and
iv. To conclude the seasonal variationin proximate, fatty acid and amino acid profile of *Monopterus cuchia.*

2.Materials and methods

2.1 Experimental fish

The experimental fish, *Monopterus cuchia* is a carnivorous and nocturnal freshwater mud eel which prefers animal based food like small fish, mollusks, aquatic insects, prawns, small frogs and worms (Miah et al., 2015). Body is slender shaped streamlined with a tapering tail inclusive deep brown body color. The main feature of the body is slimy whereas abdominal part is comparatively opaque. Eyes are small, head not conspicuous and gill-opening crescentic of which gills greatly reduce. They do not have pectoral, pelvic and dorsal fin. The dorsal and anal fins are fused with caudal fin or tail forming a single ribbon along the whole length of the fish. It has a pair of air breathing organ contain a complicated labyrinthine organ (Mittal and Agarwal, 1977).

Identification of male or female fish is so difficult but some external characteristics help to observe sexual differentiation during breeding season. It was reported that matured female is larger than matured male and the abdomen of female fish is swollen and brownish in color with rough abdominal skin. Some fishery scientists reported that mud eel pass entire summer in hole, but sometimes coming out from the hole to take oxygen (Miah et al., 2013).

Fig. 1.*Monopterus cuchia* (Cuchia, Freshwater Mud Eel) collected from Sylhet region.

M. cuchia (Cuchia) was selected for the purpose of experiment because of its following potentials-

- It is an export fishery product that is playing an important role in national economy of Bangladesh;
- In 2002, freshwater mud eel (*M. cuchia*) ranked 4th in terms of frozen food export item;
- It can generate employment directly and indirectly for more than 8,000 people in the marketing and other associated business;
- It is also commercially important due to its high demand for export in foreign market especially China (80%), Thailand (10%), Malaysia (3.5%), Japan (3.5%) and America (3%) (Hasan et al., 2012)
- In Bangladesh, the tribal ethnic people and a few other castes eat this fish due to its nutritional and medicinal values such as the prevention and treatment of coronary artery disease, diabetes, hypertension, arrhythmias, heart attack and cancer etc. (Connor, 2000; Harris, 2004).

2.1.1. Scientific classification of *M. cuchia*

Kingdom: Animalia

Phylum: Chordata

Sub-Phylum: Vertebrata

Class: Osteichthyes

Order: Synbranchiformes

Family: Synbranchidae

Genus: *Monopterus*

Species: *M. cuchia* (Hamilton, 1822)

2.2 Collection of fish sample and study period

Samples were collected from Bagha and Budhbari Bazar union of Golabganj upazila in Sylhet district, Bangladesh. Samples were collected in three season; pre-monsoon (March-May), monsoon (June-August) and post-monsoon (September-November). GPS Coordinate of Golabganj upazila is located in between 24°41' and 24°55' north latitudes and in between 91°55' and 92°06' east longitudes.

A B

Fig. 2. Storing facilities of Cuchia in A) Bagha Bazar and B) Budhbari Bazar of Golabganj upazila, Sylhet district.

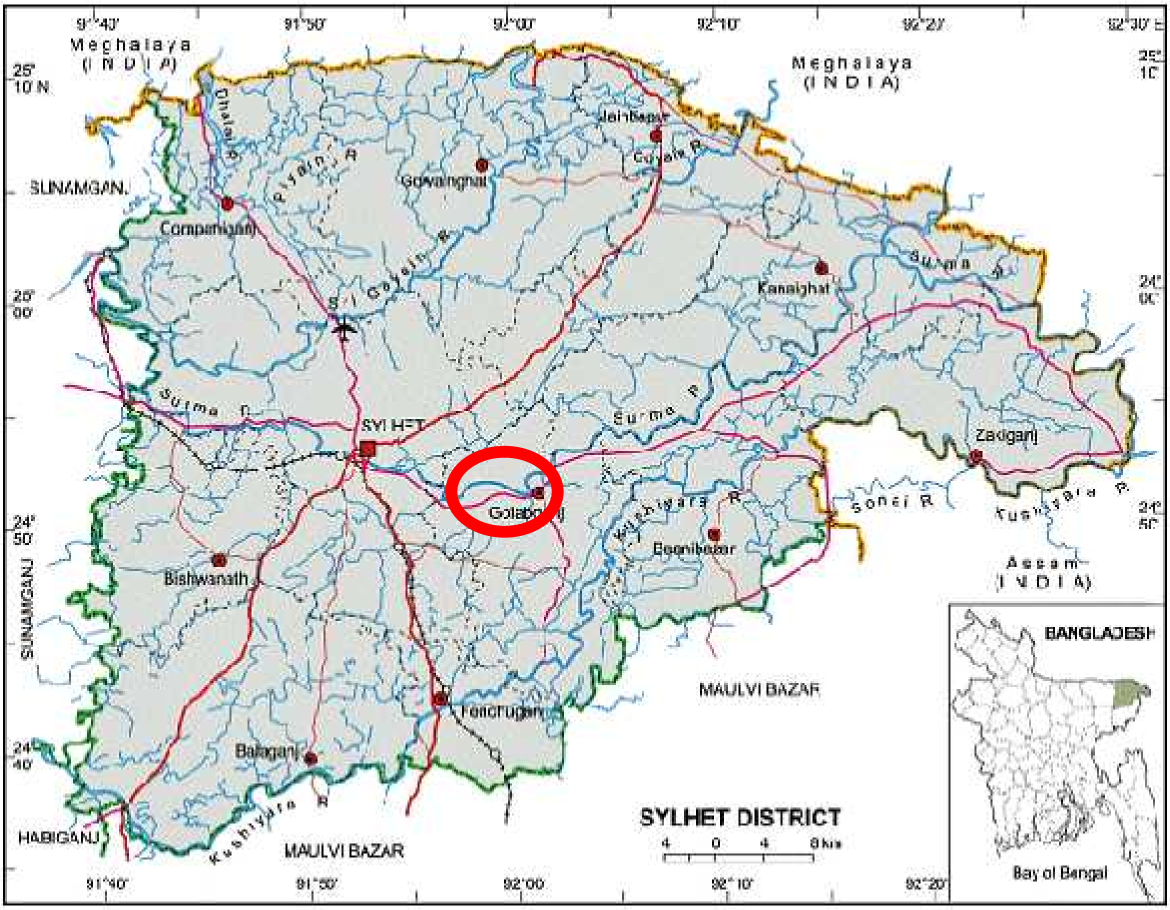


Fig.3. Map of the study area

2.3 Sample analysis

The length weight of the collected fish samples were measured using measuring tape and weight balance respectively. The samples were prepared and analyzed in both aquatic laboratory of the Department of Fisheries, University of Dhaka and BCSIR (Bangladesh Council of Scientific and Industrial Research), Dhaka, Bangladesh.

2.4 Methods of nutritional composition analysis

The proximate composition of the fish muscle was estimated the use of numerous techniques. Air oven method was used for determination of moisture content (AOAC, 2000); ash content material using Gravimetric approach; Crude protein content through the use of Micro-Kjeldhal technique, lipid content by means of (Folch and Stanly, 1957). Amino acid profile and fatty acid profile had been executed respectively through High Performance Liquid Chromatographic (HPLC) and Gas Chromatographic (GC) evaluation.

2.4.1 Determination of moisture

Principle

The sample (*Monopterus cuchia*) is dried to constant weight in the air oven. Moisture content determined as loss of weight and it was done by oven drying method (AOAC, 2000).

Procedure

Dried the empty Aluminum foil cup (15 minutes) and transferred to the desiccator to cool then

⇩

About (3-5) g prepared sample was taken in each foil cup

⇩

Weighted the foil cup with sample and noted

⇩

Dried the sample for overnight (16 hours) at 102

⇩

Removed the dish from oven and cooled in desiccator then reweighted.

Calculation

The percentage of moisture was calculated by the following equation:

$$\text{Moisture (\%)} = \frac{(W_1-W_o) - (W_2-W_o)}{(W_1-W_o)} \times 100$$

Where,

Weight of the foil cup = W_0

Weight of the foil+ Wet sample = W_1

Weight of the foil + Dry sample = W_2

Dry matter = 100 – Moisture (%)

Moisture factor = $\frac{100-\text{Moisture Content (\%)}}{100}$

2.4.2 Determination of ash

Principle

Organic matter is burned off as low temperature as possible and the remaining inorganic material is called the ash content.

Procedure

Dried the empty crucibles and transferred to the desiccator then weighted

⇩

5g of sample is transferred in the desiccator and dried overnight at 102

⇩

Sample is dried in the burner

⇩

Placed the crucibles inside the muffle furnace, as near to the center as possible and ash overnight at 550

⇩

Removed crucibles from muffle furnace and cool in a desiccator then reweighted it.

Calculation

Ash content of the fresh sample (%) $= \frac{(W_2\ W_o)}{(W_1 - W_o)} \times 100$

Where,

Weight of dry crucible = W_0

Weight of dry crucible + dry fish sample = W_1

Weight of dry crucible + ash = W_2

2.4.3 Determination of protein

Principle

The Protein content may be calculated by multiplying the nitrogen value by 6.25. Non-Protein Nitrogen may present in the material. So, it is called crude protein. Crude protein content was determined by Micro-Kjeldhal method.

The Principle of Micro-Kjeldhal method based on the conversion of nitrogen of Protein into $(NH_4)_2SO_4$

$$\text{Protein} + H_2SO_4\,(\text{conc.}) \rightarrow H_2SO_4$$

In digestion chamber NH_3 liberates by addition of excess NaOH and Steam distillation of NH_3 Produced into saturated boric acid.

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$$

$$NH_3 + H_3BO_3 \rightarrow NH_3 + H_2BO_3^-$$

Determination of the NH_3 liberated by the back titration to the endpoint with saturated HCl. An indicator in the pH of 5-6 is required Phenoptheline can be used as an indicator.

$$H_2BO_3^- + H^+ \rightarrow H_3BO_3$$

Procedure

0.5g dry powdered sample was taken in a Kjeldhal flask

⇩

1g digestion mixture and 25ml concentrated H2SO4 was added in Kjeldhal flask

⇩

Kjeldhal flask was set in the digestion chamber for 2.5-3 hours until the solution becomes colorless

⇩

Digested product was transferred to 100 ml volumetric flask and was made up to 100 ml with distilled water

⇩

At this time heat was produced and cooled it in the refrigerator for decreasing the temperature at room temperature

⇩

5 ml diluted solution, 10 ml NaOH and 100-150 ml distil water was added in Kjeldhal flask

⇩

5 ml 2% boric acid and 2 drop Phenoptheline was taken in 250 ml conical flask and transferred the Kjeldhal flask and conical flask in the Kjeldhal distillation unit

⇩

Kjeldhal flask was boiled for 30 minute and condensed water vapor was collected in the conical flask

⇩

Titrated the solution in the conical flask against 0.01 N HCl until the color becomes pink and same procedure was done for blank sample.

Calculation

The Percentage of nitrogen was calculated from the formula:

$$\text{Nitrogen} = \frac{(S-B)\times A\times 14\times C\times 100}{\text{Weight of sample}}$$

Where,

S = Titration reading for sample

B = Titration reading for Blank

A = Strength of 0.01 N HCl

C = Digest taken for distillation (dilution factor) ≈ 20

2.4.4 Determination of lipid content

Principle

A mixture of chloroform: methanol (2:1) is extracted for lipid content determination. The mixture was allowed to face overnight and lipid component became transferred to a weighted beaker and heated to dryness.

Procedure

5 g dry, homogenized fish muscle was taken in a conical flask

⇩

Then added chloroform: methanol (2:1) mixture until the sample submerged and kept it over night

⇩

Then filtered it through a filter Paper and collected into a Pre weighted conical flask

⇩

Conical flask was kept in the water bath until the solution was fully evaporated and only oil remains in the flask

⇩

Finally weighed the conical flask

Calculation

$$\text{Percentage of lipid} = \frac{(W_2 - W_1)}{\text{Weight of sample}} \times 100 \times \text{Moisture factor}$$

Where, W_2 = Dry weight of conical flask

W_1 = Weight of empty Conical flask

2.4.5 Fatty acids determination

Principle

Hydrolytic method is done due to extraction of Fat and fatty acids. Fat is extracted into ether, and then methylated to fatty acid methyl esters (FAMEs). Gas chromatography is used to measure FAMEs quantitatively. The fatty acids profile was done following gas chromatographic (GC) method (Nichols et al., 1995). Fatty acids were obtained from lipids by saponification using NaOH dissolved in methanol H_2O mixture (hydrolysis with alkali).

Procedure

Sample became methylated into fatty acid methyl ester using HCl and methanol combination, which can be easily recognized by using gas chromatography

⇩

Fatty acid methyl ester turned into separated using combination of hexane and anhydrous diethyl ether

⇩

For the organic phase aqueous NaOH became used as base wash and the top organic layer was separated

⇩

(2-3) µl of sample was injected and analyzed using Gas chromatography with capillary column and flame ionization detector

⇩

The chromatogram was used for calculation

⇩

Standard fatty acids were analyzed simultaneously

⇩

Every fatty acid within the unknown pattern turned into diagnosed based on the retention time and peak area of the standard fatty acids.

The flow chart below represents typical procedure for analysis of fatty acid:

Fat → Hydrolysis → Methylated FAMES and GC quantification.

2.4.6 Amino acids determination

Amino acids are the building blocks of proteins and serve as body builders. They are utilized to form various cell structures, of which they are key components and they serve as source of energy (Babsky et al., 1989). Amino acids profiles of *M. cuchia* have been analyzed to assess and differentiate nutritional quality within three different seasons.

Principle

The High Performance Liquid Chromatography (HPLC) was used to analyze the amino acids profile of the freshwater mud eel, *Monopterus cuchia* (Anomynous, 1993).

Procedure

0.5g fish muscle was mixed with 50 ml 6 N HCl by mortar pestle

⇩

Solution was filtered

⇩

Hydrolyzed approximately for 22-24 hours in a hydrolyzer apparatus

⇩

HCl was withdrawled from filtrate by evaporating in a water bath

⇩

Solution was made volume up to 25 ml in a volumetric flask by 0.1 N HCl
(This stock solution was used for determining amino acids)

⇩

Solution was taken across micro filter (0.45 mm) to eliminate any foreign particle if present

⇩

2-3mm of sample was placed in auto injector of HPLC

⇩

Standard amino acids were analyzed simultaneously

⇩

Based on the retention time and peak area of the standard amino acids and each amino acid in the unknown sample was identified.

2.5 Statistical analysis

The Statistical Package for the Social Sciences (SPSS) v. 20.0 software package (SPSS, SAS Institute Inc. Gary, USA) and Microsoft office excel 2007 was used for statistical analysis. The data were analyzed to determine the descriptive statistics such as mean, standard error of mean, standard deviation, minimum and maximum value. Tukey HSD test with one way ANOVA (Analysis of Variance) at 5% level of significance was done for multiple comparisons.

Plate 1

Determination of Moisture and Ash Content

A. Prepared sample for moisture content

B. Drying samples for 8-10 hours at 102℃

A. Prepared sample in Kjeldhal flask

B. Protein distillation machine

Plate 2

Determination of Crude Protein

C. Titration for Protein determination

D. Samples ready for protein determination

Plate 3

Determination of Crude Lipid

A. Prepared sample for lipid determination

B. Filtration of sample after overnight

C. Evaporation in the water bath

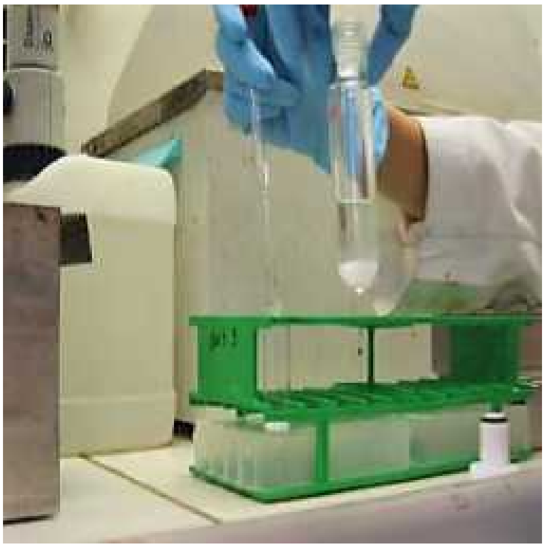
D. Sample ready for lipid determination

Plate 4

Sample preparation before run in GC (Fatty Acid Profile)

A. Extracted fat from lipid

B. Methylated sample for fatty acid profile



C. Collection of top layer after separation

D. Sample ready in vial for GC analysis

Plate 5

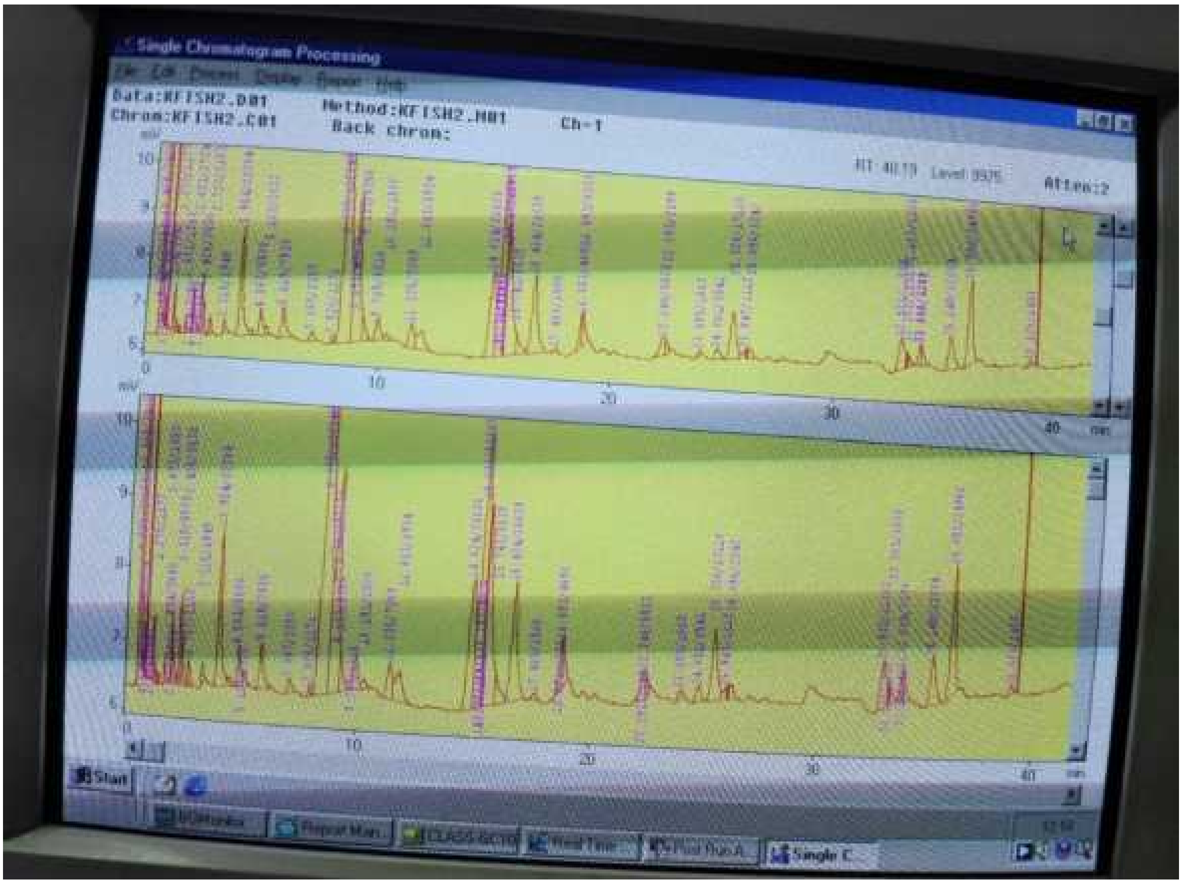
Sample run in GC (Fatty Acid Profile)

A. Injection Syringe (UL)

B. Gas Chromatography

C. Sample ready for fatty acid analysis

D. Injecting (0.2-0.3) µl sample in injection pore of GC



E. Single Chromatogram processing for fatty acids analysis by the help of pick point

Plate 6

Sample preparation before run in HPLC (Amino Acid Profile)

A. Sample preparation for Amino Acid profile

B. Sample in the heating mental for hydrolysis

C. Dilution of sample

D. Micro-filter (0.45mm)

Plate 7

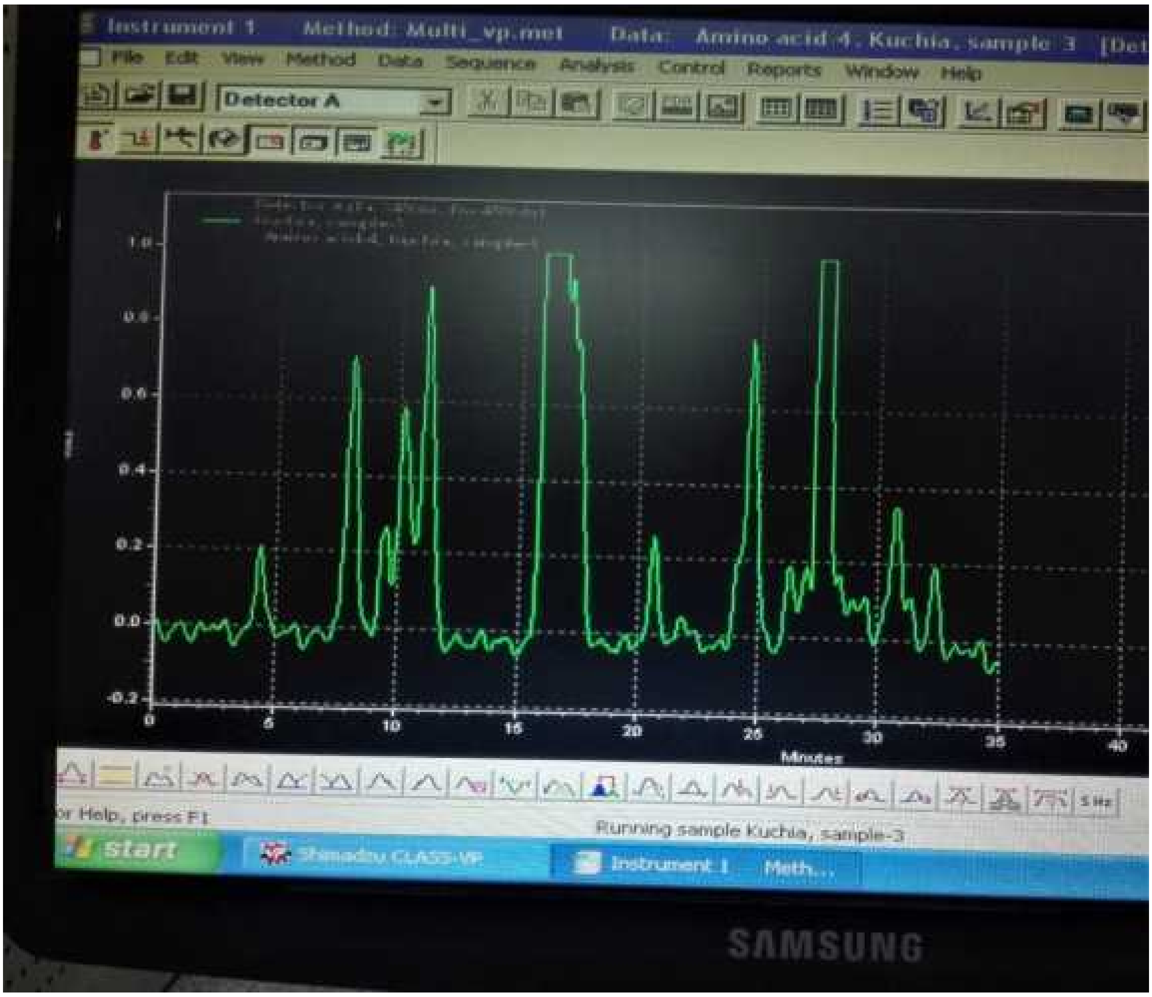
Sample run in HPLC (Amino Acid Profile)

A. Sample passed through microfilter to remove any tiny particle

B. Sample ready to run in HPLC

C. Chemicals used to run sample in HPLC

D. Sample in HPLC



E. Graph for estimation the percentage of Amino Acid by the help of these peak point

3. Results

3.1 Length and weight of freshwater mud eel, *Monopterus cuchia*

The total length and weight of*M. cuchia* were ranged from 50.2 to 74.2 cm and 151.15 to 345.32 g respectively collected in pre-monsoon period (March to May), whereas the total length and weight ranged from 56.5 to 79.3 cm and 202.07 to 463.55 g respectively collected in monsoon (June to August) and from 53.6 to 74.3 cm and 160.67 to 450.00 g respectively collected in post-monsoon season (September to November) (Appendix A-Table 2).

The mean total length for *Monopterus cuchia* was calculated as 57.71±6.73 cm and the mean total weight calculated as 216.12±64.99 g (n=10) collected in pre-monsoon, whereas the mean total length was calculated as 64.87±7.91 cm and the mean total weight calculated as 296.61±4.69 g (Mean±SD) collected in monsoon season and the mean total length and weight collected in post-monsoon were 61.18±5.97 cm and 291.13±81.86 g (Mean±SD) respectively (Table 1;Appendix A- Table 2).

Table 1: Mean length and weight of *Monopterus cuchia* among three different seasons.

Seasons	**Mean (± SD) Total Length (cm)**	**Mean (± SD) Total Weight (g)**
Pre-monsoon (March-May)	57.71±6.73	216.12±64.99
Monsoon (June-August)	64.87±7.91	296.61±4.69
Post-monsoon (September-November)	61.18±5.97	291.13±81.86

The findings of the present study revealed that, there were no significant differences ($p<0.05$) in length and weight of *Monopterus cuchia* among three different seasons (pre-monsoon, monsoon and post-monsoon) (Appendix A-Table 2).

3.2 Nutritional composition

A comparative study was undertaken to determine the moisture, ash, protein, lipid, amino acid and fatty acid profile in freshwater mud eel, *M. cuchia* of Sylhet district, Bangladesh. Depending on the season, results showed a wide variation in proximate, amino acid and fatty acid profile of experimental fish.

3.2.1 Moisture content

Mean (±SD) moisture content of the freshwater mud eel, *Monopterus cuchia* were 78.87±0.32%, 79.64±0.35% and 79.88±0.29% collected in pre-monsoon, monsoon and post-monsoon season respectively (Figure 4). Seasonally the highest value was found in post-monsoon (79.88±0.29%) and on the other hand, the lowest value was found in pre-monsoon season (78.87±0.32%) of moisture content in *M. cuchia* (Figure 4). Among these three seasons, the moisture content ranged between 78.30-80.15% (Appendix B-Table 3).

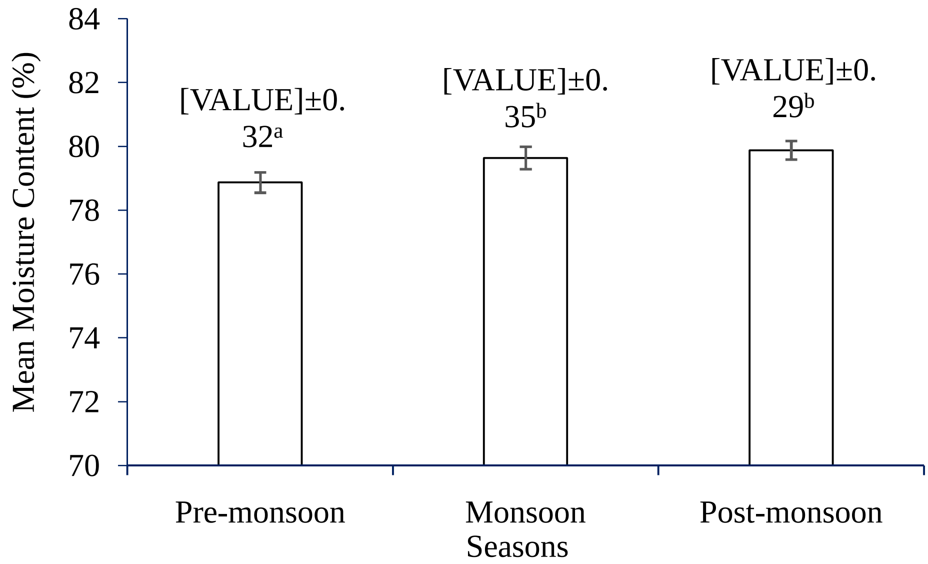


Fig. 4. Variation of moisture content (%) of *M. cuchia* among three different seasons (Pre-monsoon, Monsoon and Post-monsoon). Different letters denote significant differences ($p< 0.05$) and same letter denotes no significance ($p>0.05$).

Result also represented that moisture content differed significantly ($p < 0.05$) not only between pre-monsoon and monsoon season but also pre-monsoon and post-monsoon season of *M. cuchia*. In post-monsoon season, *M. cuchia* showed higher amount of moisture than pre-monsoon and monsoon season. But no significant difference ($p > 0.05$) in moisture content of the experimental fish was observed between monsoon and post-monsoon season (Figure 4; Appendix B-Table 5).

3.2.2 Ash content

In pre-monsoon, it was found that *M. cuchia* contains 1.10±0.04% ash and on the contrary 1.15±0.09% and 1.10±0.09% ash was found in monsoon and post monsoon respectively (Mean±SD) (Figure 5). Seasonally the highest ash content was recorded

1.15±0.09% in monsoon and the lowest value was found 1.10±0.04% in pre-monsoon in freshwater mud eel, *M. cuchia* (Figure 5). Ash content was varied from 1.00-1.27% among these three seasons (Appendix B-Table 3).

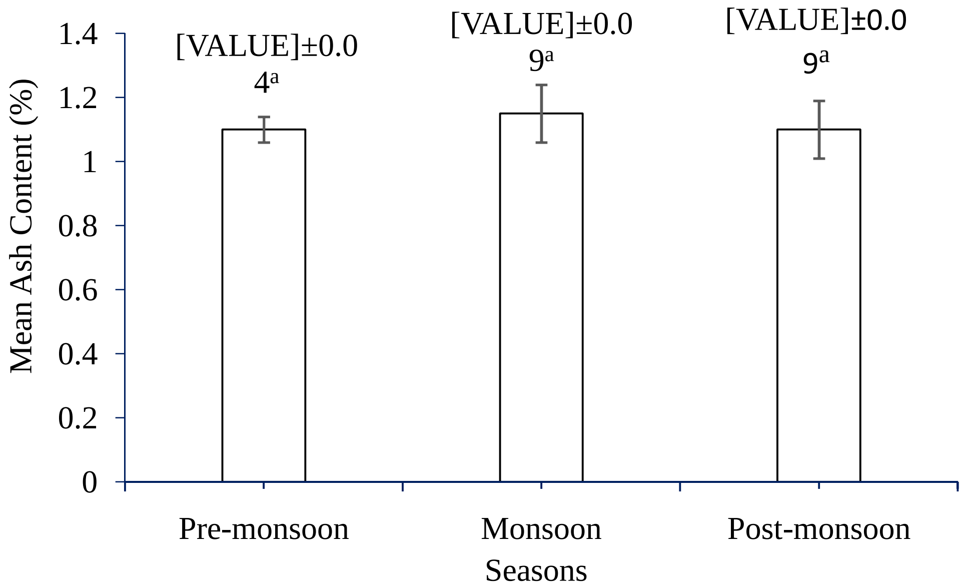


Fig. 5. Variation of ash content (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

Analysis of the data revealed that no significant difference ($p < 0.05$) was observed in ash content among these three seasons (pre-monsoon, monsoon and post-monsoon) in cuchia fish (Figure 5;Appendix B-Table 5).

3.2.3 Crude protein content

Findings described that mean crude protein of *M. cuchia* in pre-monsoon, monsoon and post-monsoon was 17.72±0.26%, 18.83±0.31 and 17.67±0.29% respectively (Mean± SD). The highest value was recorded 18.83±0.31% in monsoon and the lowest value was observed 12.13±0.01% in post-monsoon (Figure 6). Seasonally protein content varied range from 17.29-19.33 % (Appendix B-Table 3).

Analysis of the crude protein illustrated that significant difference ($p < 0.05$) was observed between pre-monsoon and monsoon season. There is also significant difference ($p < 0.05$) present between monsoon and post-monsoon season in *Monopterus cuchia* (Figure 6;Appendix B-Table 5). But there is no significant difference in crude protein content ($p < 0.05$) between pre-monsoon and post-monsoon season.

Among 30 sample size, the maximum crude protein was found in monsoon 19.33% and minimum value was 17.29% in post-monsoon (Appendix B-Table 3).

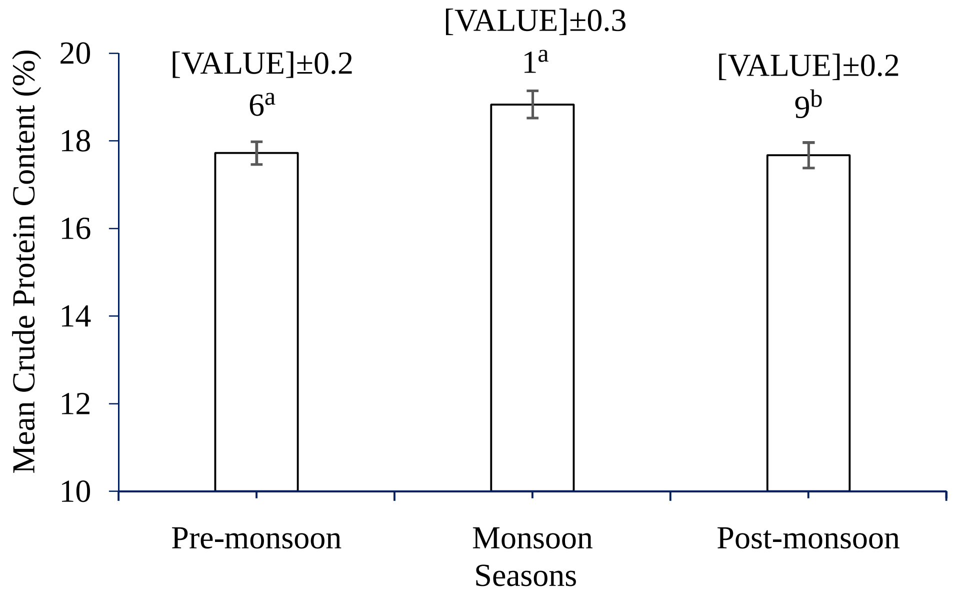


Fig. 6. Variation of Protein content (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

3.2.4 Lipid content

Result of lipid content narrated that lipid content may vary 0.25-2.38% among these three seasons (pre-monsoon, monsoon and post-monsoon) in *M. cuchia* (Appendix B-Table 3). Findings described that in pre-monsoon mean lipid content was calculated 2.24±0.06%, on the other hand 0.30±0.04% and 1.26±0.05% lipid content was presented in monsoon and post-monsoon respectively. Among these result the highest value of lipid content was marked in pre-monsoon season (2.24±0.06%) and the lowest value was pre monsoon (0.30±0.04) % (Figure 7).

The amount of lipid content of the experimental fish, *Monopterus cuchia* exposed that significant difference ($p< 0.05$) resides among these three seasons (pre-monsoon, monsoon and post-monsoon) (Figure 7; Appendix B-Table 5).

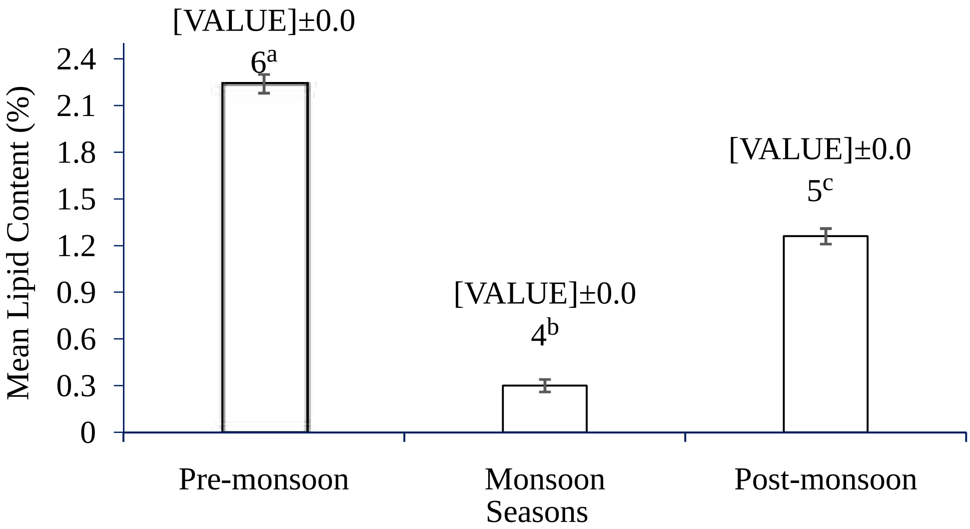


Fig. 7. Variation of lipid content (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

3.3 Fatty acids

Fatty acids are mainly two types. They are saturated fatty acid (SFA) and unsaturated fatty acid. The total SFA content of lipids were 55.786% of *M. cuchia* collected in pre-monsoon., on the other hand, 42.767% and 33.946% SFA contents were found respectively in monsoon and post-monsoon seasons (Table 2). Result expressed that *M. cuchia* contains 44.214%, 57.233% and 66.054% unsaturated fatty acid in pre-monsoon, monsoon and post-monsoon (Table 3).

3.3.1 Saturated Fatty Acids (SFAs)

Palmitic acid (C16:0) was the primary saturated fatty acid (SFA). Result showed that *M. cuchia* contains 19.808±0.002%, 22.100±0.067% and 15.977±0.002% (Mean±SEM) palmitic acid in pre monsoon, monsoon and post-monsoon, respectively (Table 2). Data also expressed that significant difference ($p < 0.05$) is observed among these three seasons (Table 2; Appendix C-Table 7).

Amount of lauric acid (C12:0) is quite high among SFAs. Findings described that lauric acid content were presented 8.274±0.002%, 3.087±0.012% and 3.772±0.002% in pre-monsoon, monsoon and post-monsoon respectively (Table 2). Data also stated that significant difference ($p < 0.05$) is resided among these three seasons (between pre-monsoon and monsoon, post-monsoon and monsoon, pre-monsoon and post-monsoon)(Table 2; Appendix C-Table 7). The highest value of lauric acid was in pre-monsoon than monsoon and post-monsoon season (Table 2).

Table 2: Saturated fatty acid composition (%) of *M. cuchia* among three seasons. Values in the same row having the different superscript are significantly different (p<0.05).

Fatty acid composition (%)	Pre-monsoon	Monsoon	Post-monsoon
Saturated fatty acids	**55.7858**	**42.7661**	**33.9452**
Caproic acid (C6:0)	4.670 ± 0.002^{a}	1.874 ± 0.002^{b}	0.302 ± 0.001^{c}
Caprylic acid (C8:0)	8.575 ± 0.004^{a}	0.987 ± 0.002^{b}	0.839 ± 0.001^{c}
Lauric acid (C12:0)	8.274 ± 0.002^{a}	3.087 ± 0.012^{b}	3.772 ± 0.002^{c}
Tridecanoic acid (C13:0)	Not detected	0.351 ± 0.001^{a}	0.494 ± 0.004^{b}
Myristic acid (C14:0)	6.152 ± 0.003^{a}	4.018 ± 0.019^{b}	2.131 ± 0.002^{c}
Pentadecyclic acid (C15:0)	0.881 ± 0.001^{a}	1.425 ± 0.0004^{b}	1.122 ± 0.002^{c}
Palmitic acid (C16:0)	19.808 ± 0.002^{a}	22.100 ± 0.067^{b}	15.977 ± 0.002^{c}
Stearic acid (C18:0)	3.982 ± 0.005^{a}	5.176 ± 0.001^{b}	6.154 ± 0.001^{c}
Arachidic acid (C20:0)	1.045 ± 0.005^{a}	1.515 ± 0.003^{b}	1.761 ± 0.001^{c}
Behenic acid (C22:0)	0.659 ± 0.002^{a}	Not detected	0.441 ± 0.002^{b}
Lignoceric acid (C24:0)	1.752 ± 0.002^{a}	2.206 ± 0.003^{b}	0.942 ± 0.005^{c}

Findings described that amount of stearic acid in pre-monsoon, monsoon and post-monsoon were 3.982±0.005%, 5.176±0.001% and 6.154±0.001% respectively (Table 2). Result narrated that significant difference ($p < 0.05$) is found among these three seasons (pre-monsoon, monsoon and post-monsoon)(Table 2; Appendix C-Table 7).

Among these result, the highest value was marked in post-monsoon and the lowest value was in pre-monsoon (Table 2). Result showed that there is a significant difference ($p < 0.05$) among these three seasons in the followings SFAs:

M. cuchia contains 6.152±0.003%, 4.018±0.019% and 2.131±0.002% myristic acid (C14:0)., 8.575±0.004%, 0.987±0.002% and 0.839±0.001% caprylic acid (C8:0)., 4.670±0.002%, 1.874±0.002% and 0.302±0.001% caproic acid (C6:0) respectively in pre-monsoon, monsoon and post-monsoon season. The amount of ligoceric acid (C24:0) in *M. cuchia* were found 1.752±0.002%, 2.206±0.003% and 0.942±0.005%., arachidic acid (C20:0) were presented 1.045±0.005%, 1.515±0.003% and 1.761±0.001%., pentadecyclic acid (C15:0) showed 0.881±0.001%, 1.425±0.0004% and 1.122±0.002% respectively in pre-monsoon, monsoon and post-monsoon seasons.

3.3.2 Unsaturated fatty acids

3.3.2.1 Monounsaturated Fatty Acids (MUFAs)

The experimental fish, *M. cuchia* contains 18.963±0.003%, 20.792±0.002% and 25.991±0.001% oleic acid (C18:1) respectively (Table 3). Analysis of all monounsaturated fatty acids revealed that significant difference were exist ($p < 0.05$) among these three seasons (pre-monsoon, monsoon and post-monsoon) in cuchia fish (Table 3;Appendix C-Table 7).

Table 3: Unsaturated fatty acid composition (%) of *M. cuchia* among three seasons. Values in the same row having the different superscript are significantly different ($p<0.05$).

Fatty acid composition (%)	Pre-monsoon	Monsoon	Post-monsoon
Unsaturated fatty acids	**44.2142**	**57.2339**	**66.0548**
Monounsaturated fatty acids	**29.2313**	**32.4819**	**37.4159**
Myristoleic acid (C14:1)	0.371±0.001^{a}	1.351±0.001^{b}	1.792±0.001^{c}
Pentadecenoic acid (C15:1)	Not detected	0.421±0.002^{a}	0.826±0.002^{b}
Palmitoleic acid (C16:1)	9.892±0.001^{a}	9.616±0.003^{b}	8.294±0.001^{c}
Oleic acid (C18:1)	18.963±0.003^{a}	20.792±0.002^{b}	25.991±0.001^{c}
Eicosenoic acid (C20:1)	Not detected	0.306±0.002^{a}	0.502±0.002^{b}
Polyunsaturated fatty acids	**14.9829**	**24.7520**	**28.6389**
Hexadecadienoic acid (C16:2)	0.641±0.001^{a}	1.384±0.001^{b}	0.680±0.001^{c}
Hexadecatrienoic acid (C16:3)	Not detected	2.910±0.001^{b}	2.155±0.001^{c}
Linoleic acid (C18:2)	6.378±0.001^{a}	5.412±0.002^{b}	17.786±0.001^{c}
Linolenic acid (C18:3)	3.980±0.0001^{a}	1.977±0.001^{b}	1.682±0.001^{c}
Eicosadienoic acid (C20:2)	Not detected	0.636±0.002^{a}	0.650±0.001^{b}
Arachidonic acid (C20:4)	2.071±0.001^{a}	3.074±0.001^{b}	2.035±0.003^{c}
Eicosapentaenoic acid (C20:5)	Not detected	0.604±0.001^{a}	0.282±0.002^{b}
Docosapentanoic acid (C22:5)	Not detected	4.016±0.001^{b}	2.206±0.002^{b}
Docosahexaenoic acid (C22:6)	1.910 ±0.0001^{a}	4.743±0.001^{b}	1.161±0.001^{c}
n-3/n-6	0.948	1.321	0.354

Myristoleic acid (C14:1) was presented in *M. cuchia* 0.371±0.001%, 1.351±0.001% and 1.792±0.001%., palmitoleic acid (C16:1) was showed 9.892±0.001%, 9.616±0.003% and 8.294±0.001% respectively (Table 3). Pentadecenoic acid (C15:1) was not detected in pre-monsoon season but presented in monsoon (0.421±0.002%) and post-monsoon season (0.826±0.002%). Eicosenoic acid (C20:1) was also not detected in pre-monsoon but presented in monsoon (0.306±0.002%) and post-monsoon (0.502±0.002%) (Table 3).

Results described significant difference ($p < 0.05$) among these three seasons (between pre-monsoon and monsoon, post-monsoon and monsoon, pre-monsoon and post-monsoon) (Table 3; Appendix C-Table 7).

3.3.2.2 Polyunsaturated Fatty Acids (PUFAs)

Result described that *M. cuchia* contains linoleic acid (C18:2) 6.378±0.001%, 5.412±0.002% and 17.786±0.001% in pre-monsoon, monsoon and post-monsoon seasons, respectively (Table 3). Among these result, the highest value was marked in post-monsoon and the lowest value was in monsoon season. Result showed that there was a significant difference ($p < 0.05$) of all polyunsaturated fatty acids among seasons (Table 3; Appendix C-Table 7).

On the other hand, hexadecadienoic acid (C16:2) was presented 0.641±0.001%, 1.384±0.001% and 0.680±0.001% whereas linolenic acid (C18:3) was 3.980±0.0001%, 1.977±0.001% and 1.682±0.001% monsoon and post-monsoon, respectively (Table 3). Findings narrated that significant difference ($p< 0.05$) exists in both linoleic acid and hexadecadienoic acid among these three seasons (pre-monsoon, monsoon and post-monsoon respectively) (Table 3; Appendix C-Table 7).

M. cuchia contains 2.071±0.001%, 3.074±0.001% and 2.035±0.003% arachidonic acid (C20:4) in pre-monsoon, monsoon and post-monsoon (Table 3). The amount of DHA (C22:6) in pre-monsoon, monsoon and post-monsoon was presented 1.910 ±0.0001%, 4.743±0.001% and 1.161±0.001%, respectively (Table 3). The value of DHA is higher in monsoon than pre-monsoon and post-monsoon season. In the case of all those fatty acid our findings concluded that significant difference is observed ($p < 0.05$) among these three seasons in cuchia fish (Table 3; Appendix C-Table 7).

Eicosapentaenoic acid (C20:5) was not detected (ND) from the experimental fish in pre-monsoon but presented in monsoon (0.604±0.001%) and post-monsoon (0.282±0.002%) (Table 3). Hexadecatrienoic acid (C16:3) was absent in pre-monsoon season but identified in monsoon (2.910±0.001%) and post-monsoon season (2.155±0.001%) respectively (Table 3).

In pre-monsoon season, both eicosadienoic acid (C20:2) and docosapentaenoic acid (C22:5) were not detected in pre-monsoon but they were presented 0.636±0.002% and 4.016±0.001% in monsoon season, were also identified from *M. cuchia* in post-monsoon respectively 0.650±0.001% and 2.206±0.002% (Table 3).

Above these Poly Unsaturated Fatty Acids (PUFAs): linoleic acid, hexadecadienoic acid, arachidonic acid, DHA, EPA, hexadecatrienoic acid, eicosadienoic acid and docosapentaenoic acid were shown a significant difference ($p < 0.05$) between pre-monsoon and monsoon, post-monsoon and monsoon, pre-monsoon and post-monsoon(Table 3; Appendix C-Table 7).The ratio of n-3/n-6 in muscle of examined fishes were 0.948, 1.321 and 0.354 in pre-monsoon, monsoon and post-monsoon season, respectively (Table 3).

3.4 Amino Acids

Amino acids profiles of *M. cuchia* was analyzed to assess and differentiate nutritional quality within three different seasons. Amount of amino acid in protein varied seasonally.

3.4.1 Arginine

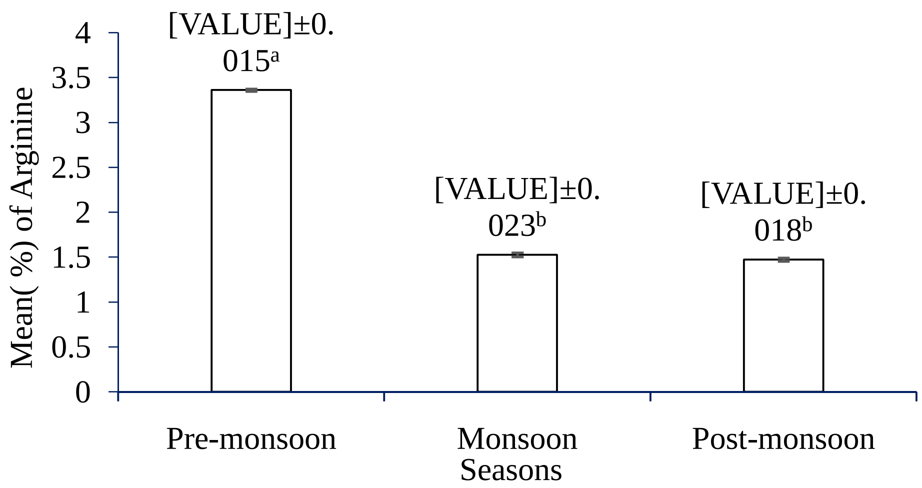


Fig. 8. Variation of arginine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

Findings described that in pre-monsoon amount of arginine ((Mean±SEM) was 3.560±0.015%, on the contrary in monsoon and post monsoon the amount were 1.526±0.023% and 1.473 ±0.018%, respectively (Figure 8; Appendix D-Table 9). Among these result the highest value was marked in pre-monsoon season as the lowest was in post-monsoon season (Figure 8). Result illustrated that significant difference ($p < 0.05$) was observed between pre-monsoon and monsoon season. There was also significant difference ($p < 0.05$) between pre-monsoon and post-monsoon season in mean amount of arginine in *Monopterus cuchia*. But there was no significant difference ($p < 0.05$) between monsoon and post-monsoon season (Figure 8; Appendix D- Table 10).

3.4.2 Glycine

Glycine content in *M. cuchia* varied based on season. Findings described that amount of glycine ((Mean±SEM) in pre-monsoon, monsoon and post-monsoon were respectively 3.107±0.018%, 1.463±0.023% and 1.313±0.023% (Figure 9; Appendix D- Table 9). The highest value was recorded in pre-monsoon season and the lowest value in post-monsoon (Figure 9). Result represented that glycine content differed significantly ($p < 0.05$) among these three in *M. cuchia* (Figure 9;Appendix D- Table 10).

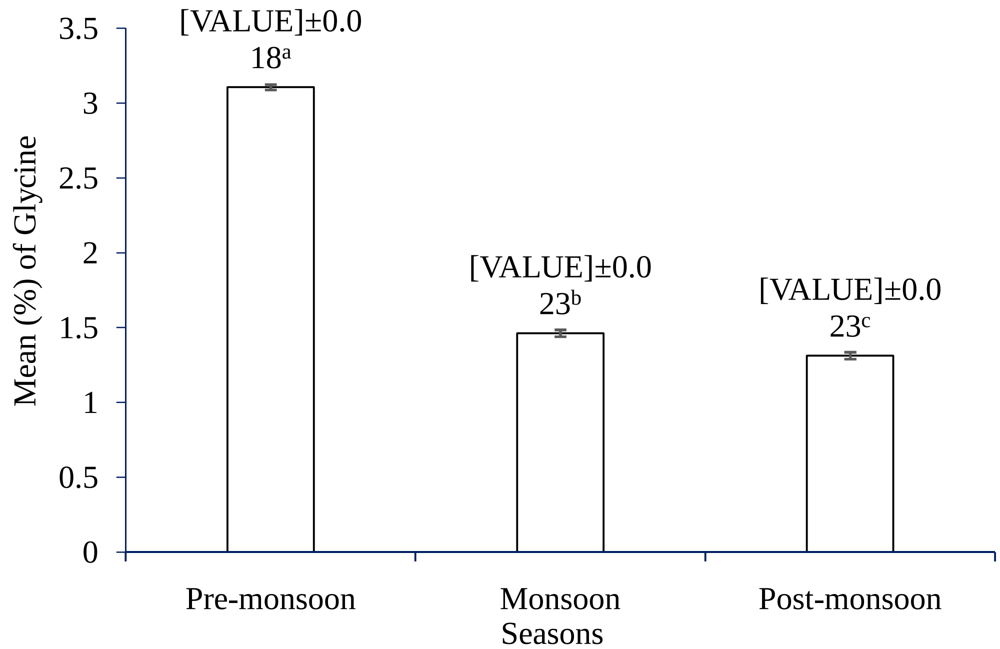


Fig. 9. Variation of glycine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

3.4.3 Histidine

Mean (±SEM) histidine content in pre-monsoon, monsoon and post-monsoon were 1.890±0.029%, 0.847±0.026% and 0.857±0.025% respectively among these three seasons.The highest value was recorded in pre-monsoon season and the lowest value in monsoon season (Figure 10; Appendix D- Table 9). Result illustrated that significant difference ($p < 0.05$) was observed between pre-monsoon and monsoon season. There was also significant difference ($p < 0.05$) present between pre-monsoon and post-monsoon season in *Monopterus cuchia*. But there was no significant difference ($p < 0.05$) in term of mean histidine content between monsoon and post-monsoon season (Figure 10;Appendix D- Table 10).

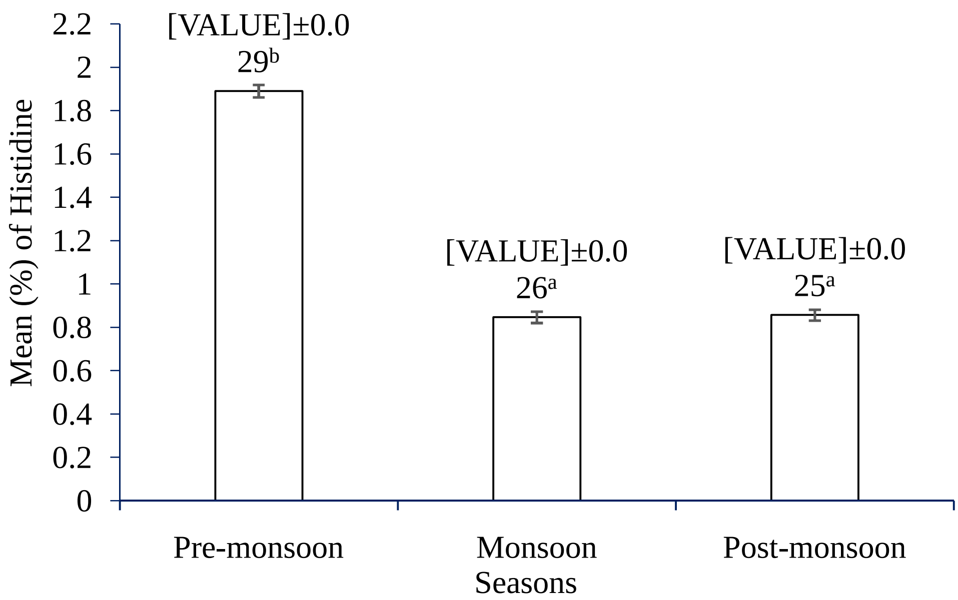


Fig. 10. Variation of histidine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

3.4.4 Isoleucine

Mean (±SEM) isoleucine content of *M. cuchia* were 1.847±0.023% and0.877±0.019%, collected in pre-monsoon and monsoon season respectively, whereas in post-monsoon season, the amount was 0.823±0.032%. Among these result the highest value was marked in pre-monsoon season as the lowest was in post-monsoon season (Figure 11; Appendix D-Table 9).Result illustrated that significant difference ($p < 0.05$) exhibits in between pre-monsoon and monsoon season. There is also significant difference ($p < 0.05$) inmean isoleucine content between pre-monsoon and post-monsoon season in *Monopterus cuchia*. But there was no significant difference ($p < 0.05$) between monsoon and post-monsoon season (Figure 11;Appendix D- Table 10).

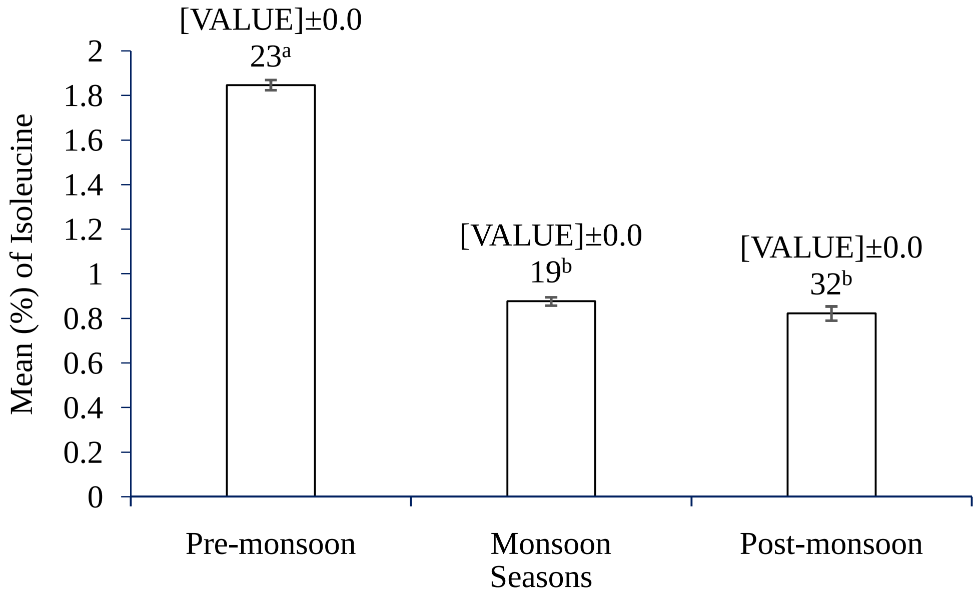


Fig. 11. Variation of isoleucine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

3.4.5 Leucine

In pre-monsoon, results illustrated that *M. cuchia* contains 2.193±0.020% leucine on the contrary in monsoon and post-monsoon the amount were 0.873±0.026% and 0.840±0.023% approximately (Figure 12; Appendix D, Table 9). Among these result the highest value was marked in pre-monsoon season as the lowest was in post-monsoon season. Result of leucine content stated that significant difference ($p < 0.05$) remains among these three seasons (pre-monsoon, monsoon and post-monsoon) in *Monopterus cuchia*(Figure 12; Appendix D-Table 10).Analysis of the arginine data illustrated that significant difference ($p < 0.05$) was observed between pre-monsoon and monsoon season. There is also significant difference ($p < 0.05$) present between pre-monsoon and post-monsoon season in *Monopterus cuchia*. But there is no significant difference ($p < 0.05$) between monsoon and post-monsoon season (Figure 12; Appendix D-Table 10).

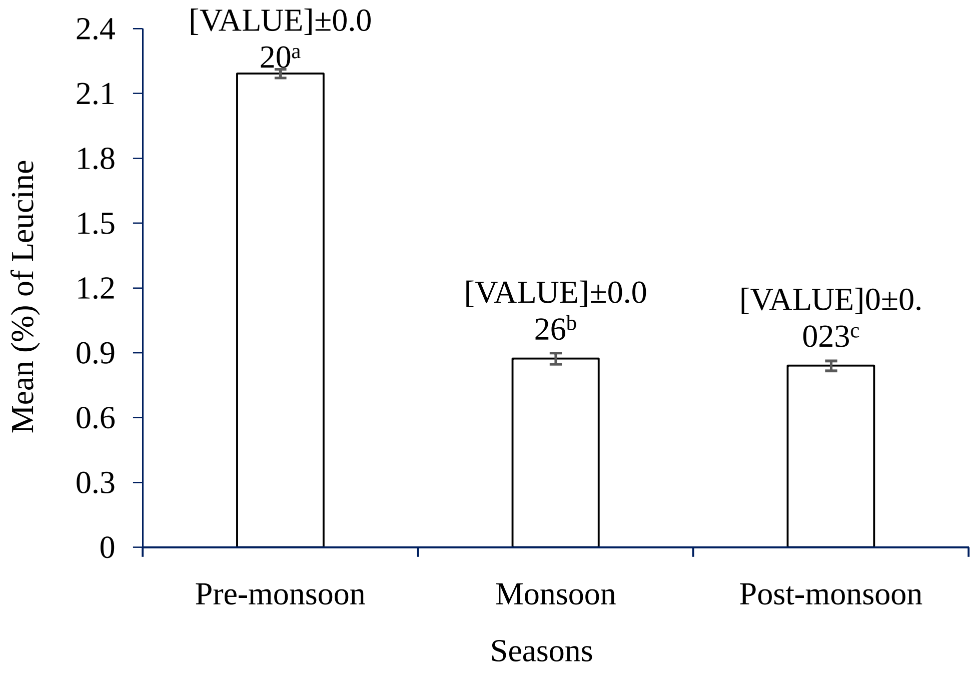


Fig. 12. Variation of leucine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

3.4.6 Alanine

Findings described that amount of alanine of *M. cuchia* in pre-monsoon, monsoon and post-monsoon was 1.677±0.015%, 0.760±0.023% and 0.650±0.015% respectively (Figure 13; Appendix D-Table 19). Among these result the highest value was marked in pre-monsoon season as the lowest was in post-monsoon season (Figure 13). Result illustrated that significant difference ($p < 0.05$) is observed between pre-monsoon and monsoon, post-monsoon and monsoon, post-monsoon and pre-monsoon in (*M. cuchia*) cuchia fish (Figure 13; Appendix D-Table 0).

3.4.7 Lysine

In pre-monsoon, results illustrated that *M. cuchia* contains 3.877±0.023% lysine on the contrary in monsoon and post-monsoon the amount were 1.760±0.017% and 1.677±0.023% approximately.Among these result the highest value was marked in pre-monsoon season as the lowest was in post-monsoon season (Figure 14; Appendix D, Table 9). Result illustrated that significant difference ($p < 0.05$) exhibits in between pre-monsoon and monsoon season. There is also significant difference ($p < 0.05$) present between pre-monsoon and post-monsoon season in *Monopterus cuchia*. But there is no significant difference ($p < 0.05$) between monsoon and post-monsoon season (Figure 14; Appendix D-Table 10).

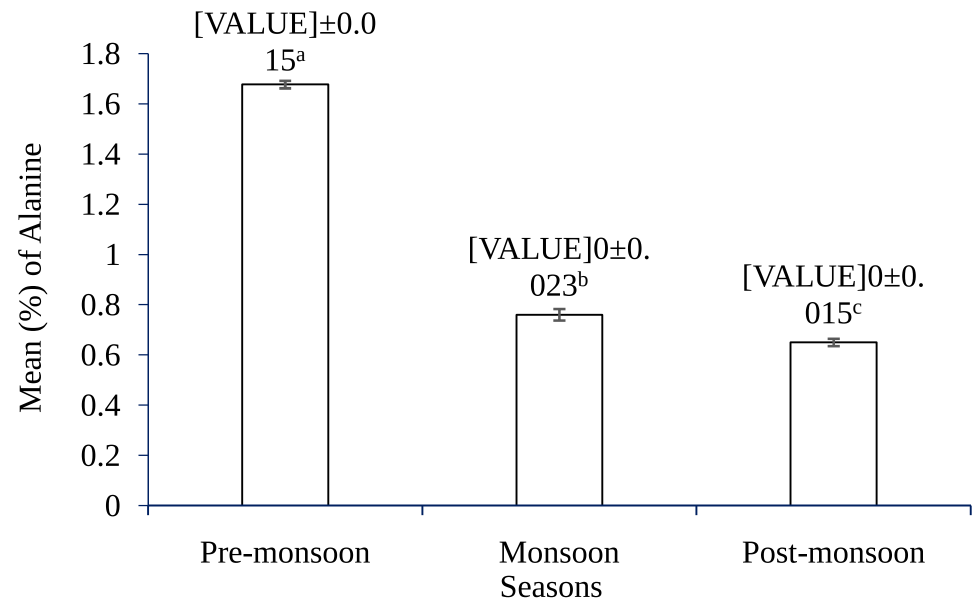


Fig. 13. Variation of alanine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

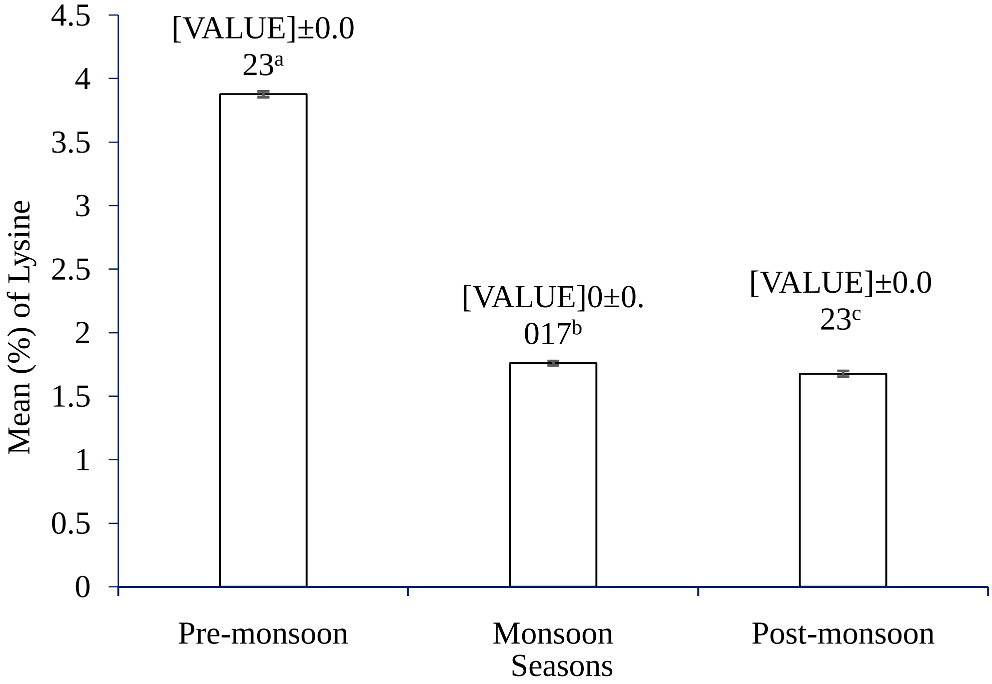


Fig. 14. Variation of lysine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences (p < 0.05) and same letter denotes no significance (p>0.05).

3.4.8 Methionine

Mean methionine content in pre-monsoon, monsoon and post-monsoon was 2.147±0.023%, 0.967±0.020% and 0.930±0.021% respectively among these three seasons. The highest value was recorded in pre-monsoon season and the lowest value in post-monsoon season. Result of methionine content stated that significant difference (p < 0.05) remains among these three seasons (pre-monsoon, monsoon and post-monsoon) in *Monopterus cuchia* (Figure 15; Appendix D-Table 10). Analysis of the arginine data illustrated that significant difference (p < 0.05) was observed between pre-monsoon and monsoon season. There is also significant difference (p < 0.05) present between pre-monsoon and post-monsoon season in *Monopterus cuchia*. But there is no significant difference (p < 0.05) between monsoon and post-monsoon season (Figure 15 and Appendix D, Table 13).

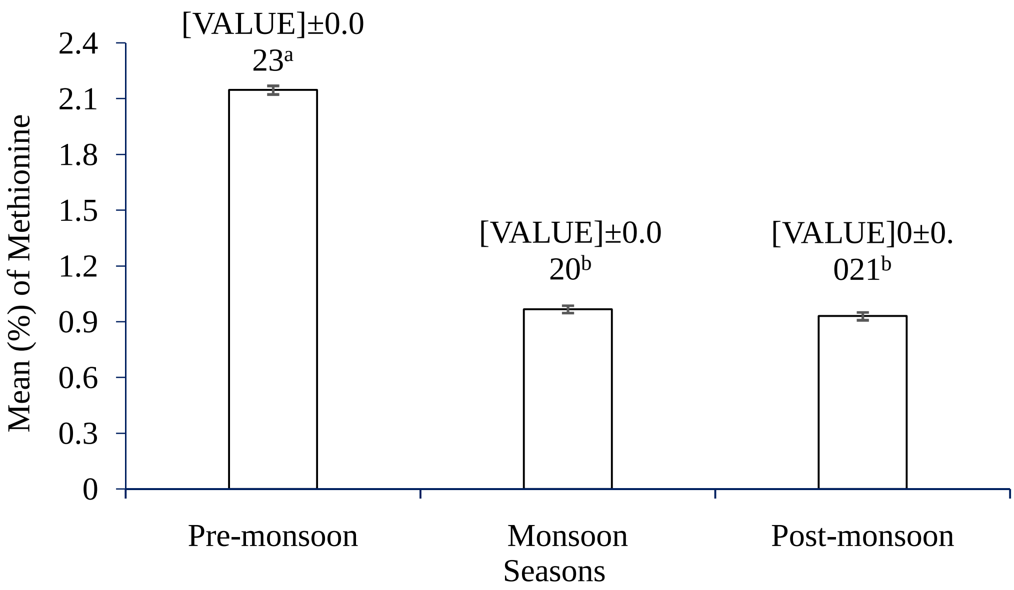


Fig. 15. Variation of methionine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences (p < 0.05) and same letter denotes no significance (p>0.05).

3.4.9 Threonine

In pre-monsoon, results illustrated that *M. cuchia* contains 1.247±0.020% threonine on the contrary in monsoon and post-monsoon the amount were 0.630±0.017% and

0.540±0.017% approximately (Figure 16; Appendix D-Table 9).Among these result the highest value was marked in pre-monsoon season as the lowest was in post-monsoon season. Result illustrated that significant difference ($p < 0.05$) is observed between pre-monsoon and monsoon, post-monsoon and monsoon, post-monsoon and pre-monsoon in cuchia fish (Figure 16; Appendix D-Table 10).

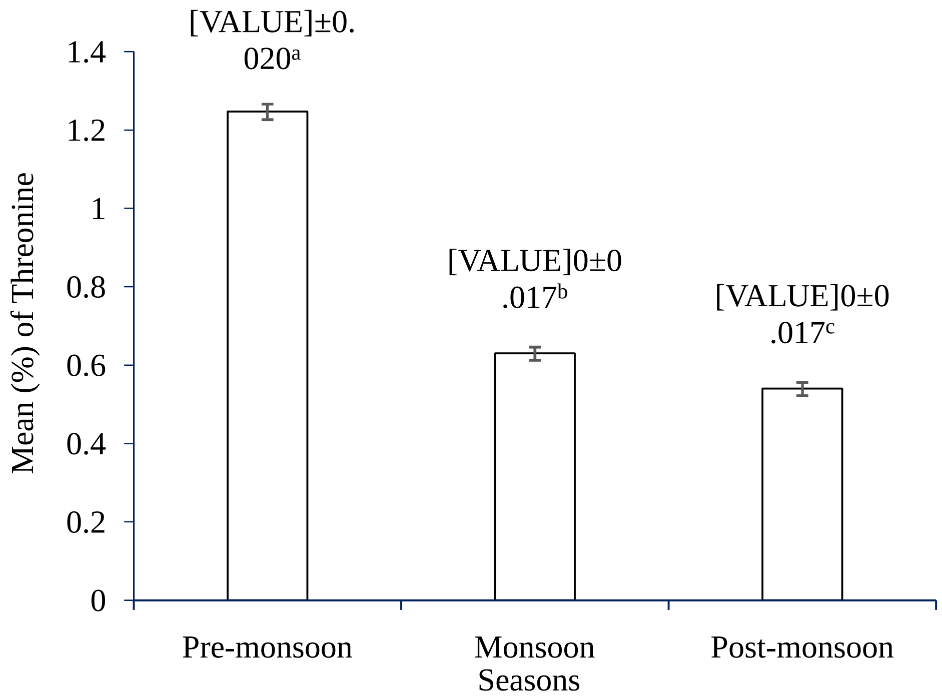


Fig. 16. Variation of threonine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

3.4.10 Valine

Findings described that in pre-monsoon amount of valine was 1.677±0.02% (Mean±SEM) of *M. cuchia* on the contrary in monsoon and post monsoon the amount were 0.717±0.015% and 0.653±0.023% respectively. Among these result the highest value was marked in pre-monsoon season as the lowest was in post-monsoon season (Figure 17; Appendix D-Table 9). Result of valine content expressed that significant difference ($p < 0.05$) shows among these three seasons (pre-monsoon, monsoon and post-monsoon) in *Monopterus cuchia*. Analysis of valine illustrated that significant difference ($p < 0.05$) was observed between pre-monsoon and monsoon season. There is also significant difference ($p < 0.05$) present between pre-monsoon and post-monsoon season in *Monopterus cuchia*. But there is no significant difference ($p < 0.05$) between monsoon and post-monsoon season (Figure 17; Appendix D-Table 10).

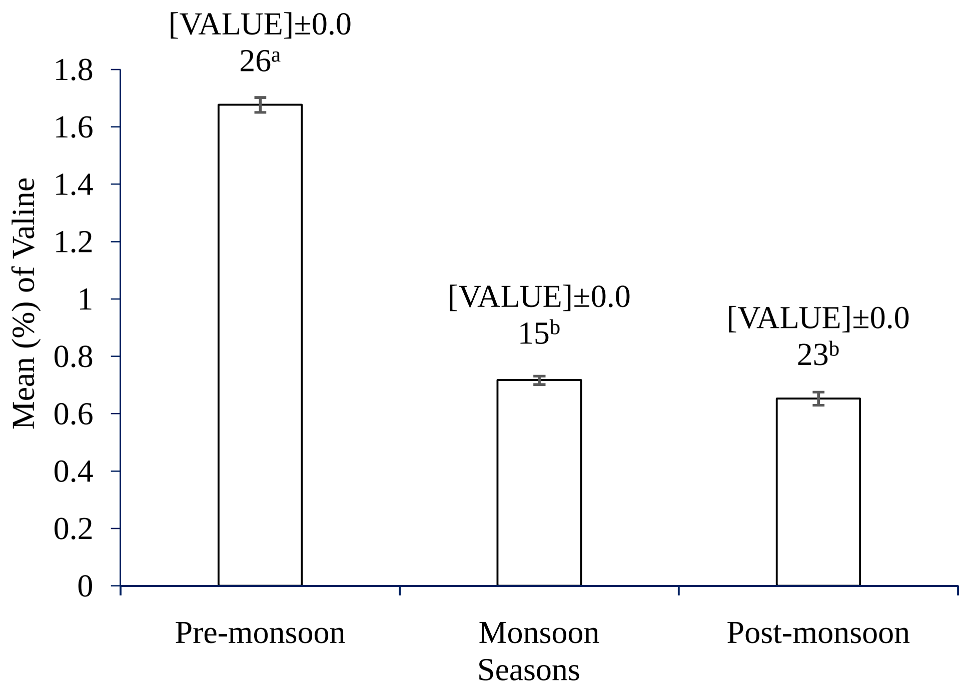


Fig. 17. Variation of valine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

3.4.11 Aspartic acid

In pre monsoon, the amount of aspartic acid in experimental fish was 2.243±0.018% whereas in monsoon, post-monsoon, the amount was 0.947±0.020% and 0.773±0.023% respectively (Figure 18; Appendix D-Table 9). Among these result the highest value was marked in pre-monsoon season as the lowest was in post-monsoon season (Figure 18). Result described that amount of aspartic acid is higher in pre-monsoon than in monsoon and post-monsoon season (Figure 18). Result illustrated that significant difference ($p < 0.05$) is observed between pre-monsoon and post-monsoon, monsoon and post-monsoon, pre-monsoon and monsoon seasons in *M. cuchia* (Figure 18; Appendix D-Table 10).

3.4.12 Tyrosine

In pre monsoon, the amount of tyrosine in experimental fish was 1.640±0.026% whereas in monsoon, post-monsoon, the amount was 0.783±0.018% and 0.673±0.026% respectively (Figure 19; Appendix D-Table 9). Result described that amount of tyrosine is higher in pre-monsoon than in monsoon and post-monsoon season (Figure 19). Result illustrated that significant difference ($p < 0.05$) is observed between pre-monsoon and post-monsoon, monsoon and post-monsoon, pre-monsoon and monsoon seasons in *M. cuchia* (Figure 19; Appendix D-Table 10).

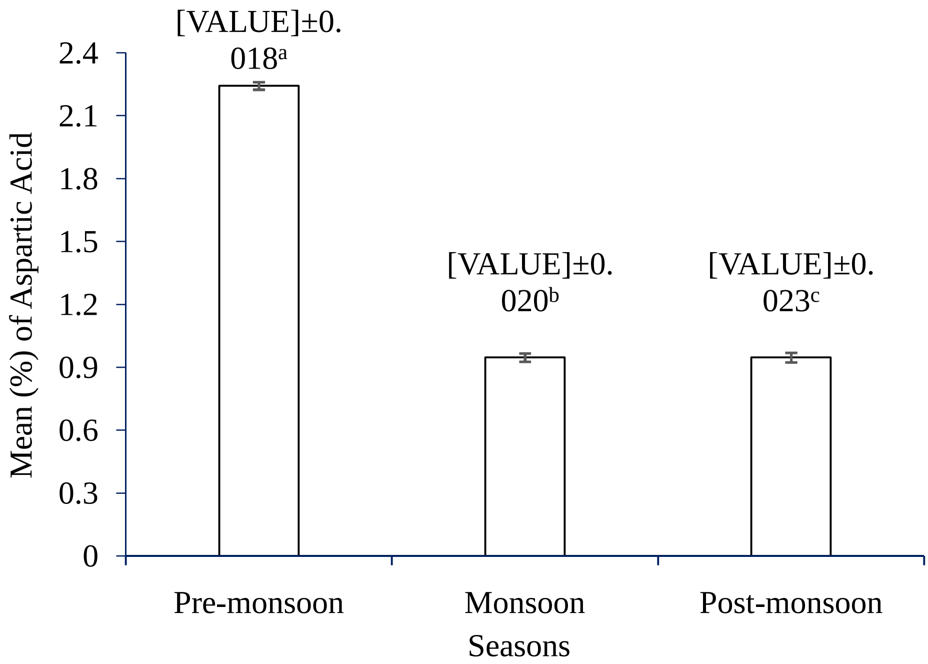


Fig. 18. Variation of aspartic Acid (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

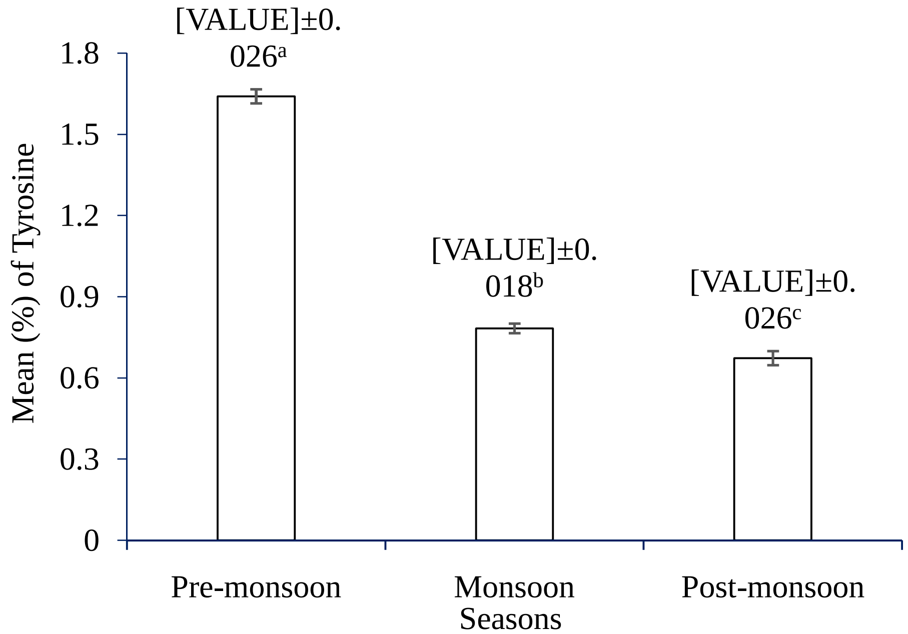


Fig. 19. Variation of tyrosine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

3.4.13 Glutamic acid

In pre monsoon, the amount of glutamic acid in experimental fish was 3.597±0.026% whereas in monsoon, post-monsoon, the amount was 1.553±0.018% and 1.553±0.018% respectively. Result described that amount of glutamic acid is higher in pre-monsoon than in monsoon and post-monsoon season(Figure 20; Appendix D-Table 9). Result illustrated that significant difference ($p < 0.05$) is observed between pre-monsoon and post-monsoon, monsoon and post-monsoon, pre-monsoon and monsoon seasons in *M. cuchia* (Figure 20; Appendix D-Table 10).

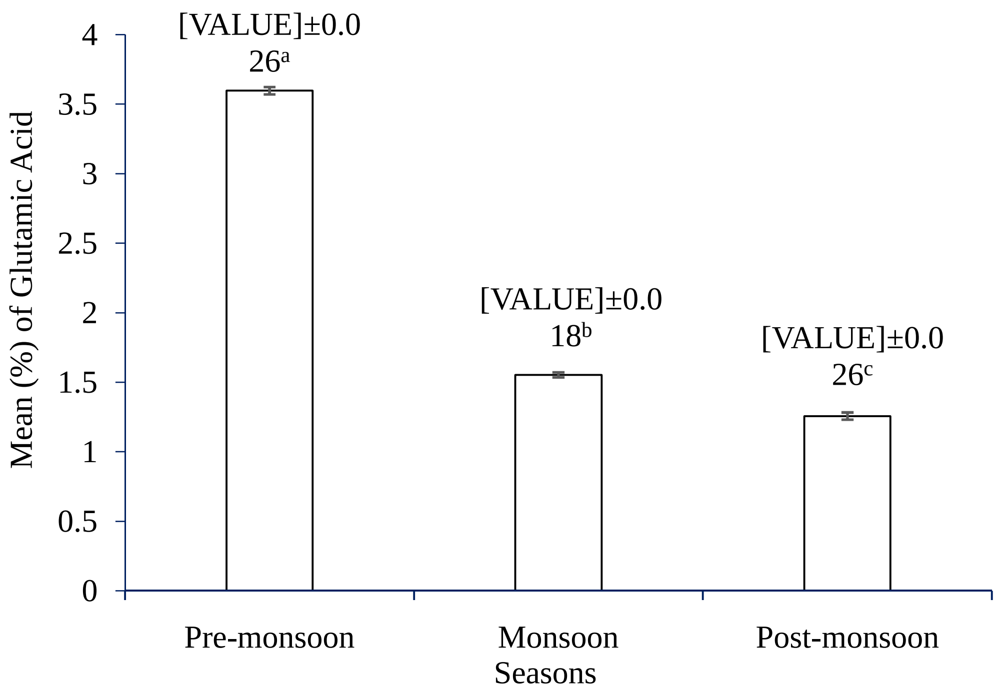


Fig. 20. Variation of glutamic acid (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

3.4.14 Serine

Findings described that in pre-monsoon amount of serine was 1.620±0.023% (Mean±SEM) of *M. cuchia* on the contrary in monsoon and post monsoon the amount were 0.740±0.023% and 0.737±0.027% respectively. The highest value was recorded in pre-monsoon season and the lowest value in post-monsoon (Figure 21). Result of serine content expressed that significant difference ($p < 0.05$) shows among these three seasons (pre-monsoon, monsoon and post-monsoon) in *Monopterus cuchia*(Figure 21; Appendix D, Table 10). Analysis of serine illustrated that significant difference ($p < 0.05$) was observed between pre-monsoon and monsoon season. There is also significant difference ($p < 0.05$) present between pre-monsoon and post-monsoon season in *Monopterus cuchia*. But there is no significant difference ($p < 0.05$) between monsoon and post-monsoon season (Figure 21 and Appendix D, Table 13).

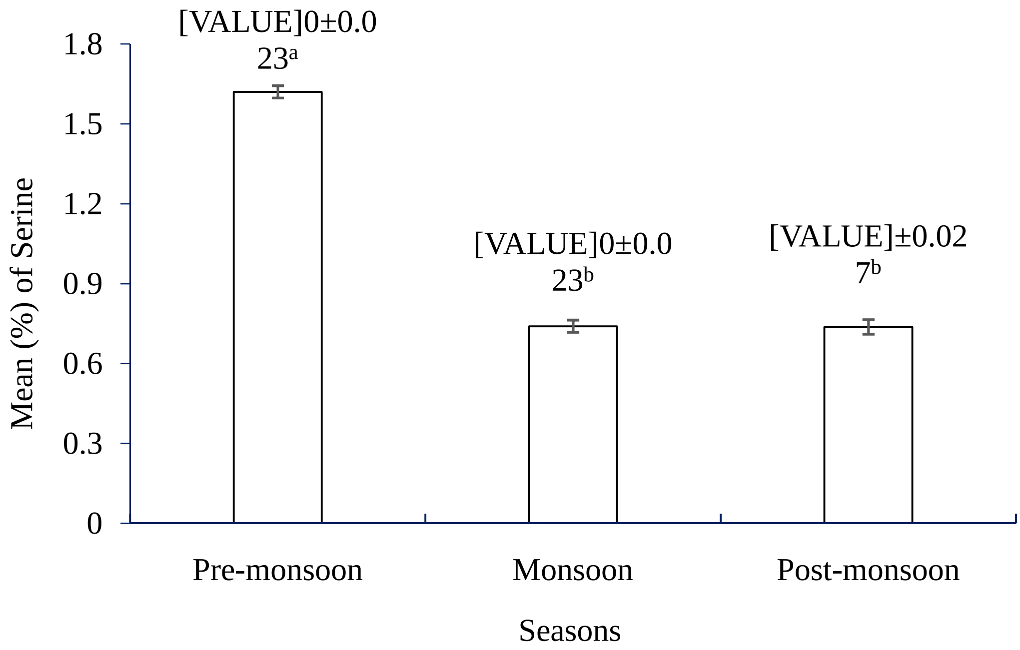


Fig. 21. Variation of serine (%) of *M. cuchia* among three different seasons. Different letters denote significant differences ($p < 0.05$) and same letter denotes no significance ($p>0.05$).

In the present study, the ratio of EAA/NEAA was observed 0.679, 0.676 and 0.706 in experimental fish (*M. cuchia*) among three different seasons as pre-monsoon, monsoon and post-monsoon. Moreover the ratio of EAA/NEAA was higher in post-monsoon season than pre-monsoon and monsoon season.

4.Discussion

Fish as a whole has a lot of food potential and can therefore be expected to provide relief from malnutrition, especially in developing countries. Fish flesh is tasty and highly digestible; it also minimizes the risk of heart diseases and increases life expectancy (Ashraf et al., 2011). Fish may be the sole accessible and/or affordable source of animal protein, well balanced essential amino acid profile, necessary minerals and fatty acids for poor households in urban or semi-urban areas. Fish is a highly proteinous food consumed by a larger percentage of populace because of its availability and palatability (Foran et al., 2005).

The *Monopterus cuchia* is a popular freshwater mud eel for tribal people in Bangladesh due to its nutritional (prevent arrhythmias, endothelial dysfunction, high blood pressure, sudden strokes and heart attack etc.) and medicinal effects (cardio-protective, anti-thrombotic, anti-atherosclerotic) and demandable for its high export value in both national and foreign markets. Biochemical composition of a species helps to assess its nutritional and edible value (Connor, 2000 and Harris, 2004).

Islam and Joadder (2005) reported that the chemical composition of fish varies greatly from species to species and one individual to another depending on age, sex, environment and season. The variation in the chemical composition of fish is closely related to feed intake, migratory swimming, sexual maturity in connection with spawning and season. Body composition is a true reflector of its feeding habits and type of food availability.

A comparative study was undertaken to determine the moisture, ash, protein, lipid, fatty acid and amino acid profile in freshwater mud eel, *Monopterus cuchia* of Sylhet district, Bangladesh. Depending on the habitat and season, results showed a wide variation in proximate composition, amino acid and fatty acid profile of experimental fish species.

4.1 Length and weight of *Monopterus cuchia*

The exact relationship between length and weight differs among species of fish according to their inherited body shape, food availability, sex and gonad development, type of habitat, growth and season. Condition is variable and dynamic. Thomas et al., (2003) reported that any other morphometric characters, the length-weight relationship can be used as a character of differentiation of taxonomic units and the relationship changes with the various development events in life such as metamorphosis, growth and onset of maturity. Length-Weight relationships can be used to predict weight from length measurements made in the yield assessment (Pauly, 1993).

The study of growth means basically the determination of the body size as a function of age.Fish can attain either isometric growth, negative allometric growth or positive allometric growth. When no change is associated with body shape as grows, an organism shows isometric growth. Negative allometric growth implies the fish becomes more slender as it increase in weight while positive allometric growth implies the fish becomes relatively stouter or deeper-bodied as it increases in length (Riedel et al., 2007).

In this study, it was found that there is a positive association between length and weight of freshwater mud eel, *Monopterus cuchia.*These findings are more or less similar to the results of Bala et al., (2009); Imam et al., (2010); Sangun et al., (2007) and Naeem et al., (2010).

4.2 Nutritional composition

Nutritional composition of fish means the composition of the fish flesh which serves to evaluate nutritional quality. Moisture, dry matter, ash, protein, lipid, vitamins and minerals are the most important components that act as sources of nutritive valu**e** of fish meat (Steffens, 2006). Knowledge of the proximate composition of fishes is essential to estimate their nutritional value. Normally fish contains 72% water, 19% protein, 8% fat, 0.5% calcium, 0.25% phosphorus and 0.1% vitamin A, D, B etc. (FAO, 1991). The measurement of some proximate profiles is often necessary to ensure that they meet the requirements of food regulations and commercial specifications. Evaluating the proximate composition of fish is the most important aspect in fish nutrition (Sutharshiny and Sivashanthini, 2011).

Alemu et al., (2013) showed that both exogenous and endogenous factors control fish body composition. The endogenous factors are genetically controlled and are associated with the life cycle of fish. Throughout the year, fish are subjected to considerable environmental changes and fluctuations in availability and compositions of feed that will affect their proximate muscle composition. Many other exogenous factors (temperature and salinity) may also affect the proximate and fatty acid compositions. Khuda, M.Q. (1964) studied the proximate composition of different species and different individual of same species. Both times he found difference. But the difference is significant in different species as compared with same species.

4.2.1 Moisture content

Moisture content is expressed as the amount of water as a percentage (%) and the remaining portion is dry matter content. The major component of fish muscle is moisture. In general the range fall between 60-80% in many fish (Murra and Burt, 2001). Moisture content may vary depending on age, fat content, habitat and feeding etc.

In our present study seasonal variation is observed in moisture content of *Monopterus cuchia* among these three seasons (pre-monsoon, monsoon and post-monsoon). The moisture content varied from (78.30-80.15%). Seasonally the highest value was found in post-monsoon (79.88±0.29%) and on the other hand, the lowest value was found in pre-monsoon season (78.87±0.32%) of moisture content in *M. cuchia.* There was a little seasonal fluctuation of the moisture content in the fresh flesh muscle samples of *M. cuchia* when compared in pre-monsoon, monsoon and post-monsoon seasons.

Stansby (1954) and Rubbi et al., (1987) described that moisture content varied from 72.2-83.6% with an average 77.64%. According to Jacquat (1961) fish contains 66-84% moisture. The mean moisture content of the freshwater species 76.71% for *Clarias gariepinus*; 76.75% for *Tilapia zillii*. The mean moisture content of the marine water species were 72.63% for *Pentanemus quinquarius* and 75.40% for *Pseudotolithus typus* (Osibona, 2011). Average value of moisture content in *A. testudineus* throughout the year was 79.11±0.65% in male and 78.99% in female (Nagris, 2006). The present findings is similar to effect of seasonal variation on the nutrient composition in selected fish species in Lake Kainji-Nigeria (Effiong and Mohammed, 2008) for proximate composition.

However, moisture content varied seasonally and also varied from species to species. Again moisture content varies depending on environment (where they survive) and season. All these findings coincide well with the present findings of moisture content in the muscle of *M. cuchia.*

4.2.2 Ash content

Finding shows that ash content varies from (1.00-1.27) % in experimental fish. In pre-monsoon, *M. cuchia* contains 1.10±0.04% ash and on the contrary 1.15±0.09% and 1.10±0.09 in monsoon and post monsoon respectively (Mean± SD).

Ates et al., (2013) analyzed proximate composition of Wild Brown Trout in Munzur River, Tunceli-Turkey. They found ash content in pre-monsoon (spring) 1.20%, monsoon (winter) 1.18% and post-monsoon (autumn) 1.15% respectively. Both scaly and nonscaly fresh water fish contains 0.85-5.11% ash (Rubbi et al., 1987). Matto et al. (1991) estimated 1.2%, 1.3%, 1.3% and 1.1% ash in *sardina pulchardus*, *Thunnus thynnus*, *Mugil cephalas and Atherina hepsetus respectively.*

Ozyurt and Polat (2006) showed their findings that *D. labrax* contains 2.18±0.14% and 1.22±0.04% lipid content in pre-monsoon and post-monsoon season. The mean ash content of the freshwater species were 1.23% for *Clarias gariepinus*; 1.11% for *Tilapia zillii*. The mean ash content of the marine water species were 1.13% for *Pentanemus quinquarius* and 1.17% for *Pseudotolithus typus* (Osibona, 2011).

There is also a little seasonal fluctuation of the ash content in the fresh flesh muscle samples of *M. cuchia* when compared in pre-monsoon, monsoon and post-monsoon seasons. This might be due to habitat, season, age, sex, geographical location etc. Body composition is a true reflector of its feeding habits and type of food availability (Ashraf et al., 2011). Above these values are more or less similar with our present findings of ash content in *M. cuchia.*

4.2.3 Crude protein content

Crude protein content is also fluctuated based on seasons (Pre-monsoon, monsoon and post-monsoon). Findings describe that crude protein contents of *M. cuchia* in pre-monsoon, monsoon and post-monsoon are 17.72±0.26%, 18.83±0.31 and 17.67±0.29% respectively (Mean± SD). The highest value is recorded 18.83±0.31% in monsoon and the lowest value is observed 12.13±0.01% in post-monsoon. Seasonally protein content varies from 17.29-19.33%.

A study was occurred in Indian pond in Panduah on *M. cuchia.* Results showed that protein value in the muscle of *M. cuchia* is higher (19.87 ± 0.57% in post-monsoon and 20.61 ± 0.76% in monsoon) than several other freshwater culturable species namely *Labeo rohita* (16.6%), *Cirrhinus mrigala* (19.6%), *Catla catla* (19.5%), *Clarias batrachus* (15.0%) (Gopalon et al., 1989). Nurullah et al., (2003) worked on Nutritional composition of mola, dhela, Chapila, Punti, tengra and kankila. They found that protein content varied between (14.08-21.70%). Irianto and Irianto (1997) studied on proximate composition of Nile Tilapia in Indonesia. The report showed that amount of protein was 20.1%. *Puntius ticto* contains 18.62% protein (Banu et al., 1985).

Rubbi et al. (1987) found 18.40±0.25% protein in prawn whereas Aykoryed et al., (1951) described 19.1% protein in shrimp. Begum and Hoque (1986) reported 17.35% protein in shrimp and Kamal et al. (2000) found 18.64% protein in *Macrobrachium rosenbergii.* Govindan (1985) showed that both marine and fresh water fish contained 9%-25% protein, where (16-19) % is regular limit. Matto et al. (1991) estimated amount of protein in *Sardina pulchardus*, *Thunnus thynnus*, *Mugil cephalas* and *Atherina hepsetus* and it was 18.6%, 18.3%, 19.6% and 18.7% respectively.

Amount of protein in fish muscle is between 15-20 per cent (Murra and Burt, 2001). Protein content may vary considering age, fat content, spawning and starvation etc. As a result, our present investigation coincides with the work.

4.2.4 Lipid content

Findings describe that in pre-monsoon mean lipid content is calculated 2.24±0.06%, on the other hand 0.30±0.04% and 1.26±0.05% lipid content presents in monsoon and post-monsoon respectively. Among these result the highest value of lipid content is marked in pre-monsoon season (2.24±0.06%) and the lowest value is in pre monsoon (0.30±0.04%).

The mean lipid content of the freshwater species were found 1.15% for *Clarias gariepinus*; 0.96% for *Tilapia zillii*. The mean lipid content of the marine water species were 0.40% for *Pentanemus quinquarius* and 1.06%for *Pseudotolithus typus* (Osibona, 2011). Kamal et al. (2000) found 1.8% lipid in *Macrobrachium rosenbergii*.

Chandrasharkar and Deosthale (1993) showed that a wide variation exists between species in lipid content and the range was 0.65-1.3%. He also reported that positive correlation remains between protein and moisture whereas inverse relationship is maintained between fat and moisture. Rubbi et al. (1987) described that lipid content varied from 0.8-1.55% in 27 fresh water species both scaly and non-scaly. Lipid content was 1.0% in *Heteropneustes fossils* and *Clarius batrachus*.

Less content of crude fat were occurred from the muscle of Oncorhynchus mykiss Estelar134 from Arunachal Pradesh and Uttarakhand in the month of June-September in our study. Similar finding of crude fat were obtained by Islam and Joadder, (2005) who carried out research on freshwater fish Gobi, *G. giuris*.

It may be inferred that during mature stage, gonad studded with the lipid hence, muscles contain less fat. Thus, it was observed that fat content of muscle is less during spawning season. These four fish species might be very good sources of fish oil, which is required for food therapy in humans. It is reported that low-fat fish have higher water content and also fat content is influenced by species, geographical regions, age, and diet (Piggott and Tucker, 1990).

Result of lipid content is narrated that lipid content in *M. cuchia* varies 0.25-2.38% among these three seasons (pre-monsoon, monsoon and post-monsoon). All these findings coincide well with the present findings of lipid content in the muscle of *M. cuchia*.

4.3 Fatty acid profile

The analysis of the fatty acid content indicates that the most abundant fatty acids of the experimental fish species are palmitic and stearic acids for saturated fatty acids, palmitoleic acids and oleic acids for monounsaturated fatty acid, linoleic, docosapentaenoic acid and docosahexaenoic acids for polyunsaturated fatty acid. Similar studies performed on tropical.

Palmitic acid (C16:0) is the primary Saturated Fatty Acid (SFA). Result shows that *M. cuchia* contains 19.808±0.002%, 22.100±0.067% and 15.977±0.002% (Mean±SEM) palmitic acid in pre monsoon, monsoon and post-monsoon respectively.

Availability of high levels of palmitic acid has been described as characteristics of fresh water fish (Ackman, 1980). In this study, Palmitic acid (C16:0) was the primary saturated fatty acid (SFA), contributing approximately 35.50%, 51.85% and 47.06% to the total SFA content in pre-monsoon, monsoon and post-monsoon season. This work coincides with our present findings. Orban et al. (2002) reported that palmitic acid (16:0), oleic acid (18:1), Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) fatty acids constituted the basic components of fatty acids of sea bass fillets. This result also partially supports our findings.

The experimental fish, *M. cuchia* contains 18.963±0.003%, 20.792±0.002% and 25.991±0.001% oleic acid (C18:1) respectively. The level of the monounsaturated fatty acid is highly dependent on the level of oleic acid, since the primary constituent of this group is oleic acid. In this study, oleic acid is identified as the dominant monounsaturated fatty acids.

The reason for the remarkable difference between the data of Shchenikova et al. (1987) may be for different species and for different geographical region and habitat. Sinanoglou and Miniadis-Meimaroglou (1998) described that amount of different fatty acid in fish lipid have direct relation to the geographical region where fishing was conducted. The high levels of oleic, palmitoleic, and arachidonic acids had been reported as a characteristic property of freshwater fish oils (Andrade et al., 1995 Osman et al., 2001). These results more or less similar to our findings. The reason may be that both are fresh water species.

Hassan et al. (2010) worked on total lipids and fatty acid profile in the liver of wild and farmed *Catla catla* fish. MUFA (C18:1) was found in considerable amounts in the liver of both and PUFA such as C18:3 (n-6) and C20: 2 (n-6) were detected in the liver of the wild fish. Our present findings are similar with this result.

Piggott and Tucker (1990) suggested that the n-3/n-6 ratio is a better index in comparing the relative nutritional value of fish oils of different species. An n-3/n-6 ratio of 1:1 is considered to be optimal for nutritional purposes (Simopoulos, 1989), particularly when n-3 fatty acids consist mainly of EPA and DHA. Based on omega-6 and omega-3 ratios, previous studies by (Steffens, 1997) showed that marine fish species are mainly composed of omega-3 and low level of omega-6. This is an opposite case for fresh water fish species which in this study are mainly composed of omega-6 and low level of omega-3 fatty acids. Rasoarahonare et al. (2005) showed that there are a number of experiments demonstrating the effects of other factors such as environmental temperature, seasonal variations, age and species on the fatty acid composition of aquatic animals especially fish. Ovissipour and Resco (2011) worked on Fatty acid and Amino acid profile of domestic and wild Beluga (*Huso huso*) and impact on fertilization ratio. They showed that the ratio of n-3/n-6 of *Huso huso* was presented 0.32 (Cultured) and 1.195 (Wild).

Harlioglu et al. (2016) showed the ratio of n-3/n-6 in sea bass from three cage farm areas was presented 1.1±0.1 (Iskele), 1.3±0.1 (Antalya) and 1.0±0.1 (Mugla). Seasonal variations in fatty acid composition of different tissues of mirror carp, *Cyprinus carpio*, in Orenler dam lake, Afyonkarahisar, Turkey (Karacali et al., 2011). They obtained the ratio of n-3/n-6 in Cyprinus *carpio* 0.86 (autumn), 0.62 (winter), 0.98 (spring) and 0.63 (summer). The ratio of ω-3/ω-6 PUFAs in total lipids of freshwater fishes ranges between 0.5 and 3.8 (Ates et al., 2013).

A dietary intake of fish with a balanced n-3/n-6 ratio is essential in the prevention and treatment of many health issues, such as heart disease, high blood pressure, arthritis, depression, inflammatory bowel disease, autoimmune disorders and many others.Results were exhibited the ratio of n-3/n-6 were 0.948 of *M. cuchia* respectively in pre-monsoon whereas in monsoon and post-monsoon these ratio were 1.321 and 0.354 respectively of *M. cuchia.* Above these circumstances, our findings are authentic.

4.4 Amino Acid profile

Amino acids are the building blocks of proteins and serve as body builders. They are utilized to form various cell structures, of which they are key components and they serve as source of energy (Babsky et al. 1989). Fish protein comprises of all the ten essential amino acid in desirable quantity for human consumption. Fish protein is very rich in such amino acid as methionine, lysine and low in tryptophan compared to mammalian protein (Nowsad, 2007).

Our present findings describe that lysine is the most abundant amino acid in *M. cuchia* that contains 3.877±0.023% lysine in pre-monsoon season on the contrary in monsoon and post-monsoon the amount were 1.760±0.017% and 1.677±0.023% approximately. Among these result the highest value was marked in pre-monsoon season as the lowest was in post-monsoon season.

Findings describe that glutamic acid is the second most abundant amino acid in *M. cuchia*. In pre monsoon, the amount of glutamic acid in experimental fish was 3.597±0.026% whereas in monsoon, post-monsoon, the amount was 1.553±0.018% and 1.257±0.026% respectively. Among these result the highest value is identified in pre-monsoon season as the lowest is in post-monsoon season.

Findings also illustrate that the amount of arginine of *M. cuchia* is 3.560±0.015% (Mean±SEM) in pre-monsoon on the contrary in monsoon and post monsoon the amounts are 1.526±0.023% and 1.473 ±0.018%. Among these result the highest value is marked in pre-monsoon season as the lowest is in post-monsoon season.

In pre monsoon, the amount of aspartic acid in experimental fish is 2.243±0.018% whereas in monsoon, post-monsoon, the amount is 0.947±0.020% and 0.773±0.023% respectively. Among these result the highest value is obtained in pre-monsoon season as the lowest is in post-monsoon season.

Nurullah et al. (2003) reported that among all the amino acids analyzed, glutamic acid content was found to be quite high whereas the other amino acids were slightly low. Iwasaki and Harada (1985) similarly reported that the main amino acids in fish muscle were aspartic acid, glutamic acid and lysine. Wesselinova (2000) also reported that the amounts and types of amino acids in fish muscle were affected by catching time (seasons) and habitat.

Barrento et al. (2010) have worked on chemical composition, cholesterol, fatty acid and amino acid in two populations of brown crab *Cancer pagurus*: Ecological and human health implication.They showed that the ratio of EAA/NEAA in brown crab *Cancer pagurus* was presented 0.80±0.01 (muscle), 0.88±0.04 (hepatopancreas) and 0.82±0.06 (gonads).

Gressler et al. (2010) also showed the ratio of EAA/NEAA in four Brazilian red algae was 0.9 (*G. domingensis*), 0.9 (*G. birdiae*), 0.9 (*L. filiformis*) and 0.8 (*L. intricate*). Amino acid and fatty acid of wild sea bass, *D. labrax*: a seasonal differentiation (Ozyurt et al., 2006). They identified the ratio of from EAA/NEAA from wild sea bass (*D. labrax*) 0.76 (autumn), 0.75 (winter), 0.77 (spring) and 0.77 (summer). Our present findings have similarity with above these works

In the present study, the ratio of EAA/NEAA is observed 0.679, 0.676 and 0.706 in experimental fish (*M. cuchia*) among three different seasons as pre-monsoon, monsoon and post-monsoon. Moreover the ratio of EAA/NEAA is higher in post-monsoon season than pre-monsoon and monsoon season. As a result, above these works are supported our present findings.

Findings from this study show that *M. cuchia* in post-monsoon season have well-balanced and high-quality protein source in the respect of EAA/NEAA ratio compare to pre-monsoon and monsoon season

5. Conclusion and recommendations

Monopterus cuchia is an export fishery product that is playing an important role in international markets. It can generate employment directly and indirectly for more than 8,000 people in the marketing and other associated business.

Findings of the study showed that seasonal variation remains in proximate, fatty acid and amino acid profile in *M. cuchia*. Seasonally the highest moisture content in *M. cuchia* was found in post-monsoon whereas the lowest value was obtained in pre-monsoon season. There was a little seasonal fluctuation of the moisture content in the fresh flesh muscle of *M. cuchia* when compared in pre-monsoon, monsoon and post-monsoon seasons.

Ash content is higher in monsoon than pre-monsoon and post-monsoon. There is no significant difference ($p < 0.05$) was observed in ash content among these three different seasons in freshwater mud eel, *M. cuchia*.

Fish lipids have been recognized as being beneficial for human health. The highest value of lipid content was marked in pre-monsoon season and the lowest value was pre monsoon. Results illustrated that significant difference ($p < 0.05$) is observed among these three different seasons in *M. cuchia*.

Fatty acid profile indicates that *M. cuchia* is more rich in Poly Unsaturated Fatty Acids (PUFAs) with balanced n-3/n-6 ratio in pre-monsoon than monsoon (n-3>n-6) and post-monsoon (n-6>n-3). The balanced n-3/n-6 ratio exhibits higher nutritional and medicinal value such asprevention and treatment of coronary artery disease, diabetes, hypertension and cancer. So it may concluded that *Monopterus cuchia* (Cuchia) is an excellent source of PUFA mainly EPA and DHA which are essential fatty acids. Human body cannot synthesize these EFAs.

The highest value of crude protein was recorded in monsoon and the lowest value was in post-monsoon season. In amino acid profile result indicates that EA/NEA ratio is the highest in post-monsoon than pre-monsoon and monsoon season. It is well known that high quality protein have higher EA/NEA ratio. So *M. cuchia* in post-monsoon season have well-balanced and high-quality protein source in the respect of EAA/NEAA ratio in pre-monsoon and monsoon season.

M. cuchia does not provide the same nutrient profile depending on the seasons. Seasonal Variation in proximate, fatty acid and amino acid due to habitat, season, age, sex, geographical location etc. Generally, this freshwater mud eel is an excellent source of protein, because of the amino acid composition and degree of digestibility.

The following recommendations are offered for related research in the field of fisheries and aquaculture focusing the different aspects of *M.cuchia in Bangladesh*.

i. Further study should be taken to analysis the proximate, fatty acids, amino acid composition including the mineral contents of the *M. cuchia* with spatial and seasonal variations between male and female with increased number of sample size;
ii. A holistic study should be taken to address the management techniques, factors affecting the habitat loss, habitat restoration and conservation, suitability of natural habitat to maintain the sustainability of the natural stock of *M.cuchia;*
iii. A study on molecular identification and characterization of the indigenous Cuchia fish should be performed.
iv. Study should be conducted to develop the captive breeding and culture methods of *M. cuchia.*

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Appendices

Appendix A

Table 1: Physical characteristics of *Monopterus cuchia* collected from three different Seasons.

Seasons	Length (cm)	Weight (g)
Pre-monsoon (March-May)	54.0	162.54
	60.5	233.23
	74.2	**345.32**
	52.4	**151.15**
	50.2	164.90
	57.1	178.80
	60.1	288.70
	56.3	255.00
	53.3	160.30
	59.0	221.23
Monsoon (June-August)	61.0	239.26
	72.2	256.97
	74.5	410.34
	79.3	**463.55**
	67.0	340.43
	56.5	**202.07**
	60.2	288.75
	60.4	250.23
	57.5	220.08
	60.1	294.23
Post-monsoon (September-November)	65.0	375.05
	66.1	315.23
	74.3	**450.00**
	56.4	260.20
	57.0	340.16
	58.2	260.00
	59.5	275.45
	61.2	242.26
	60.5	232.25
	53.6	**160.67**

Table 2: Mean length and weight of *Monopterus cuchia* collected from three different Seasons. Result showed (Mean±SD).

Seasons	Mean Total Length (cm)	Mean Total Weight (g)
Pre-monsoon (March-May)	57.71±6.73 (50.2-74.2)	216.12±64.99 (151.15-345.32)
Monsoon (June-August)	64.87±7.91 (56.5-74.3)	296.61±4.69 (202.07-463.55)
Post-monsoon (September-November)	61.18±5.97 (53.6-74.3)	291.13±81.86 (160.67-450.00)

Length

Tukey HSD

Season	N	Subset for alpha = 0.05
		1
Pre-monsoon (March-May)	10	57.710
Post-monsoon (September-November)	10	61.180
Monsoon (June-August)	10	64.870
Sig.		.071

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 10.000.

Weight

Tukey HSD

Season	N	Subset for alpha = 0.05
		1
Pre-monsoon (March-May)	10	216.1170
Post-monsoon (September-November)	10	291.1270
Monsoon (June-August)	10	296.6130
Sig.		.070

Appendix B

Table 3: Descriptive Findings about Proximate Composition of *Monopterus cuchia* among three different Seasons.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Moisture	Pre-monsoon (March-May)	10	78.8749	.32130	.10160	78.6451	79.1048	78.30	79.28
	Monsoon (June-August)	10	79.6380	.34928	.11045	79.3881	79.8879	79.10	80.05
	Post-monsoon (September-November)	10	79.8770	.28956	.09157	79.6699	80.0841	79.40	80.15
	Total	30	79.4633	.53369	.09744	79.2640	79.6626	78.30	80.15
Ash	Pre-monsoon (March-May)	10	1.0990	.03604	.01140	1.0732	1.1248	1.04	1.15
	Monsoon (June-August)	10	1.1490	.08937	.02826	1.0851	1.2129	1.00	1.27
	Post-monsoon (September-November)	10	1.1030	.08693	.02749	1.0408	1.1652	1.00	1.21
	Total	30	1.1170	.07589	.01386	1.0887	1.1453	1.00	1.27
Protein	Pre-monsoon (March-May)	10	17.7180	.26478	.08373	17.5286	17.9074	17.42	18.11
	Monsoon (June-August)	10	18.8270	.30645	.09691	18.6078	19.0462	18.35	19.33
	Post-monsoon (September-November)	10	17.6660	.29235	.09245	17.4569	17.8751	17.29	18.13
	Total	30	18.0703	.61159	.11166	17.8420	18.2987	17.29	19.33

Lipid	Pre-monsoon (March-May)	10	2.2430	.06447	.02039	2.1969	2.2891	2.15	2.38
	Monsoon (June-August)	10	.3040	.03836	.01213	.2766	.3314	.25	.37
	Post-monsoon (September-November)	10	1.2620	.04826	.01526	1.2275	1.2965	1.19	1.35
	Total	30	1.2697	.80668	.14728	.9684	1.5709	.25	2.38
Length	Pre-monsoon (March-May)	10	57.710	6.7268	2.1272	52.898	62.522	50.2	74.2
	Monsoon (June-August)	10	64.870	7.9101	2.5014	59.211	70.529	56.5	79.3
	Post-monsoon (September-November)	10	61.180	5.9710	1.8882	56.909	65.451	53.6	74.3
	Total	30	61.253	7.3053	1.3338	58.525	63.981	50.2	79.3
Weight	Pre-monsoon (March-May)	10	216.1170	64.99152	20.55212	169.6249	262.6091	151.15	345.32
	Monsoon (June-August)	10	296.6130	84.69203	26.78197	236.0280	357.1980	202.07	463.55
	Post-monsoon (September-November)	10	291.1270	81.86272	25.88726	232.5659	349.6881	160.67	450.00
	Total	30	267.9523	83.73548	15.28794	236.6850	299.2197	151.15	463.55

Table 4: Seasonal Variation in the Percentage of Nutritional Composition of *Monopterus cuchia*. Data are expressed as (Mean±SD). Different letters (a, b and c) within a row denote significant differences ($p < 0.05$) and the same letter denotes no significance.

Proximate Composition (%)	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
Moisture	78.87±0.32[a]	79.64±0.35[b]	79.88±0.29[b]
Ash	1.10±0.04[a]	1.15±0.09[a]	1.10±0.09[a]
Protein	17.72±0.26[a]	18.83±0.31[a]	17.67±0.29[b]
Lipid	2.24±0.06[a]	0.30±0.04[b]	1.26±0.05[c]

Table 5: Tukey HSD for Proximate composition. a. Means for groups in homogeneous subsets are displayed. b. Uses Harmonic Mean Sample Size = 10.000.

Homogeneous Subsets

Moisture

Tukey HSD

Season	N	Subset for alpha = 0.05	
		1	2
Pre-monsoon (March-May)	10	78.8749	
Monsoon (June-August)	10		79.6380
Post-monsoon (September-November)	10		79.8770
Sig.		1.000	.237

Ash

Tukey HSD

Season	N	Subset for alpha = 0.05
		1
Pre-monsoon (March-May)	10	1.0990
Post-monsoon (September-November)	10	1.1030
Monsoon (June-August)	10	1.1490
Sig.		.310

Protein

Tukey HSD

Season	N	Subset for alpha = 0.05	
		1	2
Post-monsoon (September-November)	10	17.6660	
Pre-monsoon (March-May)	10	17.7180	
Monsoon (June-August)	10		18.8270
Sig.		.915	1.000

Lipid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Monsoon (June-August)	10	.3040		
Post-monsoon (September-November)	10		1.2620	
Pre-monsoon (March-May)	10			2.2430
Sig.		1.000	1.000	1.000

Appendix C

Table 6: Descriptive Findings about Fatty Acids of *Monopterus cuchia* among three different Seasons.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
(C6:0) Caproic Acid	Pre-monsoon (March-May)	3	4.669533	.0043062	.0024862	4.658836	4.680231	4.6651	4.6737
	Monsoon (June-August)	3	1.873800	.0038432	.0022189	1.864253	1.883347	1.8695	1.8769
	Post-monsoon (September-November)	3	.301900	.0012530	.0007234	.298787	.305013	.3007	.3032
	Total	9	2.281744	1.9158316	.6386105	.809106	3.754383	.3007	4.6737
(C8:0) Caprylic Acid	Pre-monsoon (March-May)	3	8.575433	.0068595	.0039604	8.558393	8.592473	8.5707	8.5833
	Monsoon (June-August)	3	.987333	.0034269	.0019785	.978821	.995846	.9835	.9901

	Post-monsoon (September-November)	3	.838633	.0011504	.0006642	.835776	.841491	.8375	.8398
	Total	9	3.467133	3.8317680	1.2772560	.521776	6.412491	.8375	8.5833
(C12:0) Lauric Acid	Pre-monsoon (March-May)	3	8.274040	.0034852	.0020122	8.265382	8.282698	8.2701	8.2767
	Monsoon (June-August)	3	3.086800	.0213136	.0123054	3.033854	3.139746	3.0665	3.1090
	Post-monsoon (September-November)	3	3.772400	.0042790	.0024705	3.761770	3.783030	3.7675	3.7754
	Total	9	5.044413	2.4403698	.8134566	3.168579	6.920248	3.0665	8.2767

(C13:0) Tridecanoic Acid	Pre-monsoon (March-May)	3	.000000	0E-7	0E-7	.000000	.000000	.0000	.0000
	Monsoon (June-August)	3	.350867	.0009504	.0005487	.348506	.353228	.3499	.3518
	Post-monsoon (September-November)	3	.494433	.0062228	.0035927	.478975	.509892	.4904	.5016
	Total	9	.281767	.2203016	.0734339	.112428	.451105	.0000	.5016
(C14:0) Myristic Acid	Pre-monsoon (March-May)	3	6.152433	.0051965	.0030002	6.139525	6.165342	6.1489	6.1584
	Monsoon (June-August)	3	4.018200	.0324640	.0187431	3.937555	4.098845	3.9973	4.0556
	Post-monsoon (September-November)	3	2.130733	.0038004	.0021942	2.121293	2.140174	2.1269	2.1345
	Total	9	4.100456	1.7426181	.5808727	2.760961	5.439950	2.1269	6.1584
(C15:0)Pentadecyclic Acid	Pre-monsoon (March-May)	3	.881067	.0020232	.0011681	.876041	.886093	.8798	.8834
	Monsoon (June-August)	3	1.424700	.0008000	.0004619	1.422713	1.426687	1.4239	1.4255
	Post-monsoon (September-November)	3	1.122100	.0030265	.0017474	1.114582	1.129618	1.1187	1.1245
	Total	9	1.142622	.2359102	.0786367	.961286	1.323959	.8798	1.4255
(C16:0) Palmitic Acid	Pre-monsoon (March-May)	3	19.808300	.0028827	.0016643	19.801139	19.815461	19.8057	19.8114
	Monsoon (June-August)	3	22.100100	.1168787	.0674799	21.809757	22.390443	21.9654	22.1747
	Post-monsoon (September-November)	3	15.976733	.0033710	.0019462	15.968359	15.985107	15.9740	15.9805

	Total	9	19.295044	2.6799307	.8933102	17.235067	21.355022	15.9740	22.1747
(C18:0) Stearic Acid	Pre-monsoon (March-May)	3	3.982367	.0089913	.0051911	3.960031	4.004702	3.9723	3.9896
	Monsoon (June-August)	3	5.176100	.0022271	.0012858	5.170568	5.181632	5.1741	5.1785
	Post-monsoon (September-November)	3	6.154367	.0024379	.0014075	6.148311	6.160423	6.1516	6.1562
	Total	9	5.104278	.9420571	.3140190	4.380149	5.828407	3.9723	6.1562
(C20:0) Arachidic Acid	Pre-monsoon (March-May)	3	1.045700	.0079568	.0045938	1.025934	1.065466	1.0386	1.0543
	Monsoon (June-August)	3	1.515467	.0043466	.0025095	1.504669	1.526264	1.5122	1.5204
	Post-monsoon (September-November)	3	1.761400	.0015133	.0008737	1.757641	1.765159	1.7602	1.7631
	Total	9	1.440856	.3149523	.1049841	1.198762	1.682949	1.0386	1.7631
(C22:0) Behenic Acid	Pre-monsoon (March-May)	3	.658500	.0027074	.0015631	.651774	.665226	.6566	.6616
	Monsoon (June-August)	3	.000000	0E-7	0E-7	.000000	.000000	.0000	.0000
	Post-monsoon (September-November)	3	.441500	.0028618	.0016523	.434391	.448609	.4388	.4445
	Total	9	.366667	.2906167	.0968722	.143279	.590054	.0000	.6616
(C24:0) Lignoceric Acid	Pre-monsoon (March-May)	3	1.752300	.0026514	.0015308	1.745714	1.758886	1.7500	1.7552
	Monsoon (June-August)	3	2.205867	.0054372	.0031392	2.192360	2.219373	2.2001	2.2109

	Post-monsoon (September-November)	3	.941967	.0093751	.0054127	.918678	.965256	.9313	.9489
	Total	9	1.633378	.5545330	.1848443	1.207126	2.059630	.9313	2.2109
(C14:1) Myristoleic Acid	Pre-monsoon (March-May)	3	.371267	.0014295	.0008253	.367716	.374818	.3697	.3725
	Monsoon (June-August)	3	1.351333	.0013429	.0007753	1.347997	1.354669	1.3498	1.3523
	Post-monsoon (September-November)	3	1.791967	.0013577	.0007839	1.788594	1.795339	1.7907	1.7934
	Total	9	1.171522	.6297905	.2099302	.687422	1.655622	.3697	1.7934
(C15:1) Pentadecenoic Acid	Pre-monsoon (March-May)	3	.000000	0E-7	0E-7	.000000	.000000	.0000	.0000
	Monsoon (June-August)	3	.421100	.0028931	.0016703	.413913	.428287	.4178	.4232
	Post-monsoon (September-November)	3	.836233	.0033005	.0019055	.828034	.844432	.8335	.8399
	Total	9	.419111	.3621094	.1207031	.140769	.697453	.0000	.8399
(C16:1) Palmitoleic Acid	Pre-monsoon (March-May)	3	9.892400	.0012288	.0007095	9.889347	9.895453	9.8910	9.8933
	Monsoon (June-August)	3	9.616333	.0048952	.0028263	9.604173	9.628494	9.6109	9.6204
	Post-monsoon (September-November)	3	8.294433	.0019757	.0011407	8.289525	8.299341	8.2923	8.2962
	Total	9	9.267722	.7396949	.2465650	8.699142	9.836302	8.2923	9.8933
(C18:1) Oleic Acid	Pre-monsoon (March-May)	3	18.963067	.0045369	.0026194	18.951796	18.974337	18.9579	18.9664

	Monsoon (June-August)	3	20.792500	.0028000	.0016166	20.785544	20.799456	20.7893	20.7945
	Post-monsoon (September-November)	3	25.990733	.0016442	.0009493	25.986649	25.994818	25.9895	25.9926
	Total	9	21.915433	3.1574637	1.0524879	19.488392	24.342475	18.9579	25.9926
(C20:1) Eicosenoic Acid	Pre-monsoon (March-May)	3	.000000	0E-7	0E-7	.000000	.000000	.0000	.0000
	Monsoon (June-August)	3	.305867	.0039209	.0022637	.296127	.315607	.3022	.3100
	Post-monsoon (September-November)	3	.502100	.0026514	.0015308	.495514	.508686	.4998	.5050
	Total	9	.269322	.2191493	.0730498	.100869	.437775	.0000	.5050
(C16:2) Hexadecadienoic Acid	Pre-monsoon (March-May)	3	.640733	.0025325	.0014621	.634442	.647024	.6388	.6436
	Monsoon (June-August)	3	1.384267	.0017388	.0010039	1.379947	1.388586	1.3823	1.3856
	Post-monsoon (September-November)	3	.680067	.0019399	.0011200	.675248	.684886	.6788	.6823
	Total	9	.901689	.3623384	.1207795	.623171	1.180207	.6388	1.3856
(C16:3) Hexadecatrienoic Acid	Pre-monsoon (March-May)	3	.000000	0E-7	0E-7	.000000	.000000	.0000	.0000
	Monsoon (June-August)	3	2.909967	.0012662	.0007311	2.906821	2.913112	2.9090	2.9114
	Post-monsoon (September-November)	3	2.154533	.0015695	.0009062	2.150634	2.158432	2.1533	2.1563
	Total	9	1.688167	1.3076987	.4358996	.682980	2.693353	.0000	2.9114

(C18:2) Linoleic Acid	Pre-monsoon (March-May)	3	6.378433	.0009504	.0005487	6.376072	6.380794	6.3775	6.3794
	Monsoon (June-August)	3	5.411833	.0029092	.0016796	5.404607	5.419060	5.4088	5.4146
	Post-monsoon (September-November)	3	17.785800	.0017000	.0009815	17.781577	17.790023	17.7841	17.7875
	Total	9	9.858689	5.9600483	1.9866828	5.277390	14.439988	5.4088	17.7875
(C18:3) Linolenic Acid	Pre-monsoon (March-May)	3	3.978967	.0003055	.0001764	3.978208	3.979726	3.9787	3.9793
	Monsoon (June-August)	3	1.975867	.0009074	.0005239	1.973613	1.978121	1.9749	1.9767
	Post-monsoon (September-November)	3	1.680333	.0015567	.0008988	1.676466	1.684200	1.6787	1.6818
	Total	9	2.545056	1.0830207	.3610069	1.712572	3.377539	1.6787	3.9793
(C20:2) Eicosadienoic Acid	Pre-monsoon (March-May)	3	.000000	0E-7	0E-7	.000000	.000000	.0000	.0000
	Monsoon (June-August)	3	.631733	.0037207	.0021481	.622491	.640976	.6285	.6358
	Post-monsoon (September-November)	3	.650100	.0010536	.0006083	.647483	.652717	.6490	.6511
	Total	9	.427278	.3205628	.1068543	.180871	.673684	.0000	.6511
(C20:4) Arachidonic Acid	Pre-monsoon (March-May)	3	2.070600	.0011790	.0006807	2.067671	2.073529	2.0693	2.0716
	Monsoon (June-August)	3	3.074433	.0023714	.0013691	3.068543	3.080324	3.0719	3.0766

	Post-monsoon (September-November)	3	2.035333	.0044970	.0025964	2.024162	2.046505	2.0323	2.0405
	Total	9	2.393456	.5109682	.1703227	2.000691	2.786221	2.0323	3.0766
(C20:5) Eicosapentaenoic Acid	Pre-monsoon (March-May)	3	.000000	0E-7	0E-7	.000000	.000000	.0000	.0000
	Monsoon (June-August)	3	.603567	.0016803	.0009701	.599393	.607741	.6021	.6054
	Post-monsoon (September-November)	3	.281933	.0028746	.0016597	.274792	.289074	.2793	.2850
	Total	9	.295167	.2615457	.0871819	.094125	.496209	.0000	.6054
(C22:5) Docosapentaenoic Acid	Pre-monsoon (March-May)	3	.000000	0E-7	0E-7	.000000	.000000	.0000	.0000
	Monsoon (June-August)	3	4.015900	.0023259	.0013429	4.010122	4.021678	4.0138	4.0184
	Post-monsoon (September-November)	3	2.205767	.0030534	.0017629	2.198182	2.213352	2.2028	2.2089
	Total	9	2.073889	1.7417474	.5805825	.735063	3.412714	.0000	4.0184
(C22:6) Docosahexaenoic Acid	Pre-monsoon (March-May)	3	1.909967	.0002309	.0001333	1.909393	1.910540	1.9097	1.9101
	Monsoon (June-August)	3	4.743467	.0018556	.0010713	4.738857	4.748076	4.7417	4.7454
	Post-monsoon (September-November)	3	1.160767	.0009504	.0005487	1.158406	1.163128	1.1598	1.1617
	Total	9	2.604733	1.6365272	.5455091	1.346787	3.862680	1.1598	4.7454

Table 7: Turkey HSD for significance test in Fatty Acids of *M. cuchia* within and between groups of variables. a. Means for groups in homogeneous subsets are displayed. b. Uses Harmonic Mean Sample Size = 3.000.

(C6:0) Caproic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	.301900		
Monsoon (June-August)	3		1.873800	
Pre-monsoon (March-May)	3			4.669533
Sig.		1.000	1.000	1.000

(C8:0) Caprylic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	.838633		
Monsoon (June-August)	3		.987333	
Pre-monsoon (March-May)	3			8.575433
Sig.		1.000	1.000	1.000

(C12:0) Lauric Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Monsoon (June-August)	3	3.086800		
Post-monsoon (September-November)	3		3.772400	
Pre-monsoon (March-May)	3			8.274040
Sig.		1.000	1.000	1.000

(C13:0) Tridecanoic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	.000000		
Monsoon (June-August)	3		.350867	
Post-monsoon (September-November)	3			.494433
Sig.		1.000	1.000	1.000

(C14:0) Myristic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	2.130733		
Monsoon (June-August)	3		4.018200	
Pre-monsoon (March-May)	3			6.152433
Sig.		1.000	1.000	1.000

(C15:0)Pentadecyclic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	.881067		
Post-monsoon (September-November)	3		1.122100	
Monsoon (June-August)	3			1.424700
Sig.		1.000	1.000	1.000

(C16:0) Palmitic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	15.976733		
Pre-monsoon (March-May)	3		19.808300	
Monsoon (June-August)	3			22.100100
Sig.		1.000	1.000	1.000

(C18:0) Stearic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	3.982367		
Monsoon (June-August)	3		5.176100	
Post-monsoon (September-November)	3			6.154367
Sig.		1.000	1.000	1.000

(C20:0) Arachidic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	1.045700		
Monsoon (June-August)	3		1.515467	
Post-monsoon (September-November)	3			1.761400
Sig.		1.000	1.000	1.000

(C22:0) Behenic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Monsoon (June-August)	3	.000000		
Post-monsoon (September-November)	3		.441500	
Pre-monsoon (March-May)	3			.658500
Sig.		1.000	1.000	1.000

(C24:0) Lignoceric Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	.941967		
Pre-monsoon (March-May)	3		1.752300	
Monsoon (June-August)	3			2.205867
Sig.		1.000	1.000	1.000

(C14:1) Myristoleic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	.371267		
Monsoon (June-August)	3		1.351333	
Post-monsoon (September-November)	3			1.791967
Sig.		1.000	1.000	1.000

(C15:1) Pentadecenoic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	.000000		
Monsoon (June-August)	3		.421100	
Post-monsoon (September-November)	3			.836233
Sig.		1.000	1.000	1.000

(C16:1) Palmitoleic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	8.294433		
Monsoon (June-August)	3		9.616333	
Pre-monsoon (March-May)	3			9.892400
Sig.		1.000	1.000	1.000

(C18:1) Oleic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	18.963067		
Monsoon (June-August)	3		20.792500	
Post-monsoon (September-November)	3			25.990733
Sig.		1.000	1.000	1.000

(C20:1) Eicosenoic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	.000000		
Monsoon (June-August)	3		.305867	
Post-monsoon (September-November)	3			.502100
Sig.		1.000	1.000	1.000

(C16:2) Hexadecadienoic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	.640733		
Post-monsoon (September-November)	3		.680067	
Monsoon (June-August)	3			1.384267
Sig.		1.000	1.000	1.000

(C16:3) Hexadecatrienoic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	.000000		
Post-monsoon (September-November)	3		2.154533	
Monsoon (June-August)	3			2.909967
Sig.		1.000	1.000	1.000

(C18:2) Linoleic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Monsoon (June-August)	3	5.411833		
Pre-monsoon (March-May)	3		6.378433	
Post-monsoon (September-November)	3			17.785800
Sig.		1.000	1.000	1.000

(C18:3) Linolenic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	1.680333		
Monsoon (June-August)	3		1.975867	
Pre-monsoon (March-May)	3			3.978967
Sig.		1.000	1.000	1.000

(C20:2) Eicosadienoic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	.000000		
Monsoon (June-August)	3		.631733	
Post-monsoon (September-November)	3			.650100
Sig.		1.000	1.000	1.000

(C20:4) Arachidonic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	2.035333		
Pre-monsoon (March-May)	3		2.070600	
Monsoon (June-August)	3			3.074433
Sig.		1.000	1.000	1.000

(C20:5) Eicosapentaenoic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	.000000		
Post-monsoon (September-November)	3		.281933	
Monsoon (June-August)	3			.603567
Sig.		1.000	1.000	1.000

(C22:5) Docosapentaenoic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Pre-monsoon (March-May)	3	.000000		
Post-monsoon (September-November)	3		2.205767	
Monsoon (June-August)	3			4.015900
Sig.		1.000	1.000	1.000

(C22:6) Docosahexaenoic Acid

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	1.160767		
Pre-monsoon (March-May)	3		1.909967	
Monsoon (June-August)	3			4.743467
Sig.		1.000	1.000	1.000

Appendix D

Table 8: Amino Acid Composition (%) of *Monopterus cuchia* among three different Seasons.Data are expressed as Mean±SEM (n = 3). Different letters within a row denote significant differences ($p < 0.05$) and the same letter denotes no significances ($p>0.05$)

Amino Acids (%)	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
Aspartic Acid^{NEAA}	2.243±0.018^{a}	0.947±0.020^{b}	0.947±0.023^{c}
Threonine^{EAA}	1.247±0.020^{a}	0.630±0.017^{b}	0.540±0.017^{c}
Serine^{NEAA*}	1.620±0.023^{a}	0.740±0.023^{b}	0.737±0.027^{b}
Glutamic Acid^{NEAA}	3.597±0.026^{a}	1.553±0.018^{b}	1.553±0.018^{c}
Glycine^{NEAA}	3.107±0.018^{a}	1.463±0.023^{b}	1.313±0.023^{c}
Alanine^{NEAA}	1.677±0.015^{a}	0.760±0.023^{b}	0.650±0.015^{c}
Valine^{EAA}	1.677±0.026^{a}	0.717±0.015^{b}	0.653±0.023^{b}
Methionine^{EAA}	2.147±0.023^{a}	0.967±0.020^{b}	0.930±0.021^{b}
Isoleucine^{EAA}	1.847±0.023^{a}	0.877±0.019^{b}	0.823±0.032^{b}
Leucine^{EAA}	2.193±0.020^{a}	0.873±0.026^{b}	0.840±0.023^{c}
Tyrosine^{NEAA}	1.640±0.026^{a}	0.783±0.018^{b}	0.673±0.026^{c}
Histidine^{NEAA}	1.890±0.029^{b}	0.847±0.026^{a}	0.857±0.025^{a}
Lysine^{EAA**}	3.877±0.023^{a}	1.760±0.017^{b}	1.677±0.023^{c}
Arginine^{NEAA}	3.360±0.015^{b}	1.526±0.023^{a}	1.473±0.018^{a}
EAA/NEAA	0.679	0.676	0.706

*NEAA = Non-Essential Amino Acid, **EAA = Essential Amino Acid.

Table 9: Descriptive Findings about Amino Acids of *Monopterus cuchia* among three different Seasons.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Arginine	Pre-monsoon (March-May)	3	3.3533	.01528	.00882	3.3154	3.3913	3.34	3.37
	Monsoon (June-August)	3	1.5233	.01528	.00882	1.4854	1.5613	1.51	1.54
	Post-monsoon (September-November)	3	1.4367	.00577	.00333	1.4223	1.4510	1.43	1.44
	Total	9	2.1044	.93748	.31249	1.3838	2.8251	1.43	3.37
Histidine	Pre-monsoon (March-May)	3	1.8700	.02646	.01528	1.8043	1.9357	1.84	1.89
	Monsoon (June-August)	3	.8533	.01528	.00882	.8154	.8913	.84	.87
	Post-monsoon (September-November)	3	.8333	.02517	.01453	.7708	.8958	.81	.86
	Total	9	1.1856	.51379	.17126	.7906	1.5805	.81	1.89
Isoleucine	Pre-monsoon (March-May)	3	1.8367	.01528	.00882	1.7987	1.8746	1.82	1.85
	Monsoon (June-August)	3	.8367	.02517	.01453	.7742	.8992	.81	.86
	Post-monsoon (September-November)	3	.8167	.02517	.01453	.7542	.8792	.79	.84
	Total	9	1.1633	.50545	.16848	.7748	1.5519	.79	1.85
Leucine	Pre-monsoon (March-May)	3	2.1900	.01000	.00577	2.1652	2.2148	2.18	2.20
	Monsoon (June-August)	3	.8733	.01528	.00882	.8354	.9113	.86	.89
	Post-monsoon (September-November)	3	.8333	.01155	.00667	.8046	.8620	.82	.84
	Total	9	1.2989	.66864	.22288	.7849	1.8129	.82	2.20
Lysine	Pre-monsoon (March-May)	3	3.8667	.01528	.00882	3.8287	3.9046	3.85	3.88
	Monsoon (June-August)	3	1.7567	.02517	.01453	1.6942	1.8192	1.73	1.78

	Post-monsoon (September-November)	3	1.6533	.01528	.00882	1.6154	1.6913	1.64	1.67
	Total	9	2.4256	1.08189	.36063	1.5939	3.2572	1.64	3.88
Methionine	Pre-monsoon (March-May)	3	2.1433	.01528	.00882	2.1054	2.1813	2.13	2.16
	Monsoon (June-August)	3	.9767	.02082	.01202	.9250	1.0284	.96	1.00
	Post-monsoon (September-November)	3	.9333	.01528	.00882	.8954	.9713	.92	.95
	Total	9	1.3511	.59465	.19822	.8940	1.8082	.92	2.16
Threonine	Pre-monsoon (March-May)	3	1.2367	.01528	.00882	1.1987	1.2746	1.22	1.25
	Monsoon (June-August)	3	.6233	.02082	.01202	.5716	.6750	.60	.64
	Post-monsoon (September-November)	3	.5367	.01528	.00882	.4987	.5746	.52	.55
	Total	9	.7989	.33081	.11027	.5446	1.0532	.52	1.25
Valine	Pre-monsoon (March-May)	3	1.5267	.01528	.00882	1.4887	1.5646	1.51	1.54
	Monsoon (June-August)	3	.7033	.01528	.00882	.6654	.7413	.69	.72
	Post-monsoon (September-November)	3	.6667	.02082	.01202	.6150	.7184	.65	.69
	Total	9	.9656	.42140	.14047	.6416	1.2895	.65	1.54
Alanine	Pre-monsoon (March-May)	3	1.6933	.01155	.00667	1.6646	1.7220	1.68	1.70
	Monsoon (June-August)	3	.7600	.01000	.00577	.7352	.7848	.75	.77
	Post-monsoon (September-November)	3	.6667	.01528	.00882	.6287	.7046	.65	.68
	Total	9	1.0400	.49178	.16393	.6620	1.4180	.65	1.70
Aspartic	Pre-monsoon (March-May)	3	2.2067	.02517	.01453	2.1442	2.2692	2.18	2.23
	Monsoon (June-August)	3	.9400	.01000	.00577	.9152	.9648	.93	.95
	Post-monsoon (September-November)	3	.7767	.02517	.01453	.7142	.8392	.75	.80
	Total	9	1.3078	.67812	.22604	.7865	1.8290	.75	2.23
Glutamic	Pre-monsoon (March-May)	3	3.5933	.02082	.01202	3.5416	3.6450	3.57	3.61

	Monsoon (June-August)	3	1.5133	.01155	.00667	1.4846	1.5420	1.50	1.52
	Post-monsoon (September-November)	3	1.2633	.01528	.00882	1.2254	1.3013	1.25	1.28
	Total	9	2.1233	1.10789	.36930	1.2717	2.9749	1.25	3.61
Glycine	Pre-monsoon (March-May)	3	3.0967	.00577	.00333	3.0823	3.1110	3.09	3.10
	Monsoon (June-August)	3	1.4533	.01528	.00882	1.4154	1.4913	1.44	1.47
	Post-monsoon (September-November)	3	1.3067	.01528	.00882	1.2687	1.3446	1.29	1.32
	Total	9	1.9522	.86075	.28692	1.2906	2.6139	1.29	3.10
Serine	Pre-monsoon (March-May)	3	1.6167	.01528	.00882	1.5787	1.6546	1.60	1.63
	Monsoon (June-August)	3	.7367	.02517	.01453	.6742	.7992	.71	.76
	Post-monsoon (September-November)	3	.7333	.01528	.00882	.6954	.7713	.72	.75
	Total	9	1.0289	.44115	.14705	.6898	1.3680	.71	1.63
Tyrosine	Pre-monsoon (March-May)	3	1.6267	.01528	.00882	1.5887	1.6646	1.61	1.64
	Monsoon (June-August)	3	.7533	.01528	.00882	.7154	.7913	.74	.77
	Post-monsoon (September-November)	3	.6667	.01528	.00882	.6287	.7046	.65	.68
	Total	9	1.0156	.46006	.15335	.6619	1.3692	.65	1.64

Table 10: Turkey HSD for significance test in Amino Acids of *M. cuchia* within and between groups of variables. a. Means for groups in homogeneous subsets are displayed. b. Uses Harmonic Mean Sample Size = 3.000.

Homogeneous Subsets

Arginine

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	1.4367		
Monsoon (June-August)	3		1.5233	
Pre-monsoon (March-May)	3			3.3533
Sig.		1.000	1.000	1.000

Histidine

Tukey HSD

Season	N	Subset for alpha = 0.05	
		1	2
Post-monsoon (September-November)	3	.8333	
Monsoon (June-August)	3	.8533	
Pre-monsoon (March-May)	3		1.8700
Sig.		.564	1.000

Leucine

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	.8333		
Monsoon (June-August)	3		.8733	
Pre-monsoon (March-May)	3			2.1900
Sig.		1.000	1.000	1.000

Leucine

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	.8333		
Monsoon (June-August)	3		.8733	
Pre-monsoon (March-May)	3			2.1900
Sig.		1.000	1.000	1.000

Lysine

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	1.6533		
Monsoon (June-August)	3		1.7567	
Pre-monsoon (March-May)	3			3.8667
Sig.		1.000	1.000	1.000

Methionine

Tukey HSD

Season	N	Subset for alpha = 0.05	
		1	2
Post-monsoon (September-November)	3	.9333	
Monsoon (June-August)	3	.9767	
Pre-monsoon (March-May)	3		2.1433
Sig.		.050	1.000

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Threonine

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	.5367		
Monsoon (June-August)	3		.6233	
Pre-monsoon (March-May)	3			1.2367
Sig.		1.000	1.000	1.000

Valine

Tukey HSD

Season	N	Subset for alpha = 0.05	
		1	2
Post-monsoon (September-November)	3	.6667	
Monsoon (June-August)	3	.7033	
Pre-monsoon (March-May)	3		1.5267
Sig.		.091	1.000

Alanine

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	.6667		
Monsoon (June-August)	3		.7600	
Pre-monsoon (March-May)	3			1.6933
Sig.		1.000	1.000	1.000

Aspartic

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	.7767		
Monsoon (June-August)	3		.9400	
Pre-monsoon (March-May)	3			2.2067
Sig.		1.000	1.000	1.000

Glutamic

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	1.2633		
Monsoon (June-August)	3		1.5133	
Pre-monsoon (March-May)	3			3.5933
Sig.		1.000	1.000	1.000

Glycine

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	1.3067		
Monsoon (June-August)	3		1.4533	
Pre-monsoon (March-May)	3			3.0967
Sig.		1.000	1.000	1.000

Serine

Tukey HSD

Season	N	Subset for alpha = 0.05	
		1	2
Post-monsoon (September-November)	3	.7333	
Monsoon (June-August)	3	.7367	
Pre-monsoon (March-May)	3		1.6167
Sig.		.975	1.000

Tyrosine

Tukey HSD

Season	N	Subset for alpha = 0.05		
		1	2	3
Post-monsoon (September-November)	3	.6667		
Monsoon (June-August)	3		.7533	
Pre-monsoon (March-May)	3			1.6267
Sig.		1.000	1.000	1.000