Faculty of Resource Science and Technology

Proximate composition and Fatty acid analysis of selected commercial fishes in Sarawak

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Declaration

This Final Year Project 2022 is based on my original work except for the quotation and citation which have been acknowledged. I also declared that is has not been submitted previously or currently to any other degree at Universiti Malaysia Sarawak or any other institution.

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Abstract

Consumers believed that the nutritional content of many kinds of fish is identical; nevertheless, it differs by species. This study was conducted to compare and analyse the fatty acid content and proximate composition of three commercially fishes in Sarawak, namely *Tenualosa toli* (Terubuk), *Channa striatus* (Haruan) and *Xenopterus naritus* (Buntal Kuning). In general, the moisture content varied between 6 and 77%, the ash content varied between 1 and 5%, and the protein content varied between 20 and 87%. In this study, the chemometric analysis showed the fatty acid content of eicosapentaenoic acid and arachidonic acid *Tenualosa toli* and *Xenopterus naritus* were (2.70%, 0.73 %) and (0.16 %, 0.61 %) respectively while the content of arachidonic acid in Channa striatus was 1.47%. The dominant saturated fatty acid in this study is palmitic acid with a percentage of 41.10%.

Keywords: Proximate composition, Fatty acid analysis, Eicosapentaenoic acid, Arachidonic acid, Saturated fatty acid.

Abstrak

Pengguna percaya bahawa kandungan pemakanan pelbagai jenis ikan adalah sama; namun, ia berbeza mengikut spesies. Kajian ini dijalankan untuk membandingkan dan menganalisis kandungan asid lemak dan komposisi proksimat tiga ekor ikan komersial di Sarawak iaitu Tenualosa toli (Terubuk), Channa striatus (Haruan) dan Xenopterus naritus (Buntal Kuning). Secara amnya, kandungan lembapan berbeza antara 6 dan 77%, kandungan abu berbeza antara 1 dan 5%, dan kandungan protein berbeza antara 20 dan 87%. Dalam kajian ini, analisis kemometrik menunjukkan kandungan asid lemak asid eicosapentaenoic dan asid arakidonik Tenualosa toli dan Xenopterus naritus masing-masing adalah (2.70%, 0.73 %) dan (0.16 %, 0.61 %) manakala kandungan asid arakidonik dalam Channa striatus ialah 1.47%. Asid lemak tepu yang dominan dalam kajian ini ialah asid palmitik dengan peratusan 41.10%.

Kata kunci: Komposisi proksimat, Analisis asid lemak, asid Eicosapentaenoic, Asid arakidonik, Asid lemak tepu.

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AA	Arachidonic Acid
^0C	Degree celcius
Cm	Centimeter
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic Acid
g	Gramme
GCMS	Gas chromatography–mass spectrometry
LC	Long Chain
m	Meter
mm	Milimeter
Ω	Omega
SFA	Saturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid

1.0 Introduction

For the vast majority of people, fish is an essential part of their daily diet. Norimah *et al.,* (2008) reported that according to the results of the Malaysian Adult Nutrition Survey (MANS) conducted in 2008, 51% and 34% of people in rural and urban areas were familiar with the consumption of fish. Fish has demonstrated health benefits including anti-oxidation, anti-inflammation, wound healing, neuro protection, cardio protection and hepatoprotection (Chen *et al*., 2022). Oftentimes, consumers overlook the nutritional value of a fish in favour of the freshness, colour, taste and availability.

Inside the muscles of a fish, the key nutrients found are water, protein, and fat, while vitamins, carbohydrates, and minerals are minor components. Analyzing a proximate composition examination of the specimen of a fish; ash, protein, moisture, and lipid constitution, are necessary for determining the nutritional value of fish and optimizing its use in manufacturing. In order to ensure the quality of various fishing goods and value-added products derived from by-products, it is necessary to know the product's immediate composition.

It is commonly perceived that the composition of polyunsaturated fatty acids (PUFAs) in fish varies depending on the species (Muhamad and Mohamad, 2012). Moisture, protein, fat, and ash content of fish vary considerably according to species, food, age, sex, season, and living condition (Obodai, 2009; Osibona, 2011; Ondo – Azi et al., 2013). In addition, fish muscles and their by-products may have distinct nutritional contents. Hence, it is important to take into account both the fat content and the proportions of PUFAs in fish when they are recommended as a health supplement (Rahnan *et al*., 1995). Fish comprises a massive quantity of Ω-3 fatty acids, principally docosahexaenoic acid (DHA), linoleic acid, and eicosapentaenoic acid (EPA), making its lipids particularly significant (Pal et al.,2018). Ω-3 and Ω-6 polyunsaturated fatty acids (PUFAs) significantly decrease cardiac related disease and promote wound healing (Gibson,1983; Conner, 1997). Fish protein contains an abundance of amino acids such as methionine and lysine, while its tryptophan level is minimal (Nowsad,2007). Amino acids are commonly found in fish muscle and have a crucial function in the generation of cell walls, growth of the human body, and infant development.

The muscle tissue and liver were used in this study to obtain the protein, lipid, fish moisture, and ash content of three commercial fish in Sarawak to determine the proximate and fatty acid composition while demonstrating its benefits to consumers. Locals regard the roe and flesh of *T. toli* and *X. naritus* as delicacies, while *C. striatus* is frequently employed for medicinal purposes.

Fish is an important source of high-quality animal proteins, and its accessibility and affordability are superior to those of other animal protein sources (Alam *et al*., 2012). In a study conducted by Osman *et al.*, (2001), Malaysians, in general, believed that different types of fish have the same nutritional value. The purpose of this study is to provide information on the proximate values and fatty acid composition of three commercially important fish species in Sarawak: *Tenualosa Toli* (Ikan Terubuk), *Channa Striatus* (Ikan Haruan), and *Xenopterus Naritus* (Ikan Buntal Kuning).

The objectives of the study were:

a) To conduct proximate analyses on the muscle of *T. toli*, *C. striatus,* and *X. naritus,* including moisture content, protein content, and ash content.
b) To distinguish and analyse fatty acids in the liver of *T. toli, C. striatus,* and *X. naritus*.
c) To determine the amount of polyunsaturated fatty acids (PUFAs) present in the oil extracted from *T. toli, C. striatus,* and *X. naritus.*
d) To conduct a chemometric analysis of fatty acids found in the muscle and liver oils of *T. toli*, *C. striatus,* and *X. naritus.*

2.0 Literature Review

2.1 Proximate composition in Fishes

Extensive research on the nutritional composition of fish have been published, and the outcomes of the research have been utilised to create nutritional requirements for fish. The term "proximate composition" is often used in the food and feed industries, and its principal components include moisture, ash, fat, protein, and carbohydrate content stated as a percentage (Pal *et al.*,2018). Understanding the chemical components of marine organisms is important for evaluating raw material quality, storage stability, and industrial applications (Gokolu, 2021).

In order to quantify food value in terms of energy units, it is essential to conduct a comprehensive analysis of the species' proximate composition (Qasim, 1972). In a study on fish bioenergetics and the effect of pollutants, the proximate composition of a fish serves as a significant indicator of the morphology of fish morphology for routine fisheries analysis (Ahmed and Shah,2017; Cui and Wootton,1988).

The very first and fundamental step in determining the total nutritional content of a fish is by examining its moisture content as it is an effective demonstration of a food's caloric protein and fat content (Ahmed *et al.*,2021; Barua *et al.*,2012). Ahmed *et al.*, 2021 reported that fatty fishes with a high protein constituent have a lower moisture content thus an increased number of calories per gramme. However, the values vary considerably based on the species, size, sexual status and season (Ahmed *et al.*,2021).

Protein comes in second in the proximate composition after moisture because of the presence of essential amino acids (EAA), which are essential for the well-being, growth, and health of fishes. Unlike mammalian protein, fish protein contains a higher concentration of amino acids including lysine and methionine (Begum *et al.*, 2012). Fat comes in third place as one of the important components in the muscle of a fish and it contributes to the formation of cells and tissue membranes. Additionally, it is also a major source of metabolic energy (Ahmed *et al.,* 2021). Ash content is linked to minerals found in the fish as the total inorganic content of fish is expressed in the mineral composition.

2.2 Fatty Acids Content in Fishes

Oils and fats are naturally occurring components of fatty acids, which are classed based on their chemical structure into three groups: saturated, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (PUFA). Because they cannot be metabolised by the human body, polyunsaturated fatty acids (PUFAs) are important nutrients that must be acquired through diet or supplementation (Kris – Etherton et al., 2002). In a study conducted by Tasbozan and Gokce (2017), diet, species, and environmental factors, such as season, temperature, geographical characteristics, and salinity, among others, were shown to influence the composition of fatty acids in fish. Lipids are a heterogeneous chemical class considered to be the best source of dietary energy and a source of important fatty acids (Ahmed et al.,2021).

2.3 Commercial Fishes in Sarawak

2.3.1 *Tenualosa Toli*

Tenualosa toli is native to the Indo-West Pacific region, which stretches from India to the Java Sea and South China Sea (Jawad et al.,2011). T. toli, also known locally as Terubok, is a member of the Clupeidae family, which includes allies, menhadens, shads, pilchards, sardines and herrings. *T. toli* is grouped as an Actinopterygii, a ray-finned fish that can grow up to 60 cm in length and is typically found in brackish waters with a depth of 10 metres (Nurasyikin et al, 2006). The estuaries of the Lassa, Saribas, and Lupar Rivers in Sarawak are considered the "core Terubok's regions" since the majority of the *T. toli* population is found in these water bodies (Rahim et al., 2014). The *T. toli* population is reportedly localised in the Daro and Mukah regions (Aziz et al.,2015). Moreover, according to Rahim et al. (2014), the primary locations for the species include the estuaries of Lassa, Saribas, and Lupar in Sarawak.

In Malaysia, the Toli shad is highly after for consumption and its commercial worth (Blaber et al., 1997). The culture of this fish is currently developing fast in Bangladesh and India; therefore, it is shipped to Malaysia to meet demand (Hossain et al.,2018). In Sarawak, the fish is often sold for its roe, which can garner competitive prices (Blaber et al.,2003).

Figure 2.1: A lateral view of *T. toli*.

2.3.2 *Xenopterus Naritus*

The yellow pufferfish, *Xenopterus Naritus*, is a member of the Tetradontidae family. The South China Sea is home to the euryhaline species, *X. naritus*. The species is easily distinguished by its notable yellowish gold colouration, particularly at the lower abdomen of the body, and its torpedo-shaped body (Mohd *et al*., 2015). *X. naritus* can be found in abundance in Sarawak along the estuaries of the Saribas River, Sadong River, Lupar River and Rajang River (Ahmad Nasir *et al*., 2017). Although many consider this species to be a trash fish, it is popular among the locals in Sarawak and is caught by artisanal fishermen. Furthermore, the species is commercialized and used to make a variety of products (Mohd *et al*., 2015). Due to the high demand for salted yellow puffer fish roe, an annual festival called "Yellow Puffer Fish Festival" is held in Betong, Saribas to commemorate the significance of the species in the community.

X. naritus is extensively dispersed in Southeast Asian countries, China and India (Gambang and Lim, 2004). The species is restricted to the southern shore of Sarawak, Malaysia, from Tanjung Datu in Sematan to Mukah in the state's centre. (Ahmad Nasir *et al*.,2017). *X. naritus* is a migratory species that can be found spawning on the upper part of the Saribas River nearby Manggut Village, approximately 90 kilometres away from the estuary of Saribas (Ahmad Nasir *et al*.,2017).

A handful of pufferfish species are notorious for containing complex neurotoxins, specifically tetrodotoxin (TTX). These toxins are normally found in different tissues of a pufferfish, namely the liver, skin muscle, gonad and stomach (Mohammed *et al*.,2013). Chou *et al.*, (1994) reported that food poisoning involving the consumption of pufferfishes depended on the concentration of TTX in the fish tissues. Bojo et al. (2006) performed a study to determine the toxicity of the fish. The study discovered that the sample's toxicity ranged from 0.7 to 4.5 MU/g, which is generally accepted because it is below 10 MU/g. In Sarawak, Malaysia, two death incidents were recorded as a result of yellow puffer species ingestion attributed to the consumption of the salted yellow puffer roes, a local delicacy (Muliadi and Muhammad Raduan, 2008).

Figure 2.2: A lateral view of *X. naritus*.

2.3.3 *Channa Striatus*

The snakehead fish Channa striatus, a native species to many tropical and subtropical countries, belongs to the Channidae family. It is known to locals as "ikan haruan". *C. striatus* can grow up to 100cm in length and 3000g in weight (Muhamad and Mohamad, 2012). The carnivorous freshwater species possess special characteristics such as air-breathing (Mohsin and Ambak, 1983). *C. striatus* can be recognized by other snakeheads by colour patterns, spots, blotches, and lower jaw morphology. It can be observed that the species possess an abrupt colour transition where its upper body is darker in colour with diagonal stripes and a white margin on its anal fin only. When compared to *C. melasoma*, the *C. striatus* possess a jawline that converges towards the snout while the *C. melasoma* is slightly divergent. *C. striatus* is a slimy fish due to its natural secretion of mucus to protect itself in stressful environments (Zuraini *et al.*, 2006).

C. striatus can live in a wide range of water bodies, from small drainages to ricefields, river systems, and lakes in tropical and subtropical Asian countries spanning Pakistan and India to Southeast Asia and Southern China (Haniffa *et al.,* 2014). In Malaysia, locals believe that the flesh of the fish speeds up the process of wound healing and can be used to treat injuries while improving health. Ginseng compound is mixed with the essence of *C. striatus* as a post–operative remedy to facilitate wound healing particularly after major surgery (Haniffa *et al.,* 2014). A wide array of products derived from the extracts of *C.striatus* are available in the market, ranging from creams to liquid forms.

Figure 2.3: A lateral view of *C. striatus*.

3.0 Materials and Methods

3.1 Sample collection

Samples of *T. toli*, *C. Striatus* and *X. naritus* were purchased from local fishermen in the area of Kota Samarahan. Samples were kept in a Styrofoam ice box and transported to Ecotoxicology Lab, UNIMAS. The fishes were dissected and filleted and the muscle and liver were obtained.

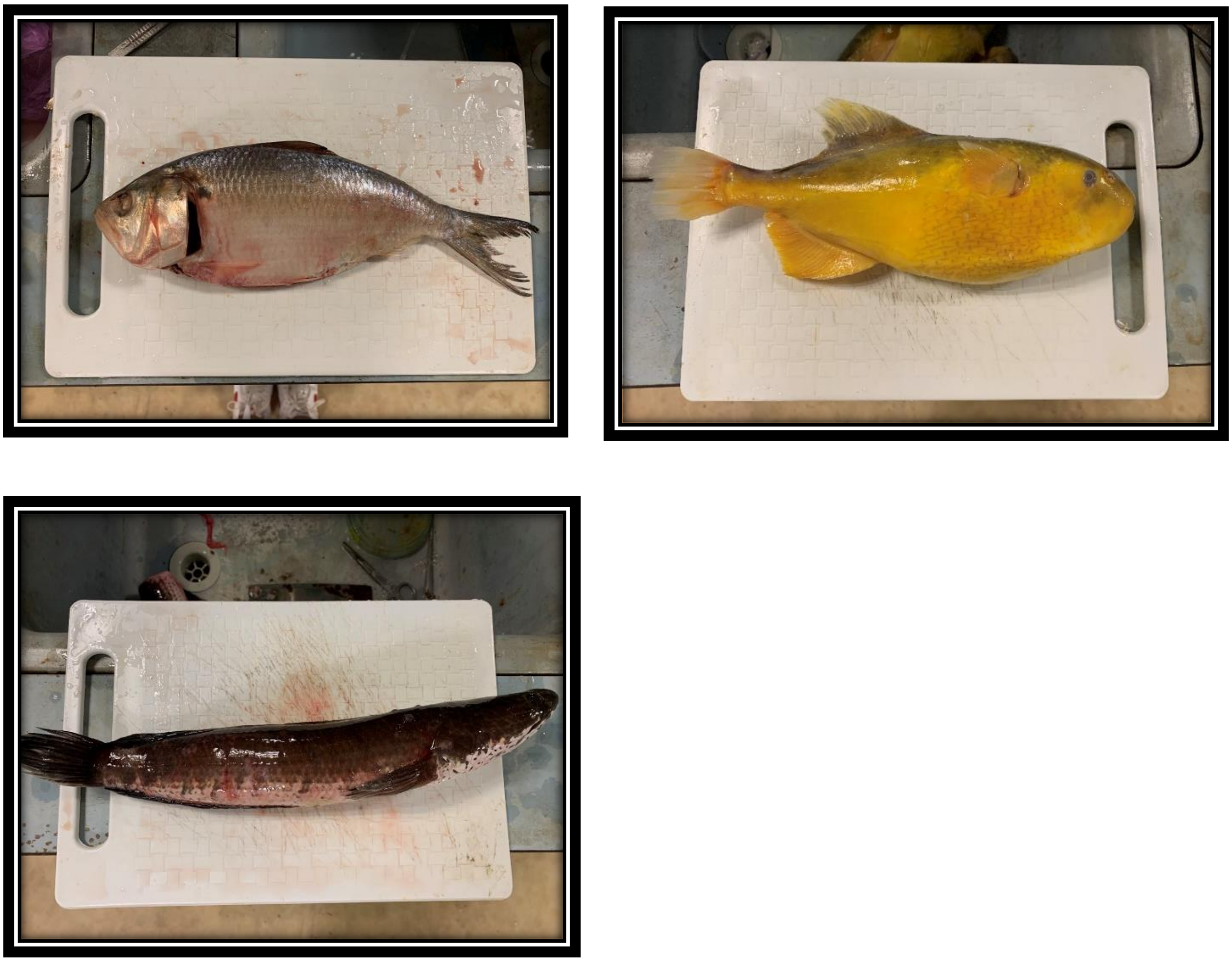

Figure 3.1: The three species of fishes selected for this study.

3.2 Proximate Analysis

3.2.1 Moisture content analysis

Content of moisture in *T. toli, C. striatus* and *X.naritus* were determined according to AOAC method with slight modifications. 90g of the minced fish fillets were placed into a silica dish in the oven at 120 °C for 12 hours, then allowed to cool in a desiccator and weighed. Drying and weighing of the samples were continued until a constant weight was achieved. The moisture content was then calculated using the equation below:

$$Moisture\ content : \frac{Weight\ of\ fresh\ sample - weight\ of\ dry\ sample}{Weight\ of\ fresh\ sample} \times 100\%$$

Figure 3.2: Formula used to calculate moisture content.

After determining the moisture content, the dried samples were ground using a dry grinder. The fine powders obtained will be used for ash and crude protein analysis.

3.2.2 Ash content

The ash content of *T. toli, C. striatus* and *X. naritus* will be determined using the AOAC (2000) methods with slight modifications. About 1g of each sample and a crucible were weighed separately. The crucible filled with 1g of samples was then placed inside the muffle furnace at a temperature of 550ºC overnight. After 12 hours, the crucibles were taken out and the weight was recorded. To determine the ash content, the formula below was used:

$$Ash\ \% = \frac{W3 - W1}{W2 - W1} \times 100$$

W1 = Weight of dry crucible

W2 = Weight of dry crucible with sample (after moisture analysis)

W3 = Weight of dry crucible and dried sample

Figure 3.3: Formula to calculate the ash content in the three species.

3.2.3 Crude protein analysis

The crude protein content of T. toli, C. striatus, and X. naritus fish minced slices will be determined using the AOAC (2000) technique with slight adjustments as suggested by Kjeltec 2100. (Foss Analytical, Denmark). Prior to this, 1 g of dried sample was weighed and inserted into digestion tubes. In each tube, two Kjeltabs Cu 3.5 catalyst salts will be placed. About 12 ml of concentrated sulphuric acid, H2SO4, will be added with caution to the test tube, which will then be gently shaken. The digestion procedure will be conducted for 60 minutes using a pre-heated (420 degrees Celsius) digestion block of the 2066 Digestor (Foss Tecator, Sweden) in order to obtain a clear blue/green solution. 20 minutes will be allotted for the chilling of the digested samples. Then, a distillation technique will be carried out. With 0.5N hydrochloric acid (HCl), the distillation will be titrated until a pinkish reaction was completed.

3.3 Extraction of fatty acid.

For the extraction of fatty acids, the wet rendering technique was used. The liver of each fish sample was placed into a beaker and distilled water was added in 1:1 ratio. The wet rendering technique was carried out at a temperature of 80 °C and 90 °C, combined with extraction periods of 15-, 25-, 35-, and 45-minutes. Boiling method was applied for extraction process. After implementing each mixture of wet rendering extraction treatments, the sample was boiled and filtered to obtain a liquid fraction. Water and oil fractions were separated by centrifuging the liquid fraction at 3000 rpm for 4 minutes.

3.4 Fatty acid analysis

3.4.1 Derivatisation of Fish Oils

Prior to the gas chromatographic analysis, fatty acids were derivatized into fatty acid methyl esters (FAMEs) to increase the volatility of the fatty acid compounds. Exactly 20mg of oil sample were dissolved in 2mL n-heptane followed by 4mL of 2M methanolic KOH. The tube were vortexed at room temperature for two minutes. Afterwards, the solution was centrifuged for ten minutes at 3000 rpm. The solution was allowed to rest for ten minutes to isolate the clear FAME solution from the hazy layer at the bottom. The top layer was cautiously retrieved and will be analysed on a Gas Chromatography.

3.4.2 Gas Chromatography Analysis

On a GC-MS Shimadzu QP equipped with a 30-meter-long glass column M, 0.25 mm diameter and 0.25 μm thickness with CP-Sil 5CB stationary phase and a pre-programmed oven temperature of 60-220 °C with a temperature rise rate of 10°C /min, derivatized fish oil will be analysed. The carrier gas is pressurised 12 kPa Helium with a total flow rate of 30 mL/min and a split ratio of 1:50. Fatty acid in the samples was identified by comparing retention times and peak areas of FAMEs standards prepared and run under identical conditions to those of the actual samples.

Figure 3.4: Gas chromatography used in analysing FAMEs.

3.5 Statistical Analysis

SPSS (Statistical Package for the Social Sciences) version 16.0 for Windows were utilised to evaluate the collected data. Before carrying out the entire analysis, the information was transformed into a normal distribution. ANOVA was used to identify the difference in the means of crude protein content, moisture content, ash content and fat content between *T. toli, C. striatus,* and *X. naritus.* The Duncan multiple range test were subsequently employed to assess the differences between the samples gathered. The T-test was applied to the fatty acid data to assess the differences between the species' mean fatty acids. The entirety of the proximate compositions was examined in triplicate and reported as a mean on a dry weight percentage basis. When $p<0.05$, the mean SD of triplicate measurements were judged substantially different.

4.0 Results and Discussion

4.1 Proximate Analysis

Table 4.1 shows the proximate composition of 3 different commercialized species in Sarawak. Contents of moisture in the muscle tissue of each species ranged from 6 -77%. *X. naritus* showed the highest value of moisture content with 77.36%, slightly higher compared to *T. toli* with 65.89%. The moisture content of muscle tissue for *C. striatus* was 6.86% which is significantly lower compared to *X. naritus* and *T. toli*. Eswar *et al* (2014) reported that an increased moisture content provides the fish with an advantage by stabilising the organisms during movement.

	Proximate composition		
Species	Moisture content	Ash content	Protein content
T.toli	65.89 ± 3.01	1.12 ± 0.43	20.37 ± 1.89
C. striatus	6.86 ± 0.64	4.82 ± 0.17	87.93 ± 0.19
X. naritus	77.36 ± 1.13	5.48 ± 0.05	87.91 ± 2.94

Table 4.1: Proximate composition of 3 different species investigated.

The ash content for muscle tissues investigated varies from 1.12% to 5.48% with *X. naritus* possessing the highest ash value (5.48%) while *T. toli* had the lowest ash value (1.12%). The ash content in a fish muscle is associated with the mineral composition of fish. In general, the total mineral content of wet fish muscle is between 0.6% and 1.5% of the entire fish body weight (Ahmed *et al.*,2021). Variations in the ash content values are contributed by factors such as seasonal conditions and the size of the species examined (Eswar *et al.,* 2014).

The highest protein content was found in *C. striatus* (87.93%) followed by *X. naritus* (87.91%) while *T. toli* (20.37%) had the lowest protein content when compared with the