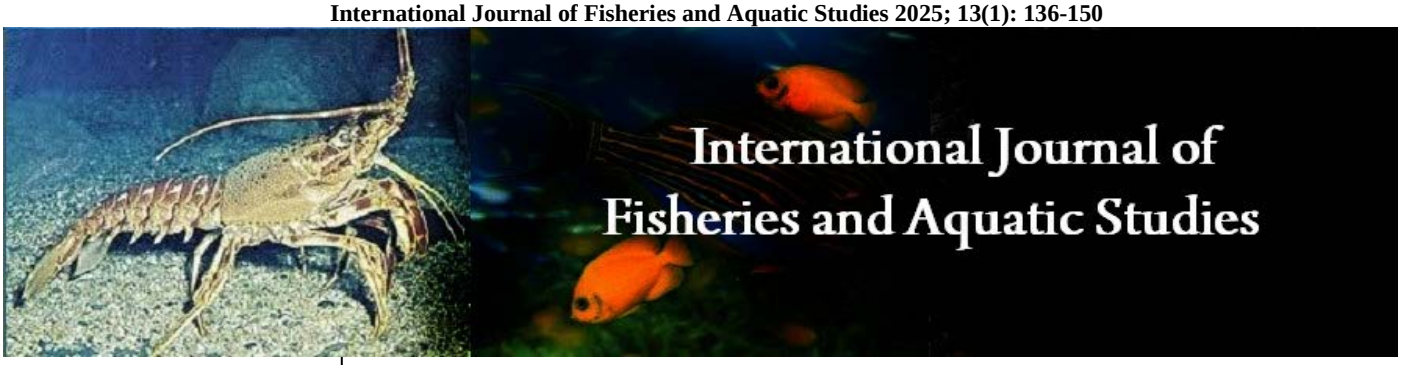

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Study on proximate composition of certain food fishes from marine, estuarine and freshwater environment fishes in tirurangadi taluk, malappuram, Kerala, India

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Abstract
Fishes are the rich source of nutrients, providing an important complement to the predominantly carbohydrate-based staple diet of many people in Kerala Fishes arcuate different from other animal food sources, because they provide calories with high quality proteins, which contain all essential amino acids in easily digestible from and are beneficial nutritional sources. The five major constituent in the muscle of fish are water, protein, lipid (fat or oil) ash (minerals) and carbohydrate. The analysis of these five basic constituents is often referred to as proximate analysis.

Keywords: Freshwater, estuarine, marine, poly unsaturated fatty acid

Introduction
Fish is consumed by many animal species, including humans. Fish has been an important part of the diet of humans in almost all countries in the world and it is the cheapest sources of animal proteins and availability and affordability is better for fish in comparison to other animal protein sources (Mohanty *et al.*, 2013) [2]. Animal proteins are considered as the complete source of protein because they contain all the ten animal amino acids. Because of this it is considered that at least one third of the total requirement of the protein in the daily diet must come from animal source (Ferdose and Hossain, 2011). More than 50% of Indian population is fish eating and in some states like Assam and other North-Eastern states, West Bengal, Odisha, Goa and Kerala, more than 90% of the population that consume fish. Fish is very important due to the presence of therapeutically important polyunsaturated fatty acids, calcium, iodine, vitamins, and several other nutrients. Aquaculture is an important part of food security, particularly, for many poor people in developing countries which make up about 22% of overall animal protein consumption (Mohanty *et al.*, 2013) [2].
Proximate composition such as protein contents, carbohydrates, lipids, moisture contents and ash percentage is often necessary to ensure that they meet the requirements of food regulations and commercial specifications (Waterman, 2000) [5]. Owing to its special nutritive status, awareness about fish as a healthy food is gaining importance. Proximate composition of a species helps to assess its nutritional and edible value. Variation in the composition of fish may occur within same species depending upon the fishing ground, age, fishing season, sex of the individual and the reproductive status. The percentage composition of the five major constituents of fish *viz*. water, protein, lipid, carbohydrate and ash (minerals) is referred to as proximate composition (it may be noted that the term does not indicate any degree of inaccuracy in the analysis). These five components account for about 96-98% of total tissue constituents in most cases. Protein content variation is affected by breeding capability, Planktonic diet and climatic changes in year. The highest and lowest protein content associated to breeding, spawning, biological regression and resting stages respectively (Kor, 1995) [1].
Lipid content in fishes varies in the range of 0.2-20%. Fish is a rich source of omega 3 poly unsaturated fatty acid (PUFA) especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Ackman, 1999). They provide a range of health benefit that are not present in any other food, including prevention of coronary heart disease,

improvement of retina and brain development, reduction of incidence of breast cancer, rheumatoid arthritis, multiple sclerosis and inflammation. Omega 3 fatty acids are associated with the synthesis of eicosanoids, thromboxane and leukotrienes.

The ash content in various kinds of fish varies from 0.5-3.5% Fish is a good source of vitamin B complex and the species with good amount of liver. Oils are good source of fat-soluble vitamins A and D. Fish is particularly a good source of minerals like calcium, phosphorus, iron, copper and trace elements like selenium and zinc. Composition tables for fish often include a value for total ash. Since ash consists largely of a number of different minerals, and the total rarely exceeds 1-2 per cent of the edible portion, this figure has also been omitted, except from the table of fish products. The most important mineral salts are that of calcium, sodium, potassium, phosphorous, iron, chlorine, while many others are also needed in trace amounts. The deficiency in these principle nutritional mineral elements induce a lot of malfunctioning; as it reduces productivity and causes diseases, such as inability of blood to clot, osteoporosis, anemia etc.

The carbohydrate content in fish varies from 0-0.5% it is clear that it is present only in trace amount (Mohanty, *et al.*, 2013) [2]. The habitat and food intake of these species are equally diverse. Some species are exclusively marine, while some are confined to freshwater and estuarine habitats. Some survive in marine as well as freshwater environments. Some marine species migrate to fresh water for spawning whereas many freshwater species enter the sea for spawning. These widely different environmental conditions of temperature, salinity, pressure, availability of food etc. have profound influence on the biochemical composition.

Proximate composition are critical for many applications and investigations on these lines had been carried out, Proper knowledge on the biochemical composition of fish finds application in several areas. Today there is an ever-increasing awareness about healthy food and fish is finding more acceptances because of its special nutritional qualities. In this context a proper understanding about the biochemical constituents of fish has become a primary requirement for the nutritionists and dieticians (Nimisha and Sheeba, 2012) [3]. Fish is an easily perishable commodity and deterioration in quality is due to the changes taking place to the various constituents like proteins, lipids etc. Information on the biochemical constituents will help a processing technologist to define the optimum processing and storage conditions, so that the quality is preserved to the maximum extent.

Materials and Methods

30 different fish samples were collected randomly from 3 different ecosystems directly by the catch of fisherman during the month of last December 2019 to 15th of January 2020. Fresh water fishes were collected from Palathingal nearby Tirurangadi, where many local people depending on fishing in Kadalundy river for their daily needs. Estuarine fishes were collected from Kadalundy where the river Kadalundy merges to Arabian ocean. Kadalundy is an area of wide varieties of plants and animal diversity. Marine fishes were collected from Parappanangadi, which is one of the major fishing harbor in Malappuram district, Kerala. Selected food fishes for the nutritional analysis are:

Table 1: Total number of fish samples collected from the three different ecosystems (Freshwater, Estuarine and Marine) of Tirurangadi Taluk, Malappuram District, Kerala

Marine fishes	Estuarine fishes	Fresh water fishes
1. *Rastrelliger kanagurta*	1. *Mugil cephalus*	1. *Puntius filamentosus*
2. *Sardinella longiceps*	2. *Xenentodon cancila*	2. *Channa striata*
3. *Otolithes ruber*	3. *Arius arius*	3. *Horabagrus brachysoma*
4. *Nemipterus japonicas*	4. *Onigocia pedimaculata*	4. *Puntius sarana*
5. *Cynoglossus arel*	5. *Leiognathus equulus*	5. *Bunaka gyrinoides*
6. *Epinephelus diacanthus*	6. *Scatophagus argus*	6. *Nandus nandus*
7. *Gerres filementosus*	7. *Etroplus suratensis*	7. *Etroplus maculatus*
8. *Atule mate*	8. *Lutjanus argentimaculatus*	8. *Anabas scandens*
9. *Stolephorus indicus*	9. *Hemiramphus xanthopterus*	9. *Heteropneustes fossilis*
10. *Ambrassis natalensis*	10. *Sphyraena obtusata*	10. *Mystus montanus*

The nutrient parameters analyzed were moisture content, ash, protein, fat and carbohydrate. After removing the scales, clean fish muscle including skin were taken for further analysis.

Determination of protein

Estimation of protein by Lowry's method (Lowry *et al.*, 1951) [7].

Determination of moisture content

A sample of 1 gm was spread in an aluminium cup uniformly and dried in a hot air oven for 5 hours at 105ºC. Sample was transferred to a dessicator to be cooled and weighed. The differences in weight represented the loss of moisture and were expressed as a percentage of oven dried sample (AOAC, 2000).

$$\text{Calculation: Moisture \%} = \frac{W1-W2}{W2} \times 100$$

W1= Weight of sample before drying (g)
W2= Weight of sample after drying (g)

Determination of ash content

A muffle furnace was used to analyze a 5 g sample for total ash (AOAC, 2000). The sample was heated in a silica crucible over low Bunsen flame with half covered lid. When fumes were no longer produced, the crucible and lid were placed in muffle furnace and heated at 550ºC overnight. After complete heating the lid was placed on the crucible to prevent loss of fluffy ash and cooled in a desiccator. Weight was taken when the sample turned grey.

$$\text{Ash\%} = \frac{\text{Weight of Ash (g)}}{\text{Weight of Sample (g)}} \times 100$$

Determination of fat content

Total fat in the sample was estimated in a Soxhlet apparatus

(AOAC, 2000). In a filter paper 1 g of sample was weighed and wrapped carefully. The wrapped sample was introduced into the extraction thimble of the Soxhlet apparatus. 250 mL of petroleum ether was filled into the round bottom flask and placed on the heating mantle. The sample was heated about 14 hours at a heat rate of 150 drop/min. The solvent was evaporated by using vacuum condenser. The bottle was incubated at 90°C until solvent was completely evaporated and the bottle completely dried. The bottle was transferred to a desiccator with partially covered lid and allowed to cool. The bottle and its dried content were reweighed.

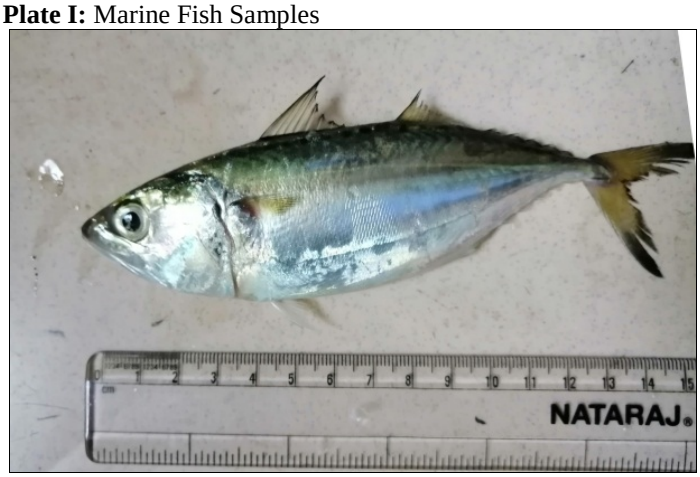
$$\text{Calculation: } \frac{\text{Weight of fat (g)}}{\text{Weight of sample (g)}} \times 100$$

Determination of available carbohydrate

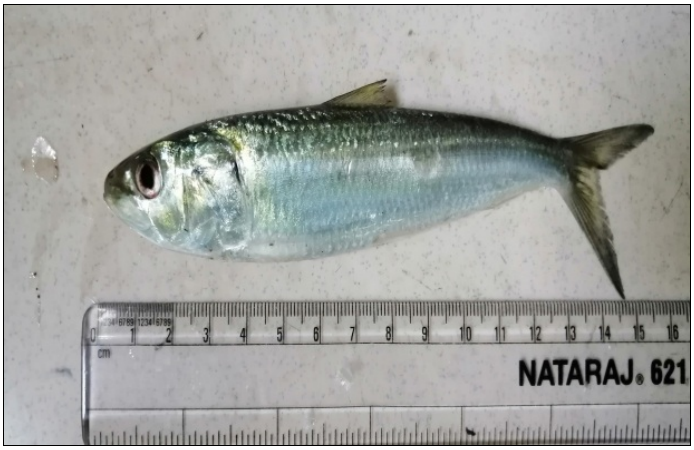
Having estimated all the other fractions by proximate analysis, the available carbohydrate content was obtained by difference (James, 1995).

Calculation: Available carbohydrates (%) = 100 - (Protein + Ash + Moisture + Fat)

Plate I: Marine Fish Samples



Rastrelliger kanagurta



Sardinella longiceps

Otolithes ruber

Nemipterus japonicus

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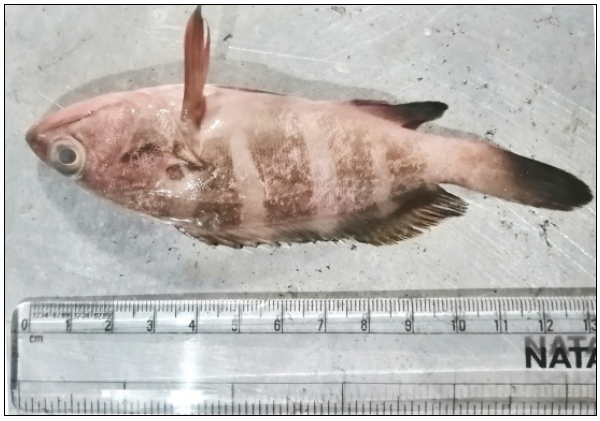
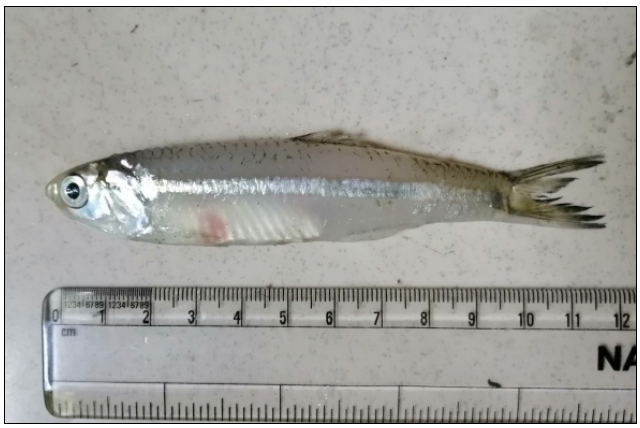
Cynoglossus arel



Epinephelus diacanthus

Gerres filamentosus



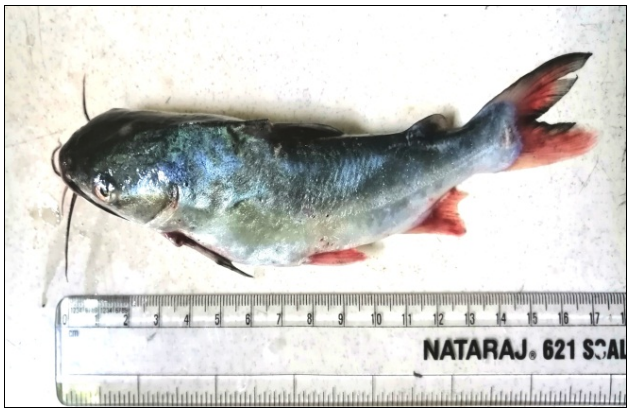
Atule mate Stolephorus indicus

Ambassis natalensis

Plate II: Estuarine fish samples

Mugil cephalus

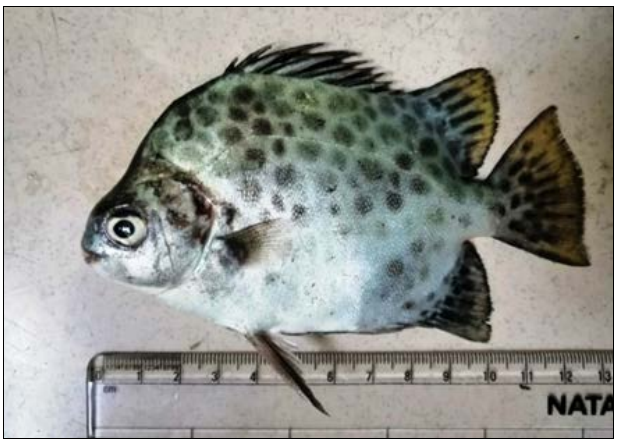
Xenentodon cancila



Arius arius

Onigocia pedimaculata

Leiognathus equulus



Scatophagus argus

Etroplus suratensis

Lutjanus argentimaculatus

Hemiramphus xanthopterus

Sphyraena obtusata

Plate III: Fresh Water Fishes

Puntius filamentosus

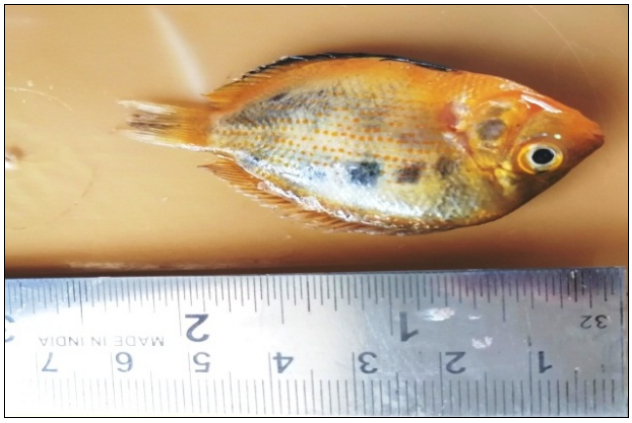
Channa striata

Horabagrus brachysoma

Puntius sarana

Bunaka gyrinoides

Nands nandus



Etroplus maculatus

Anabas testudiensis

Heteropneustes fossilis

Mystus montanus

Results and Discussion

Fish is a highly nutritious food consumed by the people where larger percentage do eat because of its availability, flavor, palatability while few percentages do so because of its nutritional value. The present study elucidated the level of moisture, protein, fat, ash and carbohydrate showed variation among the thirty fish species from three different ecosystems of marine estuarine and fresh water. Moisture content and protein can be considered as the major constituent in proximate composition because, the moisture content in this study was within the range of about 70% to 82% and the protein content among the fish samples varied widely within the range of 11% - 20%. The fat content of fish samples ranged between1% - 10% which shown significant variation among the fishes and they have been known to contain polyunsaturated fatty acids. The percentage ash content (1.5% - 5.5%) in the fishes analyzed is an indication of ample mineral content of fishes. Carbohydrates was present only in minute quantities and within the range of 0- 0.65%. Here the proximate composition of individual fish sample from each ecosystem was examined and the result as follows:

Marine Fishes: The results of proximate composition of ten marine fish samples namely *Rastrelliger kanagurta*, *Sardinella longiceps*, *Otolithes ruber*, *Nemipterus japonicus*, *Cynoglossus arel*, *Epinephelus diacanthus*, *Gerres filementosus*, *Atule mate*, *Stolephorus indicus* and *Ambrassis natalensis* are represented in Table 1.

Table 2: Proximate composition of Marine fish samples

Fish species	Moisture content (%)	Protein (%)	Fat (%)	Ash content (%)	Carbohydrate (%)
1. Rastrelliger kanagurta	70.23	17.55	9.06	2.98	0.18
2. Sardinella longiceps	70.73	18.69	8.02	2.31	0.25
3. Otolithes ruber	77.89	13.66	3.13	5.18	0.14
4. Nemipterus japonicus	78.21	13.61	3.68	4.31	0.19
5. Cynoglossus arel	80.96	11.67	2.61	4.70	0.06
6.Epinephelus diacanthus	77.94	14.03	3.87	4.05	0.11
7. Gerres filementosus	73.44	17.35	5.72	3.31	0.18
8. Atule mate	75.48	16.47	6.04	1.92	0.09
9. Stolephorus indicus	81.60	13.42	1.84	2.61	0.53
10. Ambassis natalensis	79.52	13.57	2.94	3.34	0.63

Fig. 1 represents the percentage of moisture content in selected marine fishes, which shows variation from 70% to 81% of the total weight. *S. indicus* had the highest water content of 81.6% and the least water content was recorded in *R. kanagurta*, which was 70.27%. *S. longiceps* also shows least moisture content of 70.73%. *E. diacanthus* (77.94%) and *O. ruber* (77.89%) were having almost similar moisture content. 80.96% were found in *C. arel*, it was close to the value of *S. indicus*.

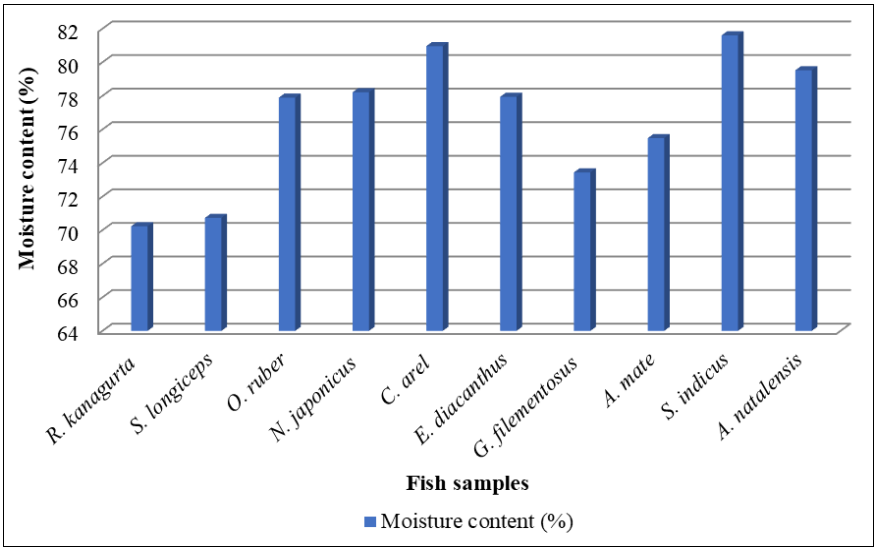


Fig 1: Moisture content of marine fish samples

In Fig. 2 Protein content in selected marine fishes shows variation from 11.67% to 18.69%. *S. longiceps* were found to be high in protein and the least protein content was observed in *C. arel*. *R. kanagurta* and *G. filementosus* had high percentage of protein and it is 17.55% and 17.35% respectively. *O. ruber* and *N. japonicus* were found to be almost similar in their protein content of 13.6%

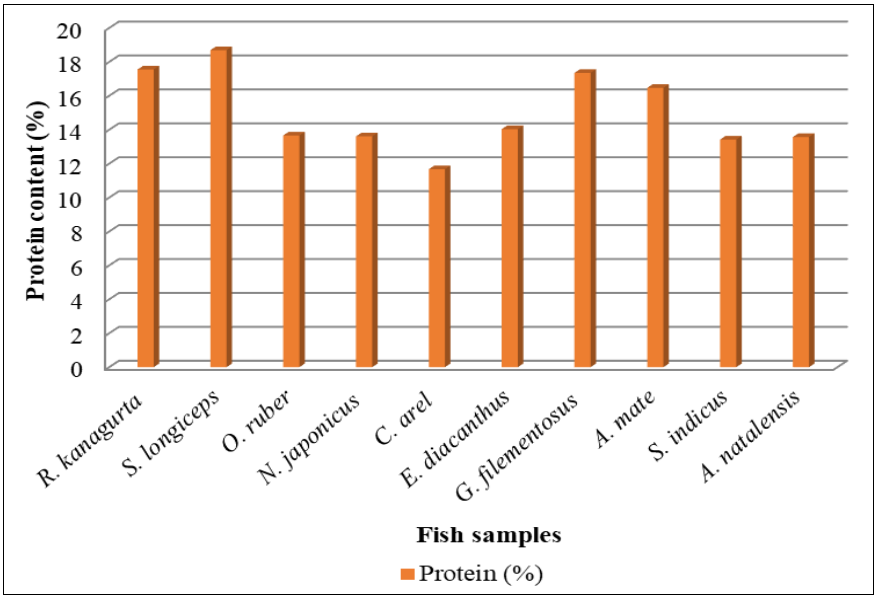


Fig 2: Protein content of marine fish samples

Fat content of selected marine fishes had been represented in Fig. 3, which shows significant variation from 1.84% to 9.06%. High fat content of 9.06% was recorded in *R. kanagurta* while the least value was recorded in *S. indicus.*Similar to mackerel *S. longiceps* also had higher fat content of 8.02% and both are considered as oily fishes. *C. arel*, *A. natalensis*, *O. ruber*, *N. japonicus* and *E. diacanthus* are the other fishes with comparatively low fat content which are 2.61%, 2.94%, 3.13%, 3.68% and 3.87% respectively.

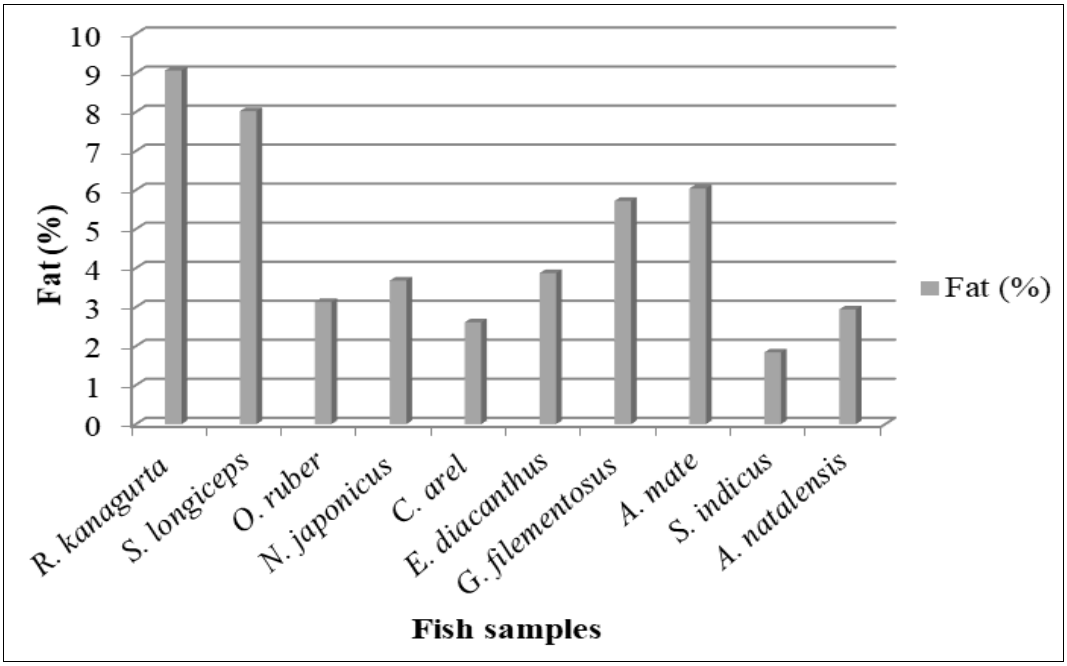


Fig 3: Fat content of marine fish samples

In Fig. 4, Percentage of ash content in marine fish samples vary between1.92 to5.18. High percentage of ash content 5.18 was recorded in *O. ruber*. *C. arel* (4.70%) and *E. diacanthus* (4.05%) also had comparatively high ash content. The least percentage of 1.92 was recorded in *A. mate*. *S. longiceps* (2.31%) were the other fish with less ash.

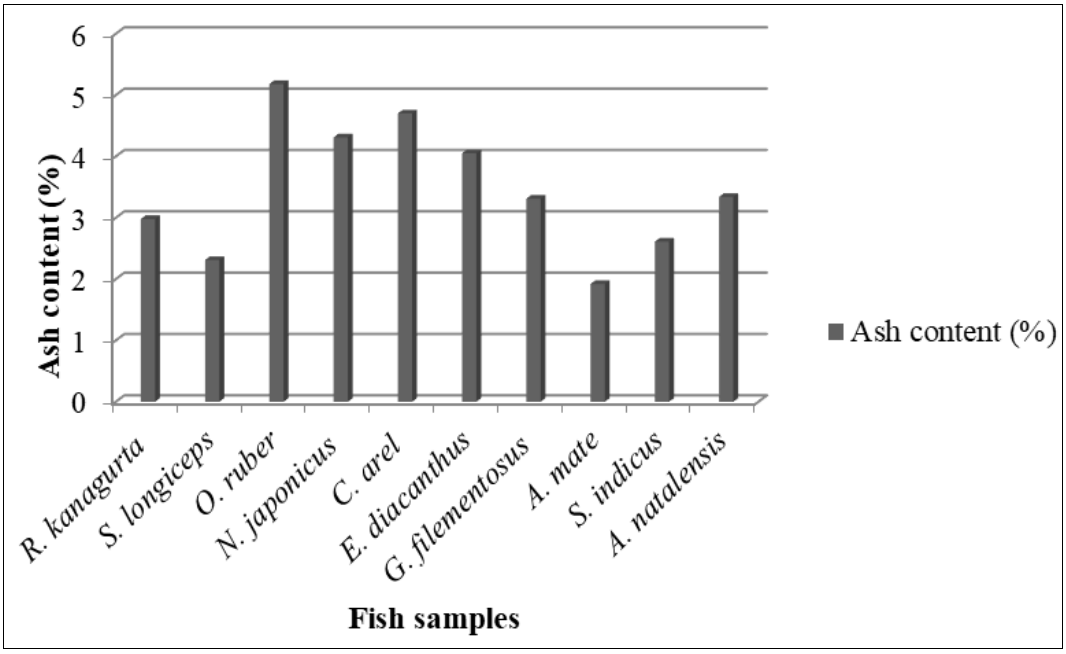


Fig 4: Ash content of marine fish samples

Percentage of carbohydrate had been represented in Fig. 5, which depicts that carbohydrate was present only in trace amount and which shows variation from 0.06% to 0.63%. *A. natalensis* had the highest level of 0.63% and *S. indicus* (0.53%) also had comparatively higher value. *R. kanagurta* and *G. filamentosus* were had the similar percentage of carbohydrate (0.18%). The lowest value was recorded in *C.s arel* (0.06). *A. mate* (0.09%) and *E. diacanthus* (0.11%) were the other two fishes with the least carbohydrate content.

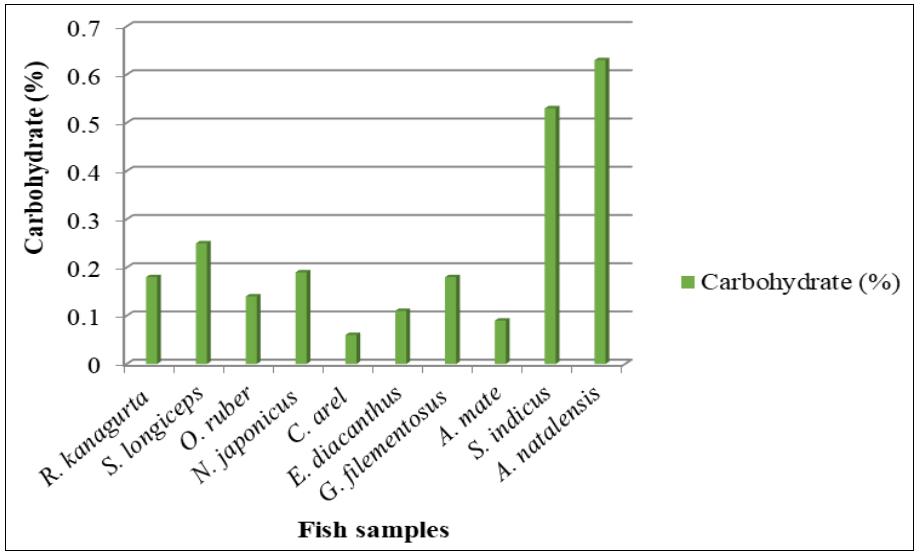


Fig 5: Carbohydrate content of marine fish samples

Estuarine fishes

Proximate composition of 10 estuarine fishes samples namely *Mugil cephalus, Xenentodon cancila, Arius arius, Onigocia pedimaculata, Leiognathus equulus, Scatophagus argus, Etroplus suratensis, Lutjanus argentimaculatus, Hemiramphus xanthopterus* and *Sphyraena obtusata* are represented in Table 1.

Table 3: Proximate composition of estuarine fish samples

Fish species	Moisture content (%)	Protein (%)	Fat (%)	Ash content (%)	Carbohydrate (%)
1. Mugil cephalus	71.68	18.73	7.88	1.6	0.11
2. Xenentodon cancila	78.68	15.21	2.34	3.38	0.39
3. Arius arius	71.50	18.54	8.03	1.91	0.02
4. Onigocia pedimaculata	80.78	11.59	2.41	4.73	0.49
5. Leiognathus equulus	73.25	17.82	3.95	4.86	0.12
6. Scatophagus argus	73.39	16.18	7.26	3.17	0.00
7. Etroplus suratensis	72.89	19.15	3.78	4.13	0.05
8. Lutjanus argentimaculatus	75.63	17.12	5.01	2.23	0.01
9. Hemiramphus xanthopterus	81.20	14.45	1.98	2.01	0.36
10. Sphyraena obtusata	80.86	13.56	1.9	3.06	0.62

The ten selected estuarine fish species had moisture content within the range of 71.50 to 81.20 percentage of total body weight, which is elucidated in Fig.6. *H. xanthopterus* recorded the highest moisture content of 81.20% followed by *S. obtusata*, *O. pedimaculata* and *X. cancila* with percentages of 80.86, 80.78 and 78.68 respectively. The least moisture content was observed in *A. arius* (71.50). *M. cephalus* (71.68%) also had low moisture content.

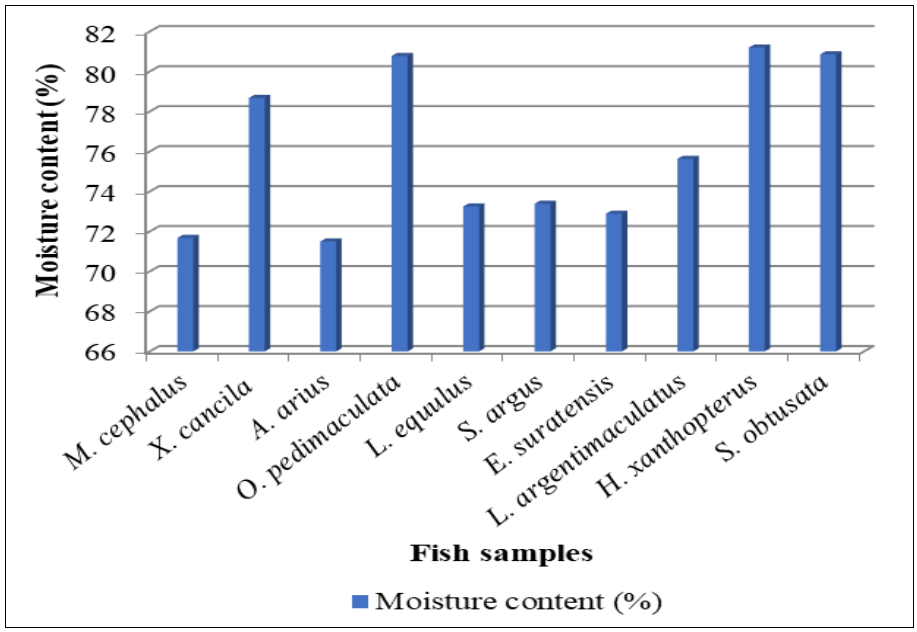


Fig 6: moisture content of estuarine fish samples

Fig. 7 depicts the protein content of estuarine fishes, which vary from 11.59% to 19.15% of total body weight. The higher value was recorded in *E. suratensis* (19.15%) and the least value in *O. pedimaculata* (11.59%). *M. cephalus* and *A. arius* were had almost closest protein content of 18.73% and 18.54% for each. *S. obtusata* (13.56%) was the other fish with low protein content

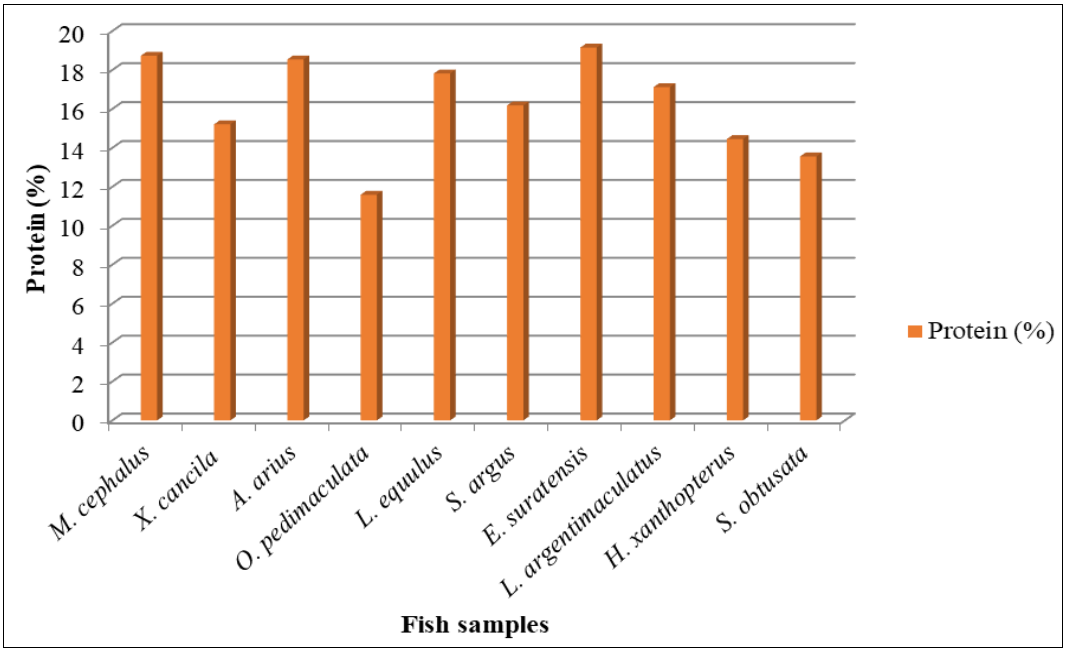


Fig 7: Protein content of estuarine fish samples

Fig. 8 elucidates the fat content of estuarine fishes in this study which are within the range of 1.90% to 8.03% of total body weight. *S. obtusata* had the lowest fat level at 1.90% while the highest was *A. arius* at 8.03%. Moderately high fat content of 7.88% was observed in *M. cephalus* similarly *S. argus* also had fat level at 7.26%., *H. xanthopterus*(1.98%) was the other fish with least fat content, which is close to value of *S. obtusata*. *X. cancila* and *O. pedimaculata* also had low percentage of fat at 2.34 and 2.41 respectively.

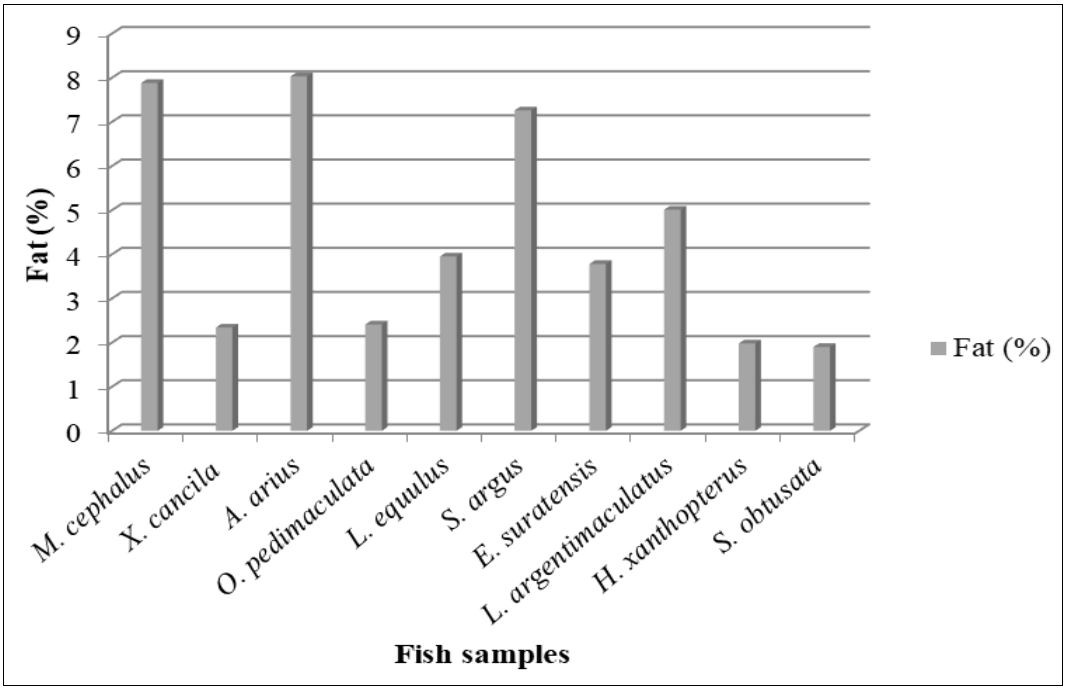


Fig 8: Fat content of estuarine fish samples

The ash content of estuarine fish samples is presented in Fig. 9 below, which shown variation from 1.6% to 4.86%. The highest value was recorded in *L. equulus* and the lowest in *M. cephalus*. *L. equulus* and *O. pedimaculata* had only slight variation in their ash level at 4.86% and 4.73% respectively. *A. arius* and *H. xanthopterus* also had low ash content at 1.91% and 2.01%.

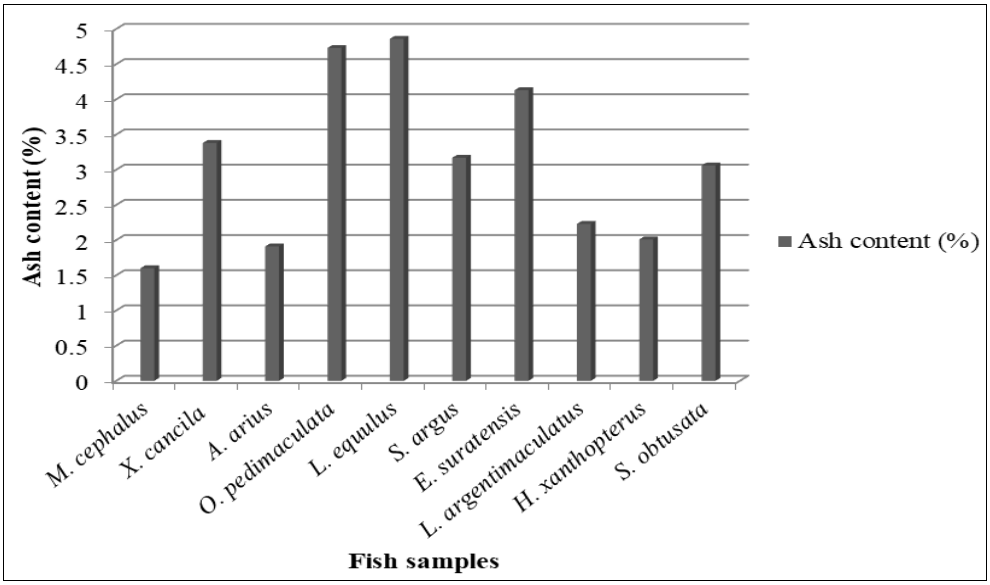


Fig 9: Ash content of estuarine fish samples

Fig. 10 depicts the percentage of carbohydrate in estuarine fish samples, which vary within the range of 0 to o.62. The highest value was estimated in *S. obtusata* (0.62%) followed by *O. pedimaculata* (0.49%), *X. cancila* (0.39%) and *H. xanthopterus* (0.36%), *S. argus* didn't had any carbohydrate in its proximate analysis and *L. equulus* (0.01%) and *A. arius* (0.02%)were the other fishes with minimum carbohydrate level.

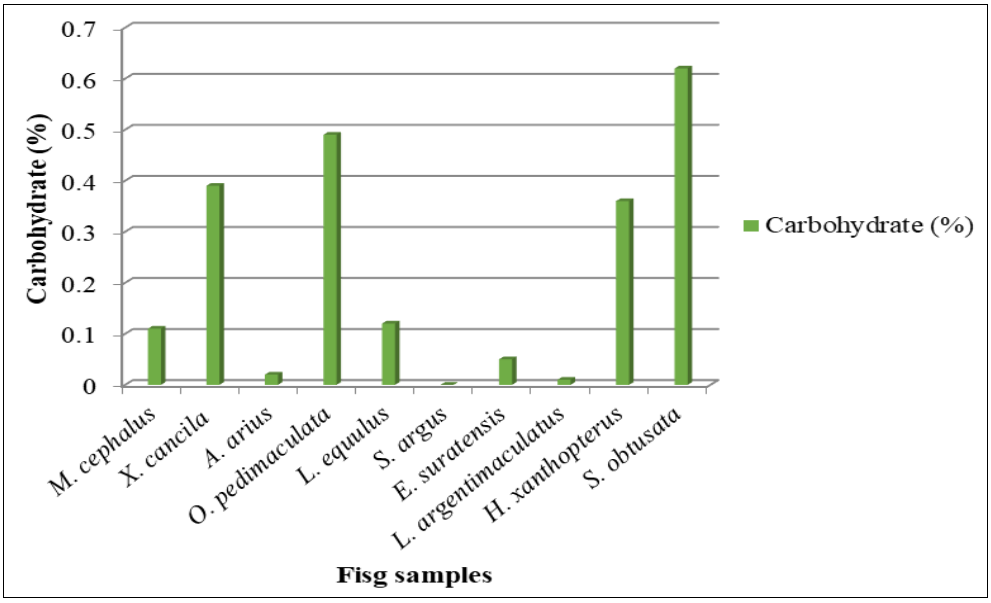


Fig 10: Carbohydrate content of estuarine fish samples

Freshwater Fishes

The proximate composition of ten fresh water fish samples namely *Puntius filamentosus, Channa striata, Horabagrus brachysoma, Puntius sarana, Bunaka gyrinoides, Nandus nandus, Etroplus maculatus, Anabas scandens, Heteropneustes fossilis* and *Mystus montanus* are given in Table 1.

Table 4: Proximate composition of fresh water fish samples

Fish species	Moisture content (%)	Protein (%)	Fat (%)	Ash content (%)	Carbohydrate (%)
1. Puntius filamentosus	72.63	16.94	6.98	3.22	0.23
2. Channa striata	74.31	18.35	3.94	3.28	0.12
3. Horabagrus brachysoma	71.17	16.31	9.94	2.51	0.07
4. Puntius sarana	72.59	15.77	7.62	3.84	0.18
5. Bunaka gyrinoides	78.78	13.29	2.97	4.45	0.51
6. Nandus nandus	76.27	14.71	3.96	4.81	0.25
7. Etroplus maculatus	72.28	17.66	5.62	4.43	0.01
8. Anabas scandens	73.98	16.22	6.38	2.83	0.59
9. Heteropneustes fossilis	80.43	13.27	2.35	3.56	0.39
10. Mystus montanus	77.89	14.81	4.96	2.07	0.27

The moisture content of fresh water fish samples within the range of 71.17% to 80.43% are represented in Fig. 11. The highest value of 80.43 was observed in *H. fossilis* and the lowest (71.17%) in *H. brachysoma*. *P. filamentosus* (72.63%), *P. sarana* (72.59%) and *E. maculatus* (72.28%) had almost closest moisture content.78.78% was observed in *B. gyrinoides.*

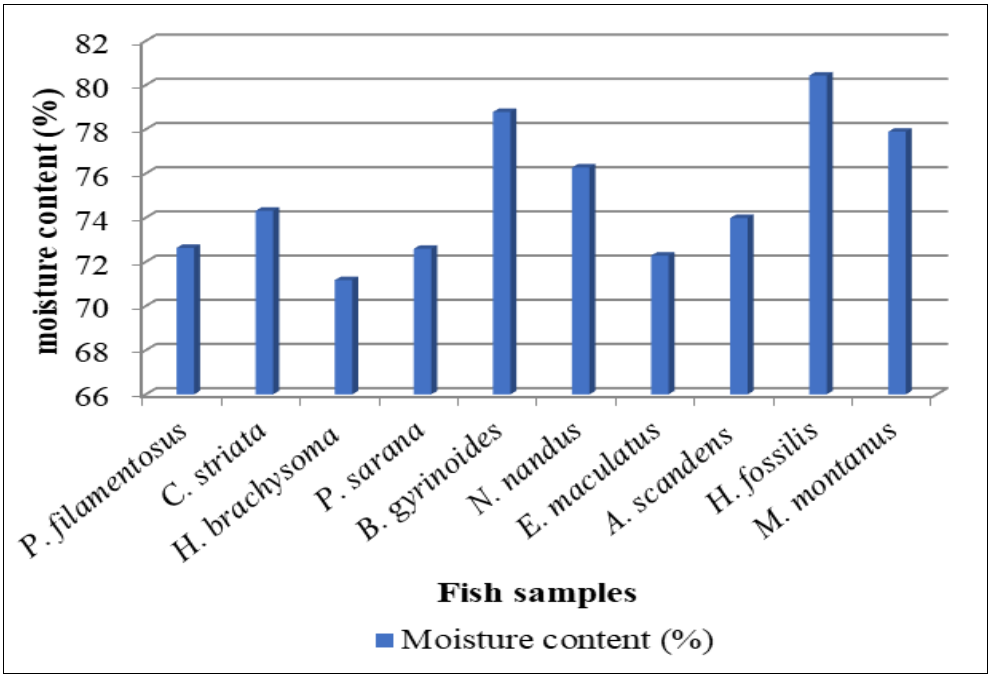


Fig 11: Moisture content of freshwater fishes

Fig. 12 represents the percentage of protein in fresh water fish samples, which vary from 13.27% to 18.35%. The lowest protein content was recorded in *H. fossilis* (13.27%) and *B. gyrinoides* (13.29%), they had slight variation about 0.02%. The highest value was recorded in *C. striata* (18.35%) followed by *E. maculatus* (17.66%) and *P.* filamentosus (16.94%)

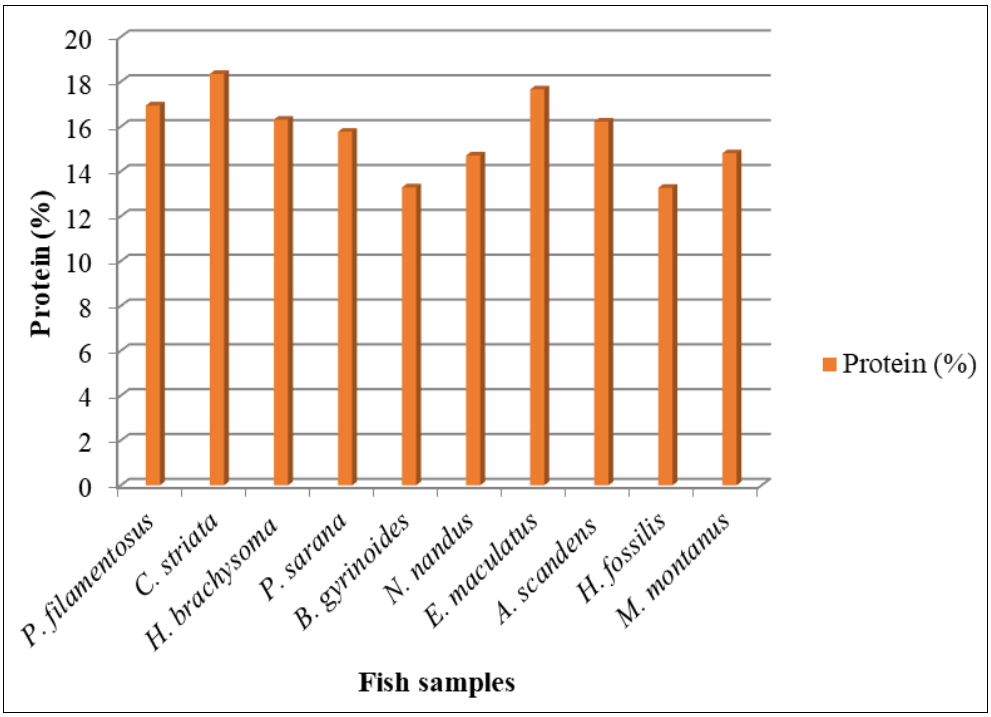


Fig 12: Protein content of freshwater fish samples

Fat content of fresh water fish samples are elucidated in Fig. 13, which shows significant variation from 2.35% to 9.94%. The highest fat content was estimated in *H. brachysoma* (9.94%) followed by *P. sarana* (7.62%). The lowest value was observed in *H. fossilis* (2.35%) and *B. gyrinoides* (2.97%) also had low fat level. *C. striata* (3.94%) and *N. nandus* (3.96%) had closest fat content in their proximate analysis.

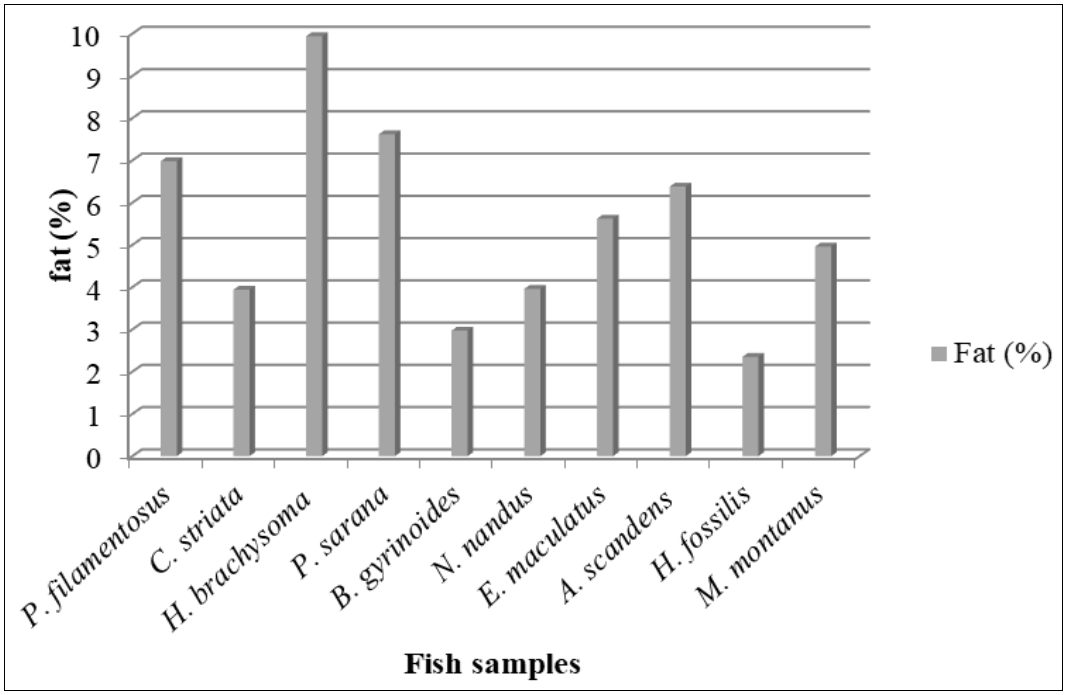


Fig 13: Fat content of freshwater fish samples

In Fig. 14 the ash content of fresh water fish samples within the range of 2.07% to 4.81 are represented. *N. nandus* (4.81%) were found to be high in ash content while the lowest value was recorded in *M. montanus* (2.07%). *B. gyrinoides* (4.45%) and *E. maculatus* (4.43%) had only a slight variation in their ash level. *P. filamentosus* (3.22%) and *C. striata* (3.28%) also had almost closest ash content.

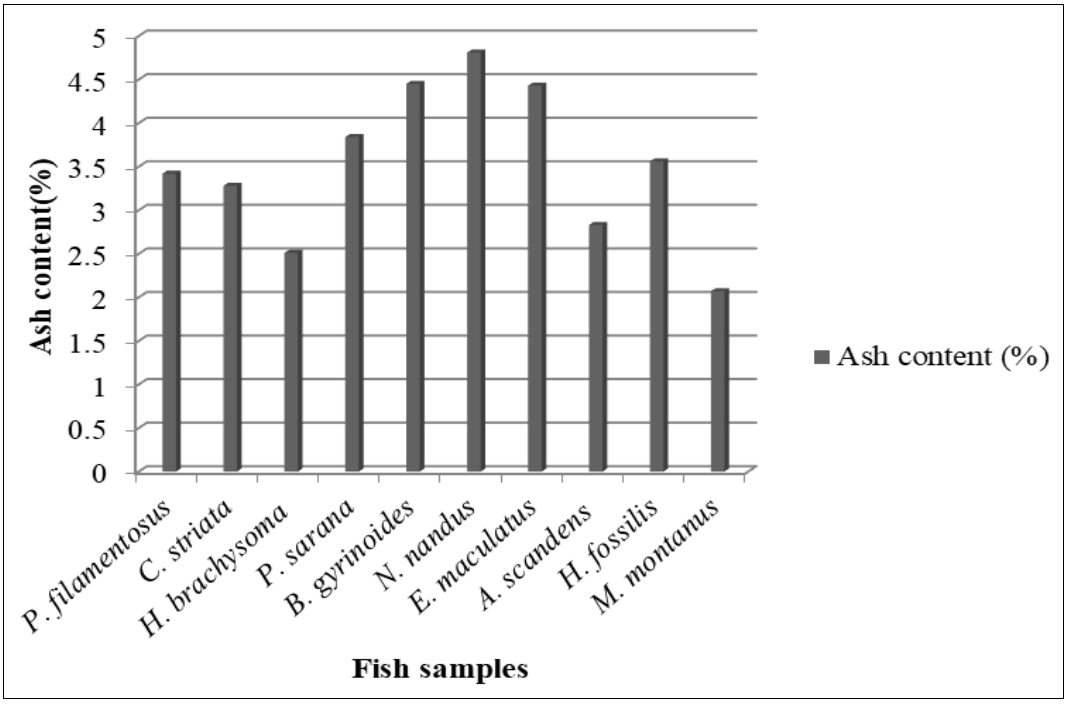


Fig 14: Ash content of freshwater fish samples

Carbohydrate content of fresh water fish samples are depicted in Fig. 15, which vary within the range of 0.01%to 0.59%. The least value was estimated in *E. maculatus* (0.01%) followed by, *H. brachysoma* (0.07%). The highest value was estimated in *A. scandens* (0.59%). *B. gyrinoides* (0.51%) also had comparatively high carbohydrate content

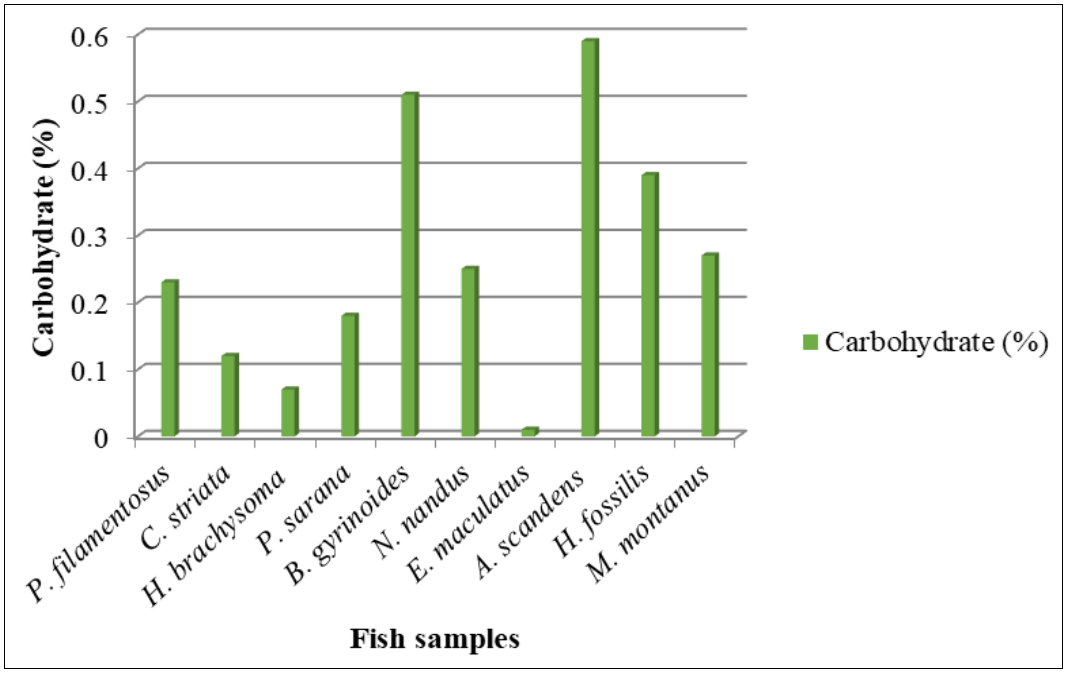


Fig 15: carbohydrate content of freshwater fish samples

In the present study, among the thirty fish samples from three different ecosystems S. *indicus* had the maximum moisture content (81.60%) while the minimum moisture was in *R. kanagurta* (70.23%) and both samples are belonging to marine fishes. Highest protein content was recorded in *E. suratensis* (19.15%) and the lowest was in *O. peimaculata* (11.59%), both are estuarine fishes. Maximum fat content was in *H. brachysoma* (9.94%), which is a fresh water fish and the marine fish *S. indicus* (1.84%) had the minimum fat. When the ash content was analyzed the higher value observed in marine fish *O. ruber* (5.18%) while the least value in *M.cephalus* (1.60%), which is an estuarine fish. In this proximate analysis Carbohydrate content was estimated only in minute quantities, the maximum value of 0.63% was recorded in a marine fish *A. natalensis*. *S. argus*, an estuarine fish was observed with no ash content.

Conclusion

Fishes are the good sources of protein, providing an important complement to the predominantly carbohydrate-based staple diet of many people in Kerala. The study of proximate composition of thirty fish species from marine, estuarine and fresh water ecosystem showed that they are rich in protein and average to high lipid contents. The present investigation revealed that these fish species are good sources of minerals. The proximate composition of commonly available fish species from three different ecosystems were studied to assess their nutritional value in order to achieve the knowledge of the risk and benefits associated with the indiscriminate consumption of these species. Researches in biochemical composition of fish are useful in several ways. Now a day there is an ever-increasing awareness about healthy food and fish is finding more acceptances because of its special nutritional qualities. Proper understanding of biochemical constituents of fish has become a prime need for the nutritionists and dieticians. The measurement of these proximate profiles is often necessary to ensure that they meet the requirement of food.

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