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Biochemical Analysis of Lipids and Proteins in three Freshwater Teleosts (*Clarias batrachus*, *Channa punctatus*, *Anabas testudineus*)

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Abstract

The study deals with the separation and estimation of protein and lipids in different tissues like liver, pancreases, gills and muscles of Clarias batrachus, Channa punctatus and Anabas testudineus. The protein and lipid contents were measured in the three species irrespective of their sex and sizes. The annual mean of protein content in liver was found to be 13.92± 2.326, 11.87 ± 0.811, 10.25± 0.52 and in pancreases 11.4±10.794 ,10.63 ± 0.494, 8.85± 0.67 gms per 100 grams of tissues in Clarias batrachus, Channa punctatus and Anabas testudineus respectively. It was also observed that the proximate composition of fish depends mostly on season, size, age, sex, reproductive cycle as well as breeding season. Similarly, the mean protein in muscle and gills was estimated to be 19.49 ± 0.732, 17.50 ± 0.865, 18.22 ± 0.931 and 6.079± 2.472, 14.779± 1.084, 7.80 ± 0.596 respectively among the above species. Lipids content was more in liver and muscles. The mean lipids of liver and muscle were 1.08 ± 0.460 , 1.10 ± 0.118, 0.88 ± 0.462 and 1.46 ± 0.262,1.44± 0.39 , 1.19 ± 0.27 respectively among the three species. Comparatively less quantity of lipids are estimated in the pancreases and gills. The mean lipids content of pancreases and gills was found to be 0.789 ± 0.371 ,1.23 ± 0.484 , 1.06 ± 0.298 and 0.81 ± 0.109 , 0.67 ± 0.192 , 1.04 ± 0.328 respectively. All biochemical parameters differ significantly (P<0.001) among the three species under investigation.

Keywords: Proteins, Lipids, Liver, Pancreas, Gills, Muscles, Clarias *batrachus*, *Channa punctatus, Anabas testudineus*.

Introduction

The chemical composition of protein and lipids is traditionally used as an indicator of the nutritional value as well as the physiological condition of fish and its habitat[1-2]. The importance of fish as source of high quality balanced and easily digestible protein, vitamins and polysaturated fatty acids is well understood[3]. Fishes are the valuable sources of high grade protein and other organic products. They are the most important source of animal protein and have been widely accepted as a good source of protein and other elements for the maintenance of healthy body[4].

Fish is a high protein food consumed by the large percentage of populace because of its high palatability, low cholesterol and tender flesh. It is the cheapest source of animal protein and other essential nutrients required in the human diet particularly in the low and middle income groups. The nature and quality of nutrients in most animals depend largely on their food type. Moreover, the feeding habit of an individual fish species greatly affects the nutritional composition of its flesh. Approximately 85-90% fish protein is digestible and all the dietary essential amino acids is found in the fish flesh. The measurement of some proximate composition including protein and fats content is often necessary to ensure the food regulatory requirements. Fish is also an important source of mineral elements required in human diets[5]. *Clarias batrachus*, *Channa punctatus* and *Anabas testudineus* are carnivorous air–breathing species, which are encountered in a rivers, ponds, pools, ditches, rice fields etc. across South Asia. It is locally known as "*Magur*", "*Gadisha*" and "*Kau*" respectively and is tropical fishes. These are widely used for traditional medicinal purposes[6]. Growth, development, biochemical and physiological state of an organism are key factors in determining species, sustainability, survivability and availability. Effect of pollutants at a higher level such as pollution, community and ecological systems are usually preceded by changes in earlier biological processes viz molecular and cellular events, which includes lipid peroxidation, antioxidant status, mixed function oxidase system and a more general physiological index such as growth[7-8] and leads to variation in proximate composition in tissues.

Aim and Objective of Present Study: The aim of the Present study was to determine the relative composition of Protein and Lipids in three different fishes.

To detect the Proximate composition of Protein and Lipids in the liver, muscle, pancreases and gills. To establish the biochemical relationship among the three different species of

fish if any. To evaluate the mean composition of Protein and Lipids in these fishes.

Materials and Methods

Collection of samples: Three specimens of *Clarias batrachus, Channa punctatus* and *Anabas testudineus* were collected from nearby Berhampur University campus rearing pond. They were treated with 5% $KmnO_4$ for five minutes for dormant disinfection. The current research had been carried out from Nov 2014-Oct 2015. The fish weighing 35-90 gms were selected for *Clarias Batrachus*, 35-150 gms for *Channa Punctatus* and 50-95 gms for *Anabas testudineus* were maintained at constant temperature of 27 ± 1°C under laboratory conditions for acclimation .

Separation of required organs: Fish sample in fresh was gutted, washed, filtered, finely minced and homogenized for chemical analysis. During each month, at the time of experiment, three different healthy species having different size and weight group were selected for the study. All the samples were analyzed in triplicates. The variation of proximate fraction of different ingredients may be due to the change in feeding behavior and to climatic changes during that season which influence the general biochemical composition of the three different fishes.

Biochemical Estimation: Protein was estimated following the method of Lowry *et al.*, [9]. Freshly weighed (100 mg) tissues of concerned organ were homogenized with 5% trichloroacetic acid in a homogenizer. The homogenate was centrifuged at 3000rpm for 10 minutes and the residue was dissolved in 0.1N NaOH. Exactly 0.2ml of this solution was made up to 1ml using 0.1N NaOH. To this, 3.5ml of Folin's reagent was added and was thoroughly mixed. After 30 minutes of rest the optical density was measured at 670nm in a Spectronic 20 spectrophotometer.

Lipids were extracted following the methods of Folch *et al.* , and its quantity was estimated by the Barnes and Blackstock[10-11].

Statistical Analysis: For the experimental parameters, the data obtained were statistically analyzed by using mean ± S.D (Standard Deviation). One way ANOVA test was used to derive significant difference between means (SPSS17).

Results and Discussion

The present investigation deals with proximate composition and seasonal variations in protein and lipid values in liver, muscle, pancreases and gills of *Clarias batrachus*, *Channa punctatus* and *Anabas testudineus*.

These result showed that a good quantity of protein is present in three different species just like the commercial fishes, so they can be safely used in food to supplement of required protein.

This result interpreted that proximate composition of protein and lipid in fish depends on season, sex and reproductive cycle (Table-1 and Figure-2 and 3).

Clarias batrachus: The proximate composition of fish protein and lipids of four different organs like liver, pancreases, gills and muscles are presented in Table-1. The proteins contents was measured to be the highest in muscles i.e 20.80gm/100 gm tissues in the month February and lowest values was observed in gills 6.38gm in June (Figure-1). Similarly the lipid contents is also highest in muscle (1.85 in November) and lowest in pancreases (0.21 in March) (Figure-2). Protein contains normally raised from January to June in liver tissue, and then started decreasing. During spawning period/captive condition normally the protein and lipid content were found to be relatively constant with minor variability. Both protein and lipid level increased in November and start decreasing again in either June or July (Figure-1 and 2).

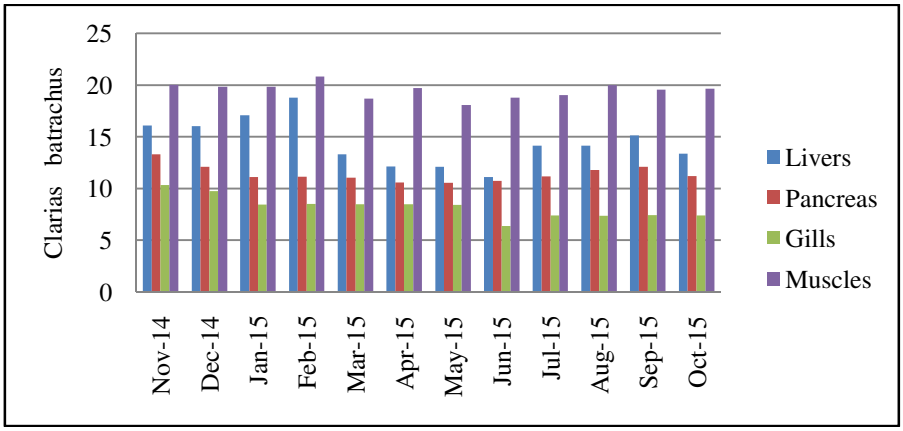

Figure-1
Monthly variable means of Proteins in livers, pancreas, gills and muscles in *C . batrachus*

Table-1
Monthly variation in Proximate composition of different tissue in *Clarias batrachus*

Months	Livers		Pancreas		Gills		Body tissues /muscles	
	Protein	Lipids	Protein	Lipids	Protein	Lipids	Protein	Lipids
Nov-2014	16.08	1.35	13.30	1.21	10.33	0.93	20.02	1.85
Dec -14	16.03	1.29	12.10	1.13	9.75	0.91	19.83	1.60
Jan -15	17.07	1.83	11.11	1.41	8.43	0.87	19.84	1.63
Feb -15	18.81	1.07	11.13	0.37	8.49	0.85	20.80	1.57
Mar -15	13.31	0.62	11.06	0.21	8.46	0.81	18.67	1.53
April -15	12.15	0.68	10.58	0.54	8.47	0.88	19.70	1.03
May -15	12.11	0.09	10.55	0.38	8.40	0.76	18.08	1.09
June-15	11.10	0.90	10.72	0.90	6.38	0.67	18.81	1.21
July -15	14.13	1.21	11.18	0.93	7.41	0.52	19.03	1.35
Aug-15	14.14	1.07	11.79	0.59	7.37	0.83	20.01	1.30
Sept-15	15.13	1.53	12.10	0.97	7.44	0.81	19.55	1.56
Oct-2015	13.37	1.31	11.19	0.83	7.40	0.87	19.63	1.80
Total	167.06	12.95	136.81	9.47	72.95	9.71	233.97	17.52
Mean	13.92	1.08	11.40	0.78	6.07	0.80	19.49	1.46
± S.D	2.326	0.460	0.794	0.371	2.472	0.109	0.732	0.262

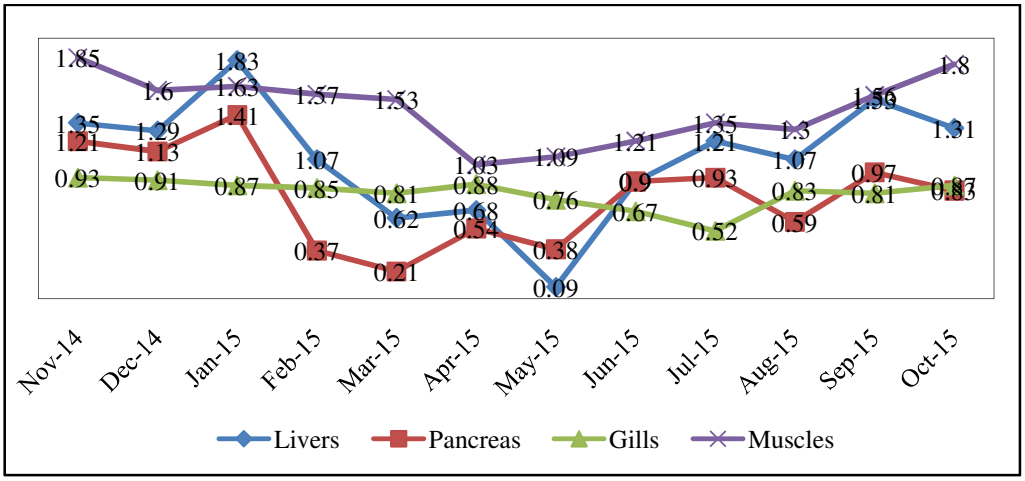

Figure-2
Monthly variable means of Lipids in livers, pancreas, gills and muscles in *C. batrchus*

Table-2
Monthly variation in Proximate composition of different tissue in *Channa punctatus*

Months	Livers		Pancreas		Gills		Body tissues /muscles	
	Protein	Lipids	Protein	Lipids	Protein	Lipids	Protein	Lipids
Nov-2014	11.07	1.07	11.15	1.83	16.03	0.79	17.03	1.83
Dec -14	12.03	1.09	10.17	1.89	16.09	0.75	18.11	1.70
Jan -15	12.98	1.31	11.31	0.78	15.81	0.69	17.86	1.70
Feb -15	12.93	1.12	10.43	0.95	13.83	0.83	18.80	1.81
Mar -15	11.80	1.21	11.49	0.59	14.80	0.47	16.55	1.05
April -15	10.53	1.11	11.03	0.83	14.63	0.83	16.40	0.94
May -15	10.93	0.95	10.49	0.96	13.05	0.85	16.03	0.81
June-15	11.37	0.97	10.43	0.87	13.08	0.39	17.65	1.81
July -15	11.32	0.99	9.93	1.33	14.19	0.63	17.64	1.80
Aug-15	12.58	1.31	10.18	1.21	14.03	0.54	17.39	1.16
Sept-15	12.36	1.05	10.80	1.58	15.08	0.37	18.24	1.11
Oct-2015	12.56	1.08	10.82	2.01	15.01	0.96	18.48	1.63
Total	142.46	13.26	127.6	14.83	177.35	8.1	210.06	17.35
Mean	11.87	1.10	10.63	1.23	14.77	0.67	17.50	1.44
± S.D	0.824	0.118	0.494	0.484	1.051	0.192	0.865	0.393

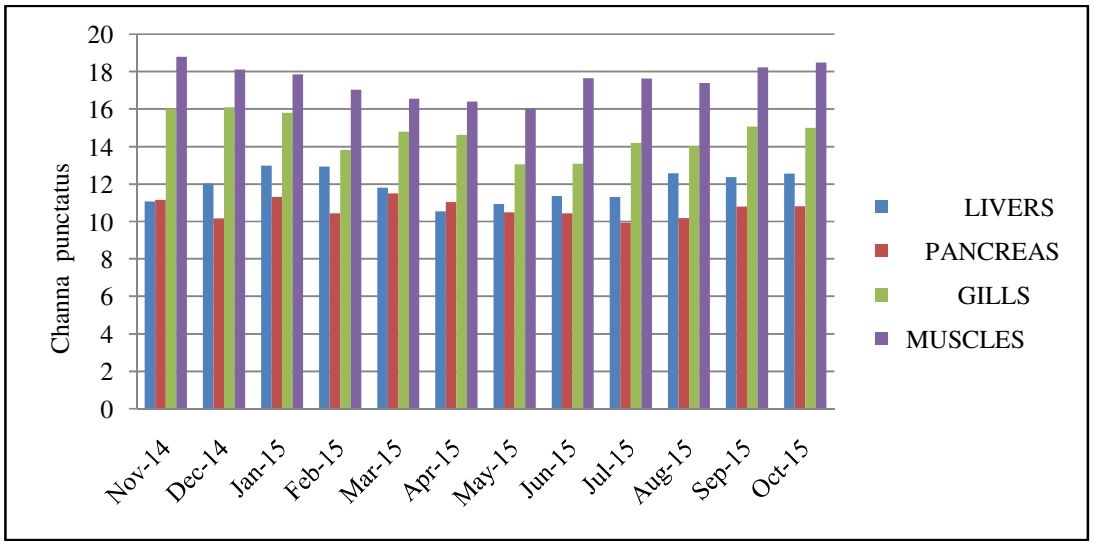

Figure-3
Monthly variable means of Proteins in livers, pancreas, gills and muscles in *C.punctatus*

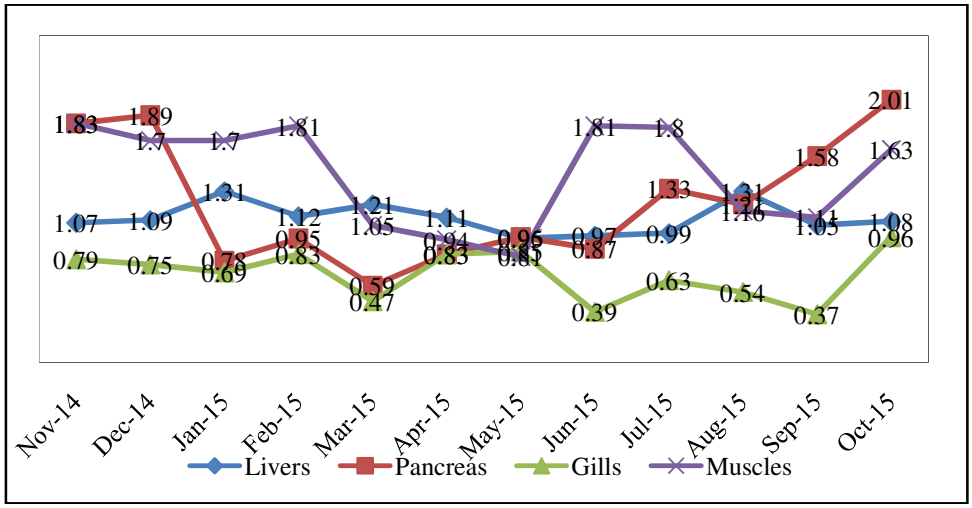

Figure-4
Monthly variable means of Lipids in livers, pancreas, gills and muscles in *C.punctatus*

Channa punctatus: The mean proximate of protein and lipid were high (18.80 and 1.83) in the November, in muscle tissues (Table-2, Figure-3). The protein content was increased from April to September and with a maximum (12.98) in January in liver tissues. The protein levels were mostly variable and start increased from May to December in both pancreas and gills. Similarly lipid contents were maximum during January and minimum in May in liver tissue i.e. in pre-spawning period/ preparatory phase. The lipids level was maximum in October and start decreasing towards March in the pancreas and gills (Table-2 , Figure-4).

Table-3
Monthly variation in Proximate composition of different tissue in *Anabas testudineus*

Months	Livers		Pancreas		Gills		Body tissues /muscles	
	Protein	Lipids	Protein	Lipids	Protein	Lipids	Protein	Lipids
Nov-2014	11.08	1.25	9.43	1.44	8.40	1.40	17.73	1.50
Dec -14	10.83	1.52	9.80	0.86	8.41	1.29	18.81	1.56
Jan -15	9.76	1.01	9.39	0.86	7.49	0.93	18.03	1.25
Feb -15	10.32	0.39	9.67	1.37	8.90	0.74	18.04	1.27
Mar -15	10.37	0.43	8.37	1.01	7.41	0.81	18.05	1.21
April -15	9.91	0.40	8.21	1.34	7.58	0.93	16.32	0.60
May -15	9.80	0.39	8.09	0.93	7.09	0.79	16.94	0.83
June-15	9.18	0.33	8.08	0.36	7.03	0.37	18.32	0.95
July -15	10.32	0.98	8.41	1.03	7.44	1.31	18.37	1.11
Aug-15	10.35	1.08	8.90	1.09	7.48	1.23	19.41	1.31
Sept-15	10.48	1.32	8.91	1.25	8.07	1.43	19.04	1.30
Oct-2015	10.67	1.46	9.03	1.27	8.33	1.30	19.63	1.37
Total	123.07	10.56	106.29	12.81	93.63	12.53	218.69	14.26
Mean	10.25	0.88	8.85	1.06	7.80	1.04	18.22	1.18
± S.D	0.520	0.462	0.670	0.298	0.596	0.328	0.931	0.275

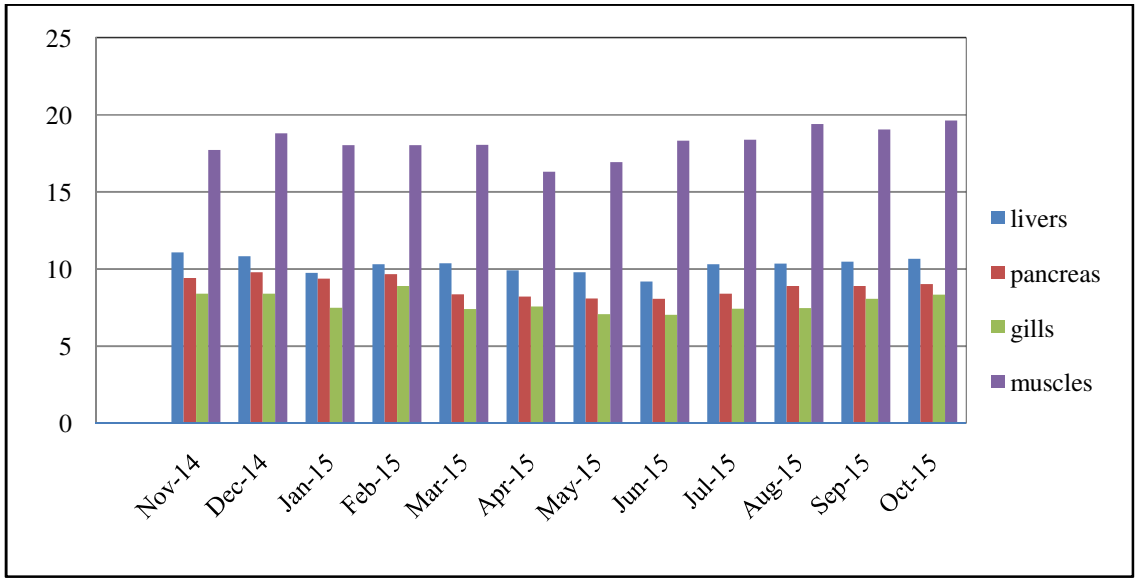

Figure-5
Monthly variable means of Proteins in livers, pancreas gills and muscles in *A.testudineus*

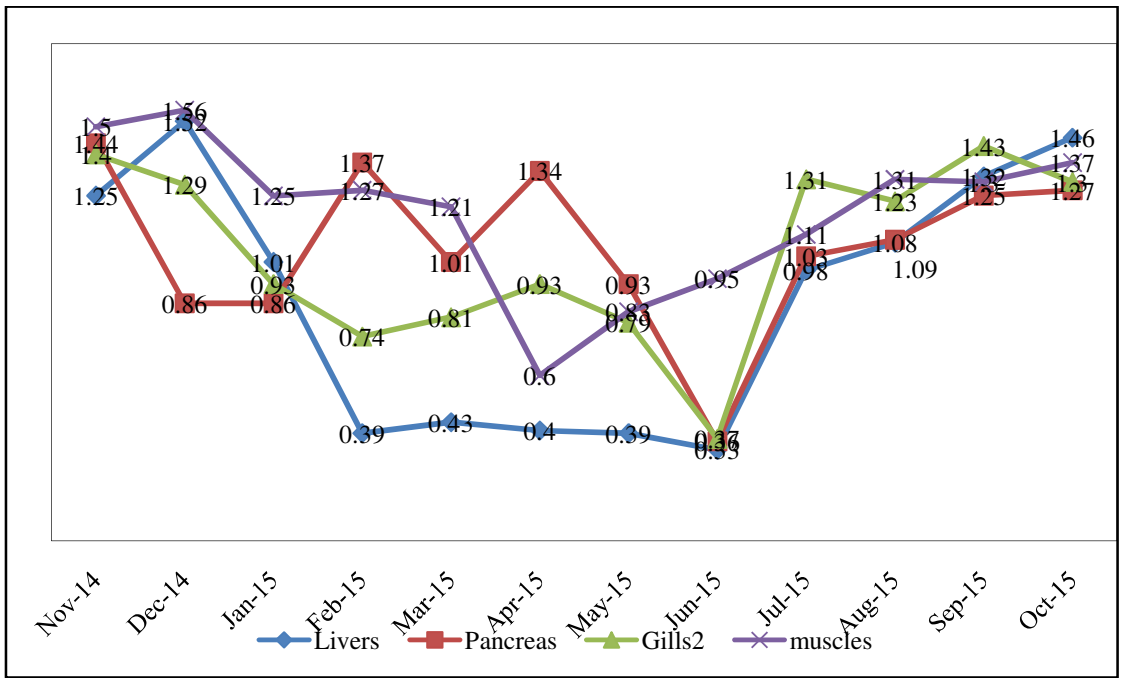

Figure-6
Monthly variable means of Lipids in livers, pancreas gills and muscles in *A.testudineus*

Anabas testudineus: Protein content in liver tissue was maximum in November (11.08) and minimum in June (9.18). In muscle tissue, it was maximum in October (19.63) and minimum in April (16.32). Protein content of pancreas was maximum in December (9.8) and minimum in May (8.09). Similarly the Protein values in gills were maximum in December (8.41) and then started decreasing thereafter (Table-3, Figure-5).

The lipid content was maximum in December in liver (1.52) and muscle (1.56) and then started decreasing slowly. Similarly the lipid level was maximum in November in pancreas (1.44) and then started decreasing. The lowest value was estimated in June (0.36). The lipid content was mostly variable with maximum in September (1.43) and minimum in June (0.37) (Table-3, Figure-6).

Table-4
Monthly variations in average means of Proximate composition of three different fishes

Months	**Liver**		**Pancreas**		**Gills**		**Body tissues /muscles**	
	Protein	Lipid	Protein	Lipid	Protein	Lipid	Protein	Lipid
C.batrachus	13.92	1.08	11.40	0.79	6.079	0.81	19.49	1.46
C.punctatus	11.87	1.105	10.63	1.23	14.779	0.675	17.50	1.445
A.testudineus	10.25	0.88	8.857	1.067	7.802	1.044	18.22	1.19

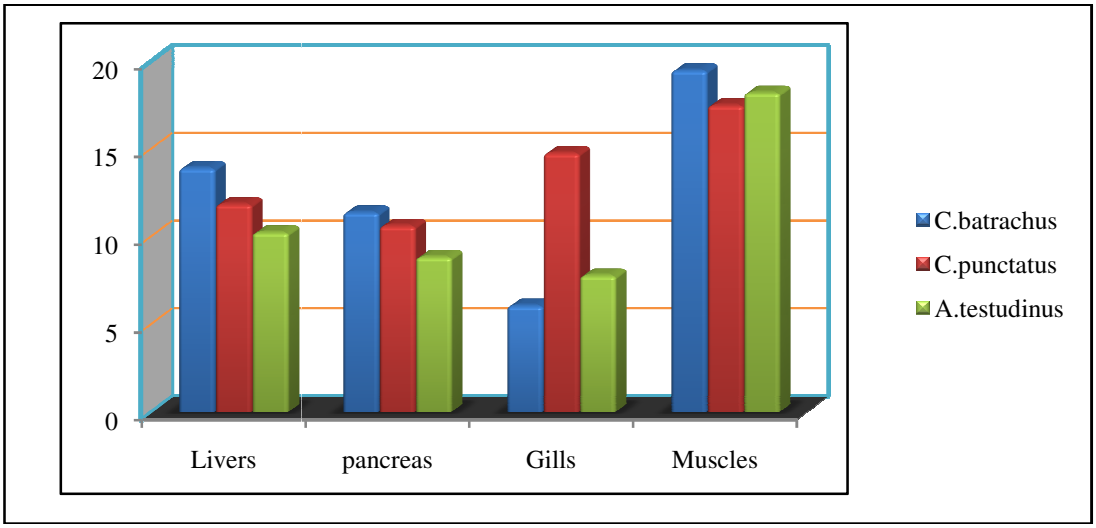

Figure-7
Mean composition of protein in three different species

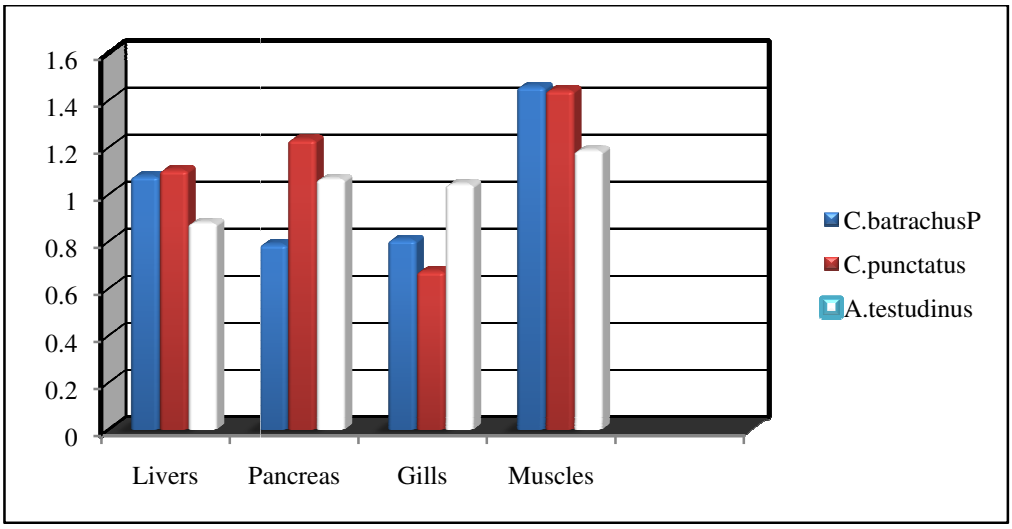

Figure-8
Mean composition of Lipids in three different species

The hepatic protein content of three different species such as *C. batrachus , C. punctatus* and *A. testudineus* were 13.92, 11.87, 10.25 and lipids 1.08, 1.105 and 0.88 respectively. The average mean proximate composition of pancreas proteins content of three different species are measured 11.4, 10.63, 8.857 and lipids 0.79, 1.23 and 1.067 respectively. The gills are the organ for breathing /gaseous exchange. It also contains a good amount of protein 6.079, 14.779, 7.802 and lipid 0.81, 0.675, 1.044 respectively (Table-4, Figure-7).

Similarly the muscles/ other body tissue contain more amounts of proteins and lipids than organs. The mean proximate composition of proteins of muscles were 19.49, 17.50, 18.22 and lipids 1.46, 1.445 and 1.19 respectively (Table-4). On comparative bases of proteins, showed increasing trends in organs of liver and pancreas of *A. testudineus, C. punctatus* and *C. batrachus* respectively (Figure-7). The *C .batrachus exhibits* maximum amount of protein in muscle as comparable to other two species. The levels of lipids is maximum in the muscles and minimum in the gills (Table-4, Figure-8).

Discussions: Fish liver is an excellent organ for the study of environmental quality biomarkers, due to its role in the animal metabolism, which include the production of protein, the oxidation, conjugation, methylation, inactivation or detoxification of substances, or rather, the excretion of pollutants was indicated by Fanta *et al.,*[12]. Protein is the most primary biochemical ingredient present in large quantity in the fish body. Ferrari *et al.,* reported that damaged muscle fibre, discontinued `H' and I band were observed in fish, *Hplias malabaricus* exposed to contamination of mercury, dark and light bands in muscles[13]. Liver is an important organ involved in metabolic processes and in the detoxification and xenobiotics , which was analyzed by Yang and Chen[14] .

Clarias batrachus: Caloric value of fish tissue protein depends on the seasonal dissolved oxygen content of aquatic ecosystem. The protein containt is 18-20% in the *C. batrachus* which is reported as Jindal and Jindal *et al.,*[15-16] . *C. batrachus* has increased the protein and lipid levels due to intake of the low cost feed and that the similar results are seen in Himadri *et al.*[17].

Channa punctatus: Biochemical composition of protein and lipid contents in tissue of liver, pancreases, gills and muscles are found to be high in natural condition. However protein and lipid content in various organs are affected from species to species and by sex, age, water temperature, degree of pollution, natural condition, seasonal variations etc was reported by Konar *et al.,* Gill *et al.,* and Dutta *et al.*[18-20]. The present study supports the above findings of protein and fatty acid quantity have similar as reported by Zden, Zuraini *et al.*[21,22]. According to Tripathy *et al.*, the increase in protein content of liver , pancreases, gills and muscles in normal condition to exposure of some chemical Impact[23]. The monthly mean proximate composition of protein and lipid content are similar observed by Singh *et al.*,[24]. The protein content of liver (1.31%), muscle (18.48%) and gill (16.09%) which was observed in the study in accordance with the authors by Tripathy *et al.* and Kawade *et al.*[25-26].

Anabas testudineus: Lipid content, an essential organic constituent of the tissues of all animals, and play a key role in energy metabolism. Lipids are the best energy producer of the body next to carbohydrates. The decreased level of tissue lipid content may be due to liver dysfunction or mobilization of glycerol or inhibition of oxidative phosphorylation and also agreed with Chezhian *et al.*[27] . Gilbert *et al.,* was reported that lipid are vital to embryogenesis, providing two third of energy by oxidation[28]. Lipid act as reversed depot of energy from where the energy is supplied as and when required. Cholesterol and lipid level were decreased in different organs of *Clarias batrachus* exposed to lead nitrate as experimented by Katti *et al.*[29].

Among all tissues, liver showed higher protein content which might be due to greater concentration in enzyme. Liver is the site of metabolism. The liver plays an important role in the synthesis of proteins. The continuous reduction of gill protein content was reported in *Anabas testudineus*. When exposed to sublethal concentration its quantity becomes variable, the same was reported by Osibona *et al.*[30].

Lipid was found more or less similar in all fishes under investigation. Lipid composition and distribution between and within the fish vary from species and are influenced by seasonal dietary variations ,and similar observation was recorded by Ackman, and Henderson *et al.,*[31 - 32]. The range of lipid content in edible part is approximately 0.5-18%. This depends on seasonal variations in feeding habits and regional differences in basic foods and nutrients. The lipid concentration in livers of *Clarias batrachus* was seen to increasing from January to June and then decreasing from July to December. The average lipids in liver are 1.079 ± 0.460. Klinger *et al.*, was also resported lower values of fat in the carnivorous fish, low fat content of muscle is recognized due to their carnivorous feeding nature. This results was similar to the result made by Klinger *et al.,* [33].

Conclusion

This investigation may be helpful in determining health status of these three fishes. The estimation of proteins and lipids will certainly detect early signs of clinical pathology with respect to their habitat. It also indicates the comparative values of two parameters like protein and fats of three different fishes. Correlation and pattern of biochemical changes in tissues like liver, pancreases, gills and muscles are found in accordance with the intrinsic and extrinsic factors.

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