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# Food and Feeding Habitat Analysis of various Fish Species Collected from River Sutlej, Punjab in relation to physico-chemical water quality parameters

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**Key words:** Microorganisms, river Sutlej, *Cirrhinus mrigala, Cyprinus carpio, Labeo rohita, Oreochromis niloticus.*

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## Abstract

A study of the food and feeding habits of fish is very important in any Fisheries research programme since feeding habitats are related with the digestive system of the fishes which differ from one species to another. In this study, food and feeding habits of selected fish species from River Sutlej has been described. The dissection and analysis of alimentary canal of various fish species samples was done for collection of different types, numbers and composition of microorganisms available in relation to the selected site of River Sutlej. The quality as well as quantity of microorganism species was analyzed with the help of Sedgwick-Rafter counting Chamber. The physico-chemical parameters of water were also analyzed and their impact was correlated with the feeding habits of fish species as well as occurrence of microorganisms found at sampling sites. The microorganisms were higher in number in gut contents of fish samples collected from River Sutlej as compared to a previous study conducted at River Ravi due to higher levels of contamination of water at the later site. Most of the microorganisms observed in gut contents of fish species were of the families Chlorophyceae, Myxophyceae and Bacillariophyceae. The results indicated that *Labeo rohita* and *Cirrhinus mrigala* were mainly herbivorous while *Cyprinus carpio* and *Oreochromis niloticus both* were found to be omnivorous.

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## Introduction

Food studies is the critical examination of food and its contexts with science and other fields. It is distinctive from other food related areas of study and tends to look beyond the mere consumption, production and aesthetic appreciation of food and tries to illuminate food as it relates to a vast number of academic fields (Bigliardi and Galati, 2013; Greene, 1998; Chen *et al.,* 1998). Many methods for analyzing fish stomach contents are listed and are critically assessed with a view to their suitability for determining dietary importance for different fish species. The appropriate alternative approaches have been proposed where difficulties in the application of these methods were observed and discussed. The modifications in these methods based on practical value are also considered. The necessity of linking measurements of dietary importance to stomach capacity have been emphasized and the effects of differential digestion upon interpretation of stomach contents outlined. The best measure of dietary importance is proposed as one where both the amount and bulk of a food category are recorded (Hyslop, 1980). Feed is prerequisite for all forms of life. A study of the food and feeding habits of fish is very important in any fisheries research programme. If the experiment was under controlled condition, it was inevitable to know the feeding habits and food of the experimental fish. The ultimate aim was for the captive breeding and larval rearing of the fish. Thus the objective behind the study was to understand the food preference of the adult and the young ones, there by culturing the preferred feeds under laboratory conditions in the futuristic studies. The live feed culture will open up a new way for aquaculture promotion (Williams *et al.,* 2017).

Analysis of gut content is widely used to ascertain the food and feeding habit of fish species. Diet of fishes represent an integration of many important ecological components that include behavior, condition, habitat use, energy intake and inter/intraspecific interactions. In a previous study, the food and feeding of the Indian mackerel *Rastrelliger kanagurta* was studied based on forty-two fishes ranging in size from 13 to 25 cm collected during the summer period, when the fish was available along the Massawa Coast. The methodologies followed in this study were occurrence method recording the stomach contents. Gravimetric method to evaluate the net weight of individuals per stomach in each food category, volumetric (Displacement) method used for estimation of various food items and also Index of Preponderance of empty stomachs was noticed. The dominant food item was Copepod. The fish (*Bregmaceros spp*), sand and fish scale also formed part of the stomach content (Nath *et. al.,* 2015). The combined study of stomach contents and stable isotopes taken by Moriniere *et al.,* 2003 has shown that the juveniles and adults of the fish species are separated ecologically and spatially for a considerable period of time and that herbivorous fishes do not change their trophic status with increasing size, whereas carnivorous fishes feed on increasingly higher trophic levels prior to their migration from the nursery habitat to the coral reef. A qualitative assessment of the fish diet conducted by Jacob and Nair, 1981 revealed that it is not confined to a varied range of aquatic fauna but encompasses altochthonous fauna. Quantitative and qualitative analysis of the diet indicate that feeding habit does not alter with size or seasonal changes. The range of prey consumed does not differ radically, qualitatively, as a function of size, but quantitatively exhibits five levels of discrimination and differential exploitation related mainly to prey size. Seasonal fluctuations in feeding are more qualitative than quantitative and seem dependent on the occurrence of food organisms. Unrevealing food and feeding habits of fishes is the center of research in aquatic biology, ecology conservation biology and fisheries. The current practice in feeding ecology of fish accredits it as descriptive ecology, relying primarily on the information of their diet, directly through gut analysis or indirectly by computing some diet based indices. Such methods often misled to the understanding of the true feeding behavior of organisms needed for more reliable and functional approach. The main objective of feeding ecology is to evaluate feeding behavior of fish. Recent developments in tools and techniques of analytical research is an opportunity to take up more reliable details by formulating affordable methodical design for recording, modulating and designing suitable approaches for better explanation of the feeding biology in fish (Saikia, 2015). The objectives of the present study included as follows: To study the physico-chemical parameters of water determining their effect on micro flora and fauna; to analyze and compare gut contents of these species and to examine the food preferences of selected Fish Fauna of River Sutlej.

## Materials and methods

*Sampling Sites*

Different selected fish species were collected using Cast nets from Head Ganda Singh site, River Sutlej as shown in Fig 1, with the help of fisherman in collaboration of the Punjab Fisheries Department.

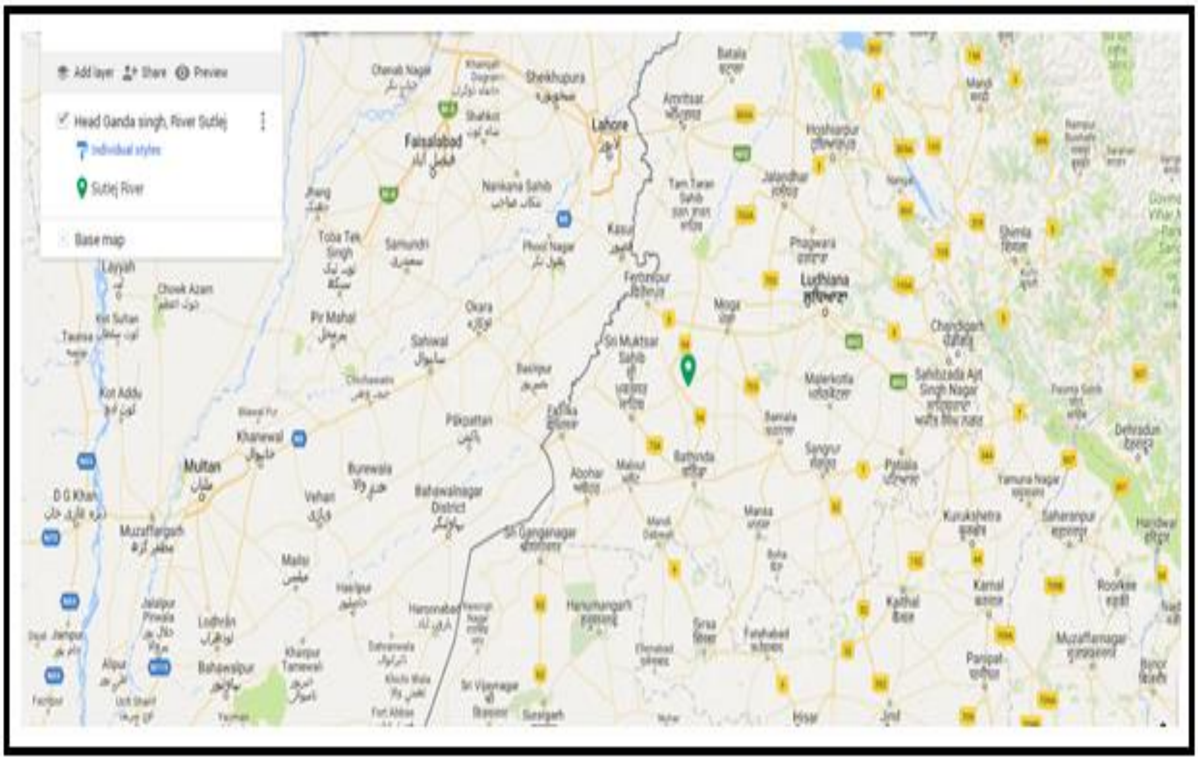


**Fig. 1.** GPS Map for Head Ganda Singh, River Sutlej sampling station.

*Details of Selected fish species*

The selected fish species for this particular study included *Labeo rohita, Cirrhinus mrigala, Oreochromis niloticus* and *Cyprinus carpio*. *Labeo rohita* (Rohu)*:* It is basically a freshwater / brackish fish found mainly in Asia (Pakistan, India, Bangladesh, Myanmer and Nepal). It may attain a maximum length up to 200 cm. It inhabits rivers; feed on plants being phytoplanktonic in nature and widely introduced outside its native range for stocking reservoirs and aquaculture being most popular as a food item.

*Cirrhinus mrigala* (Mori): It is basically a freshwater, demersal and tropical fish found mainly in Asia (Pakistan, India, Bangladesh and Nepal). It may attain a maximum length up to 99 cm. It is also phytoplanktonic in nature being column feeder (FAO, 2014; Froese and Pauly, 2014).

*Oreochromis niloticus* (Chirra): It is basically a freshwater / brackish, benhopelagic and potamodromous fish found mainly in Africa (in coastal rivers of Israel, Nile basin, various coastal basins, sewage canals, irrigation channels and various lake systems). It may attain a maximum length up to 60 cm. It feeds mainly on phytoplankton or benthic algae and mostly omnivorous in nature. It has been widely introduced for aquaculture, with many existing strains (Abdel-Fattah, 2006; Bolivar *et al.,* 2004;

Watanabe *et al.,* 2002; Beveridge and McAndrew, 2000; Pullin and Lowe-McConnell, 1982).

*Cyprinus carpio* (Gulfam): It is basically a freshwater / brackish and benthopelagic fish, introduced throughout the world and its wild stocks are only present naturally in rivers draining to the Black, Caspian and Aral Sea. It may attain a maximum length up to 120 cm. Both adults and juveniles feed on a variety of benthic organisms and plant material. It is also widely used as a food item.

*Experimental location*

These fish samples were transported to the Research and Training Institute, Fisheries Complex, Lahore using icebox containing crushed ice. On arrival, these fishes were preserved immediately in deep freezer having a temperature below -20°C to prevent digestive enzymatic activity.

*Method of Gut Dissection*

The selected caught fish were dissected and the gut was removed, uncoiled, total length noted and cut into three equal parts. The gut contents were squeezed out by applying gently pressure with fingers, washed out in glass petri dish containing distilled water and poured in separate plastic bottles with addition of 4% formalin for the preservation purpose following Prescott, 1978. The bottles were then covered with plastic sheets in addition to bottle lids in order to prevent evaporation and were carefully labelled.

*Planktons Analysis*

The preserved samples were stirred gently from a suspension since stirring may damage the appendages of planktons. One ml of sample was drawn from the bottle containing concentrate with the help of dropper. This method was used for counting planktonic organisms. This one ml sample was set on the Sedgwick-Rafter counting cell containing of glass or Plexiglas's rectangle of 50×20×1 $mm^2$ and has 10 mm depth. This cell holds exactly 1 ml of the sample. An expensive rectangular cover glass was used to cover the cells which prevented the sample from drying out through disturbances by air currents. The sample set in Sedgwick-Rafter counting cell was equally spread; to accomplish this, slip was set covered at one corner end and coverslip was moved into its proper position by capillary action.

Then planktons were counted and identified by keys given by Ward & Whipple (1959) and Atlas of Fresh Water Biota in China (Maosen *et al.,* 1995). Phytoplankton were more numerous in number and also smaller in size as compared to zooplanktons and were checked in 3 squares, chosen randomly whereas 2 rows (100 squares) were selected randomly for counting zooplanktons and analysis was done on a Sedgwick-Rafter counting cell under compound microscope with the use of 10X ocular and 10X,40X objectives. The planktons were calculated by the following formula (Michael, 1984).

No. of plankton per ml = No. of organisms counted ÷ No. of replicates taken

*Physico-chemical Analysis*

The physico-chemical water quality parameters taken under consideration included Temperature, pH, Electrical conductivity, Total Dissolved solids, Chlorides, Total Alkalinity, Free Carbon dioxide, Total, Calcium and Magnesium Hardness; were analyzed from river Sutlej following protocols of APHA, 2012.

*Statistical Analysis*

The statistical analysis on SPSS programme (Version 22) following Steel *et al.,* 1987 was applied including mean with standard deviation to find the significant differences for the parameters included in this study.

**Results and discussion**

The physico-chemical water quality parameters (including Temperature, pH, Electrical conductivity, Total Dissolved solids, Chlorides, Total Alkalinity, Free Carbon dioxide, Total, Calcium and Magnesium Hardness) analyzed from river Sutlej during this study have been recorded in Table 1.

**Table 1.** Physico-Chemical Water Quality Parameters from River Sutlej.

| Sr. No. | Physical parameters | Units | Average values ± SD |
|---|---|---|---|
| 1. | Temperature | °C | 29.7±1.78 |
| 2. | pH | | 7.25±0.43 |
| 3. | Electrical conductivity | μScm$^{-1}$ | 475.00±28.50 |
| 4. | Total Dissolved solids | mgL$^{-1}$ | 403.75±24.23 |
| 5. | Chlorides | mgL$^{-1}$ | 100.00±6.12 |
| 6. | Total Alkalinity | mgL$^{-1}$ | 260.00±15.60 |
| 7. | Free Carbon dioxide | mgL$^{-1}$ | 28.00±1.68 |
| 8. | Total Hardness | mgL$^{-1}$ | 168.00±10.08 |
| 9. | Calcium Hardness | mgL$^{-1}$ | 160.00±9.60 |
| 10. | Magnesium Hardness | mgL$^{-1}$ | 8.00±0.48 |

All the values obtained were within suitable ranges for the Aquaculture requirements for growth of various fish species. However, the water seemed to be polluted in respect to colour and odour parameters. Length-Weight record of studied Fish samples for the selected four fish species has been provided in Table 2 which comprises of the measurements including body weight, gut weight, total body length and gut length along with ratio of intestine to total length of fish of selected fish samples.

**Table 2.** Length-Weight Record of Studied Fish Samples.

| Sr. No. | Selected Fish species | Total Length | Weight | Gut Length | Gut Weight | Intestine length ratio to total fish length | Fish girth |
|---|---|---|---|---|---|---|---|
| | Units | cm | g | cm | g | | cm |
| 1 | *Labeo rohita*(Rohu) | 27.0±1.94 | 210.41±15.14 | 579.0±41.68 | 28.74±2.07 | 21.44 | 15.0±1.08 |
| 2 | *Cirrhinus mrigala*(Mori) | 27.0±1.88 | 234.26±16.87 | 331.0±23.38 | 17.24±1.24 | 12.30 | 16.0±1.15 |
| 3 | *Oreochromis niloticus* (Chirra) | 18.0±1.29 | 112.90±8.13 | 98.0±7.05 | 5.05±0.36 | 5.44 | 15.0±1.11 |
| 4 | *Cyprinus carpio* (Gulfam) | 20.0±1.45 | 114.46±2.16 | 30.0±2.16 | 2.50±0.18 | 1.50 | 15.0±0.95 |

Table 3 shows the comparative planktonic life (total number with %age) observed in the gut contents of the studied fish species.

The total quantity and types of planktonic life observed mainly consisted of four dominant phytoplankton families observed with zooplankton comparison.

The phytoplankton families included Chlorophyceae, Bacillariophyceae, Myxophyceae and Euglenophyceae. In *Labeo rohita,* the most dominant food observed was from Chlorophyceae family (58%) followed by Bacillariophyceae (26%), Myxophyceae (9%), Euglenophyceae (7%) and zooplankton were absent in the gut contents. In *Cirrhinus mrigala*, the most dominant food observed was again from Chlorophyceae family (34%) followed by Myxophyceae (25%), Bacillariophyceae (23%), Euglenophyceae (14%) and zooplankton (4%) in the gut contents. In *Oreochromis niloticus*, the most dominant food observed was from Myxophyceae (45%) followed by Chlorophyceae family (30%) Bacillariophyceae (18%), Euglenophyceae (3%) and zooplankton were absent in the gut contents.

In *Cyprinus carpio*, the most dominant food observed was zooplankton (75%) while phytoplankton were very less with Myxophyceae (19%) followed by Bacillariophyceae (6%) while Chlorophyceae (0%) and Euglenophyceae (0%) families were absent in the gut contents of this particular fish.

Fig 2 provides the Pie diagrams showing comparative account of planktonic life with respect to each fish species. Fig 3 shows selected fish species comparison with respect to the food items found from various planktonic families. Fig 4 shows a vice versa situation with selected planktonic families' comparison with respect to the selected fish species. Fig 5 - 8 represents in detail the type and total number of specific phytoplankton and zooplankton (food items) with their scientific names observed in gut contents of *Labeo rohita, Cirrhinus mrigala, Oreochromis niloticus and Cyprinus carpio* collected from river Sutlej.

**Table 3.** Comparative Planktonic Life (total number with %age) observed in the studied fish species gut contents.

| Sr. No. | Name of studied Fish species | Planktonic Families | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Chlorophyceae | | Bacillariophyceae | | Myxophyceae | | Euglenophyceae | | Zooplanktons | |
| 1 | *Labeo rohita* | 67±4.28 | 58 % | 30±1.92 | 26% | 10±0.64 | 9% | 8±0.51 | 7% | 0±0.00 | 0% |
| 2 | *Cirrhinus mrigala* | 24±1.53 | 34% | 16±1.02 | 23% | 18±1.15 | 25% | 10±0.64 | 14% | 3±0.19 | 4% |
| 3 | *Oreochromis niloticus* | 32±2.04 | 30% | 19±1.22 | 18% | 48±3.07 | 45% | 4±0.25 | 3% | 4±0.26 | 4% |
| 4 | *Cyprinus carpio* | 0±0.0 | 0% | 4±0.25 | 6% | 12±0.76 | 19% | 0±0.00 | 0% | 47±3.01 | 75% |

Many investigations and research works have been conducted previously on the same subjects taken under this study. In accordance with our study, Baker *et al.,* 2014 conducted the research on composition of gut contents of fishes.

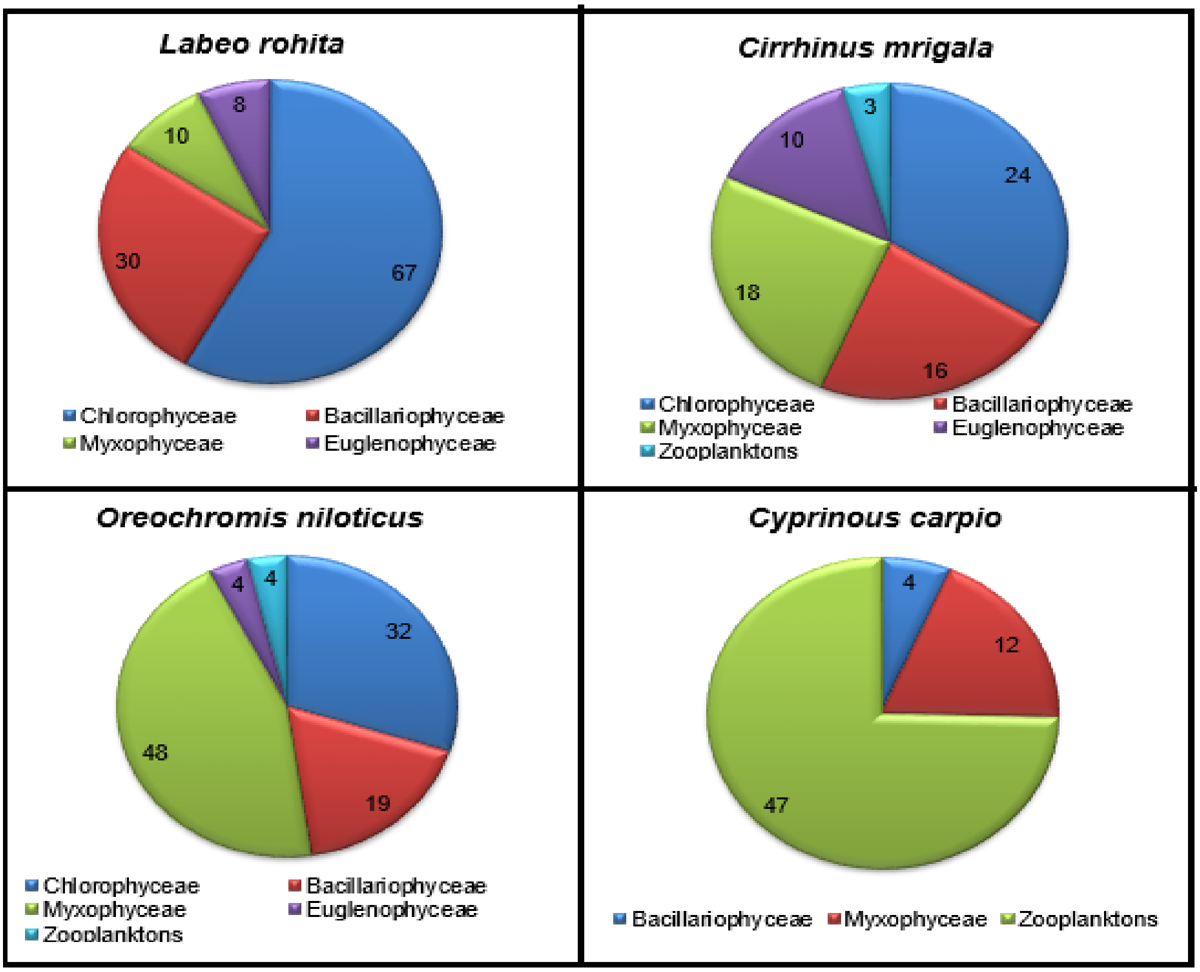


**Fig. 2.** Pie Diagrams for Comparative Planktonic Life observed in the studied fish species under consideration

They described that the description of dietary composition presented in many studies, frequency of occurrence %F provided interpretable data that overcome many of the limitations of the more detailed approaches and also provided considerable logistical and economic benefits. Nath *et al.,* 2015 also studied the food and feeding habits of Indian mackerel *Rastrelliger kanagurta* based on 42 fishes ranging in size from 13 to 25 cm.

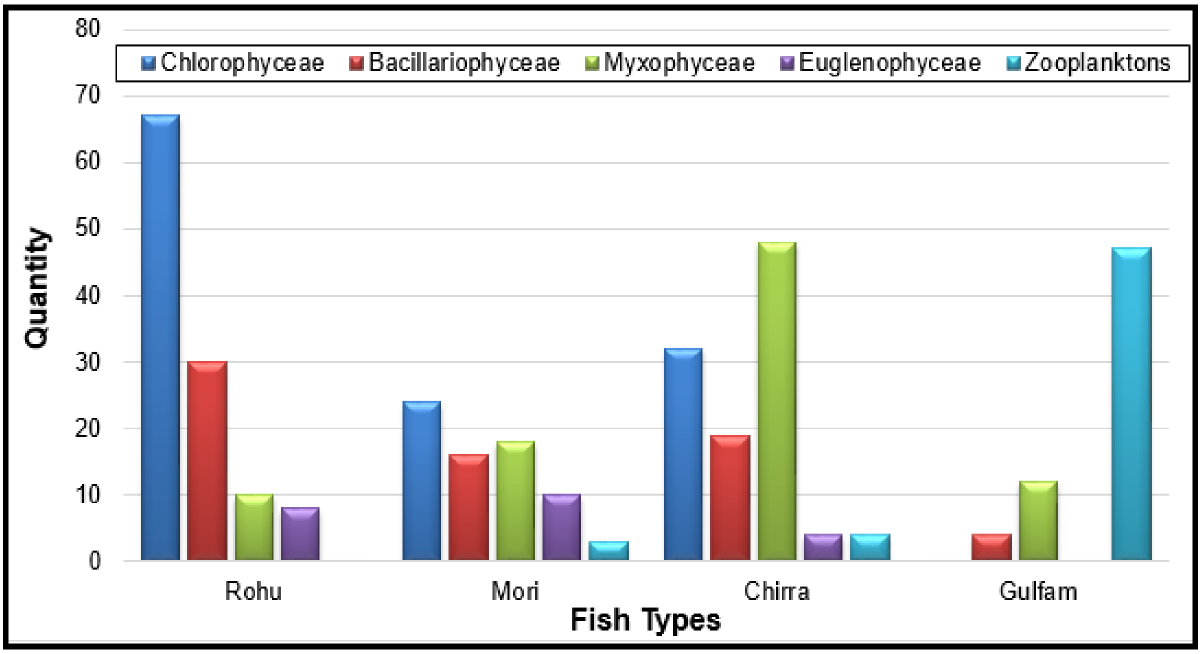


**Fig. 3.** Selected Fish Species Comparison.

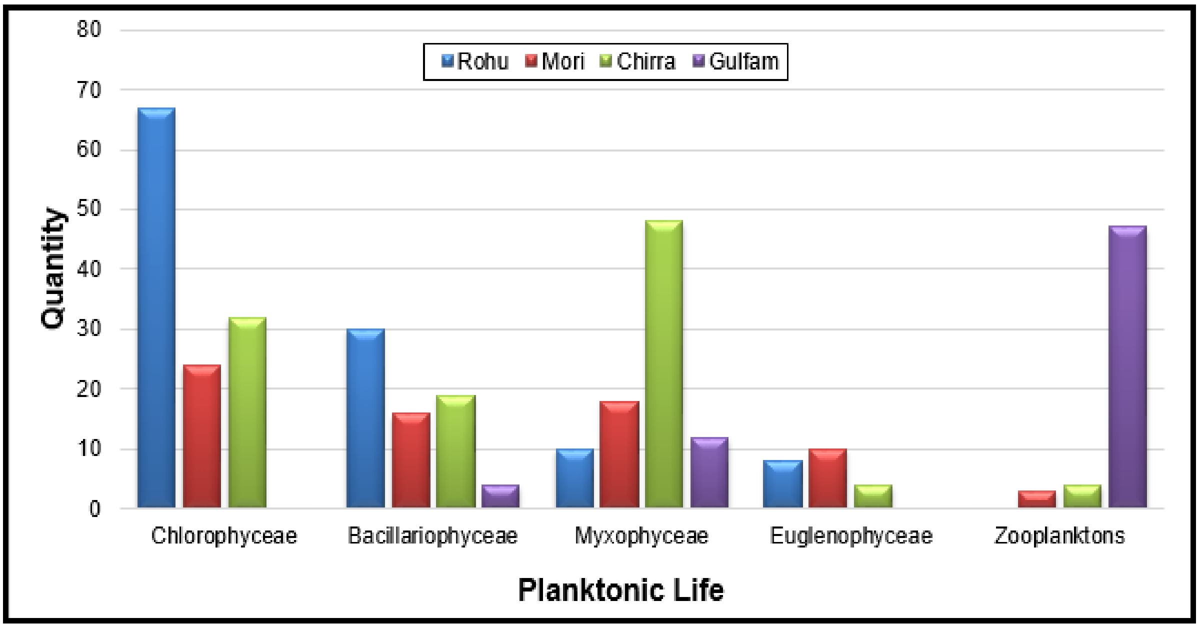


**Fig. 4.** Selected Planktonic Families Comparison.

The methodologies followed in the study were Occurrence method for recording stomach contents, Gravimetric method for evaluating the net weight of individuals per stomach in each food category, volumetric method for estimation of various food items. Index of Preponderance of empty stomachs was also observed. Copepod, sand and fish scale formed part of the stomach content. Liu *et al.,* 2016 conducted a high level advanced research on how host trophic level influences fish gut microbiota and metabolic activity. More than 985,000 quality filtered sequences from 2416 SrRNA libraries were obtained. Results revealed distinct compositions and diversities of gut microbiota. PCoA test showed that gut bacterial communities of carnivorous and herbivorous fishes formed different clusters in PCoA space. Their results indicated that host trophic level influenced the structure and composition of gut microbiota, metabolic capacity and gut content enzyme activity. Ahlbeck *et al.,* 2012 used modeling to evaluate how well different diet analysis methods describe the "true" diet of fish, expressed in mass percentages. The methods studied were both basic methods and composite indices. The basic methods performed better than composite indices. Basic methods were most robust and indicated that these methods should be used to describe energetic-nutritional sources of fish.

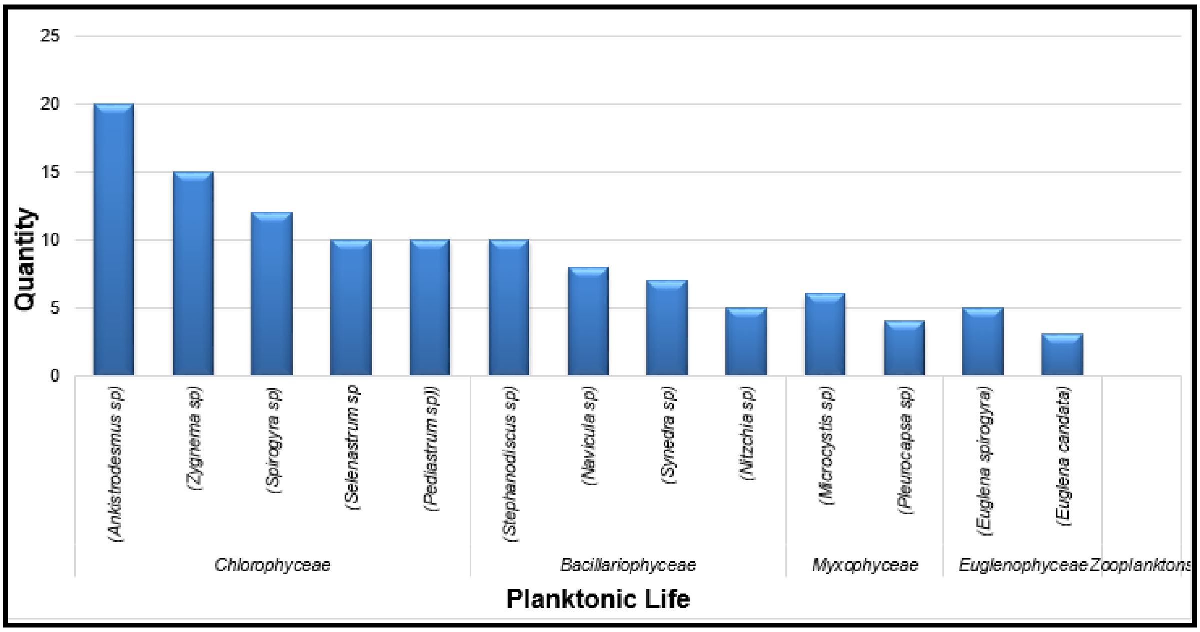


**Fig. 5.** Detailed Gut Content Results of *Labeo rohita* (Rohu) River Sutlej.

In accordance with our research, Brush *et al.,* 2012 reported about diet, carbon source and trophic position of round goby (*Neogobius melanostomus*) varied seasonally, spatially and with body size in littoral habitats of Lake Ontario. Results revealed that Bay of Quinte round goby were more reliant on terrestrial carbon, whereas littoral carbon dominated in the Kingston Basin. Stomach contents suggested dreissenids were the dominant prey item of round goby. Cortes, 1997 proposed the percent index of relative importance as a standardized measure in dietary analyses and a three dimensional graphical representation of the diet was introduced. On the basis of sampling requirements and model assumptions, the Diana and Olson-Mullen methods appeared to be the most appropriate approaches for estimating daily ration in sharks. Berg, 1979 discussed the effectiveness of different methods for investigation of stomach contents of a small, plankton-eating fish. Many nutritional indices, particularly Hynes "Frequency of Occurrence" were criticized. A logarithmic version of Shorigin's index was proposed as a replacement for Ivlev's food selection index. Corse *et al.,* 2010 extended the DNA barcoding approach to diet analysis to allow the inclusion of a wide taxonomic range of potential prey items. Thirty-four ecological clade-specific primer sets were designed to cover a large proportion of prey species found in European river ecosystem. Selected primers sets were tested on different factors using nested PCR to increase DNA detection sensitivity. Their results were consistent with the available literature on feeding behavior in these fish. Adrone *et al.,* 1973 conducted a research in which groups of trout were self-trained in about 10 days to actuate trigger and feed themselves. This capacity was retained for 3 months without the stimulus of continuous reinforcement. Conditioned populations showed a high degree of discriminatory ability towards a trigger that supplies food and one that does not. A trained population, under continuous illumination developed a feeding rhythm that occurred about 8 h.

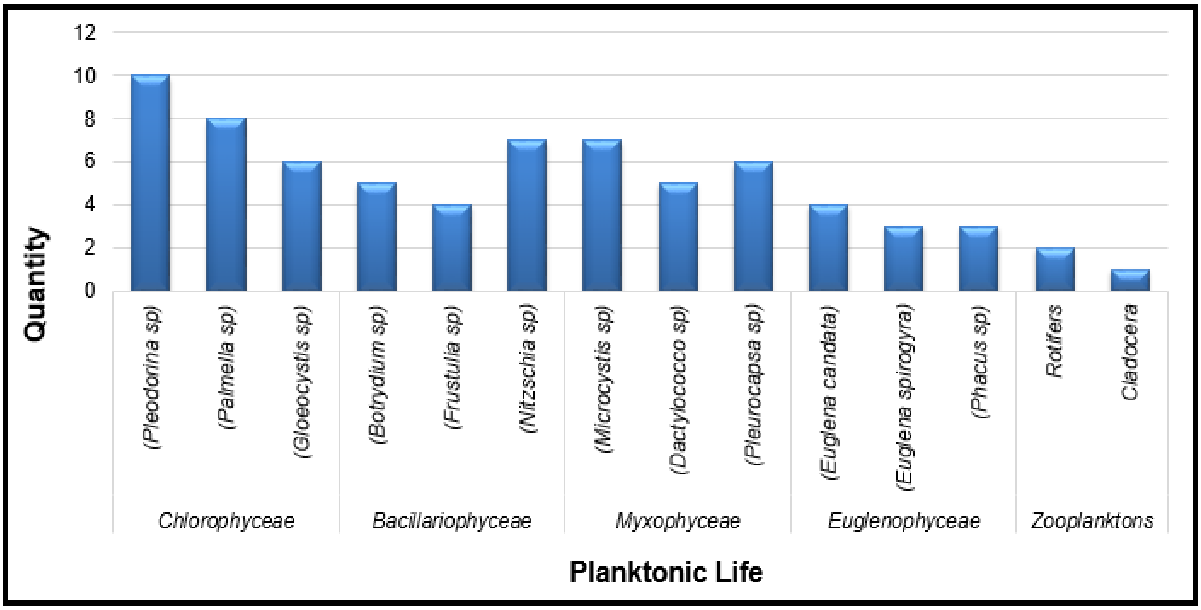


**Fig. 6.** Detailed Gut Content Results of *Cirrhinus mrigala* (Mori) River Sutlej.

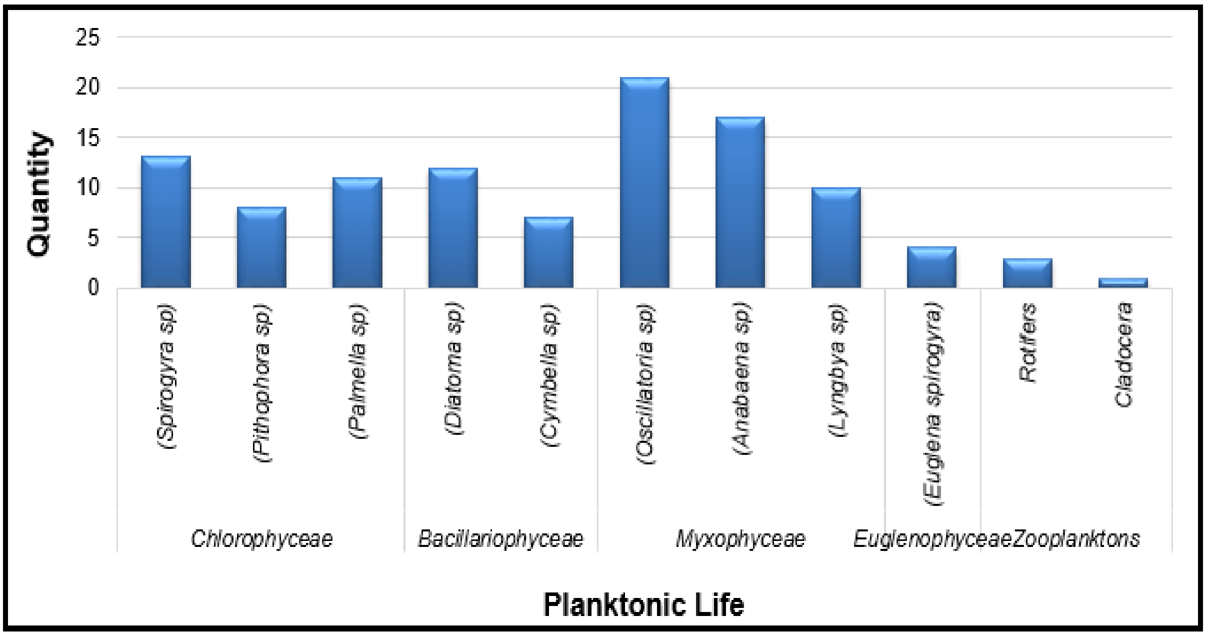


**Fig. 7.** Detailed Gut Content Results of *Oreochromis niloticus* (Chirra) River Sutlej.

In a similar study, Serdar and Ozcan, 2017 conducted the research on stomach contents of fishes of Karasu River. They observed stomach contents flooded with distilled water under a stereoscopic microscope. Contents were stored and prey items were identified. Their results showed that may fly and simulidae were found in abundance whereas chrinomid, stonefly, caddisfly, gammarus were rare in the food groups. The condition factor varied between 0.820-1.621. Adams and Sterner, 2000 conducted a series of laboratory studies varying dietary nitrogen content and measuring corresponding variations in organismal; 415 N values. They investigated the relationship between the; 415N values of the anomopod crustacean, *Daphnia magna*, and the C:N ratio of its food, the green algae, *Scenedesmus acutus*. To their knowledge, this was the first controlled study of nitrogen balance and d15N values in animals. Johannsson *et al.,* 2001 stated that the stable isotope analysis of the potential prey and predator can be combined with gut content analysis to quantify the diet. The diet of Mysis relicta was examined in Lake Ontario in different seasons using both techniques. Mysids fed on the bottom during the day and in the pelagia and on the bottom at night. They stated that daily consumption estimates were similar to those estimated from previous bioenergetics modeling. Huber *et al.,* 2003 estimated the microbial density of rainbow trout intestine by direct microscopic counts and by culturing on tryptone soya agar (TSA).16S rDNA gene sequences of 146 bacterial isolates and three sequences of uncultured bacteria were identified. In most samples, the aerobic count (on TSA) was 50-90% of the direct (DAPI) counts.10-75% of the microbial population in samples with low aerobic counts hybridized (FISH) with the probe constructed against this not-yet cultured bacterium. Azzurro *et al.,* 2007 attempted the research to give timely information on the resource partitioning between the lessepsian migrant *Siganus luridus* and two ecological native analogues.

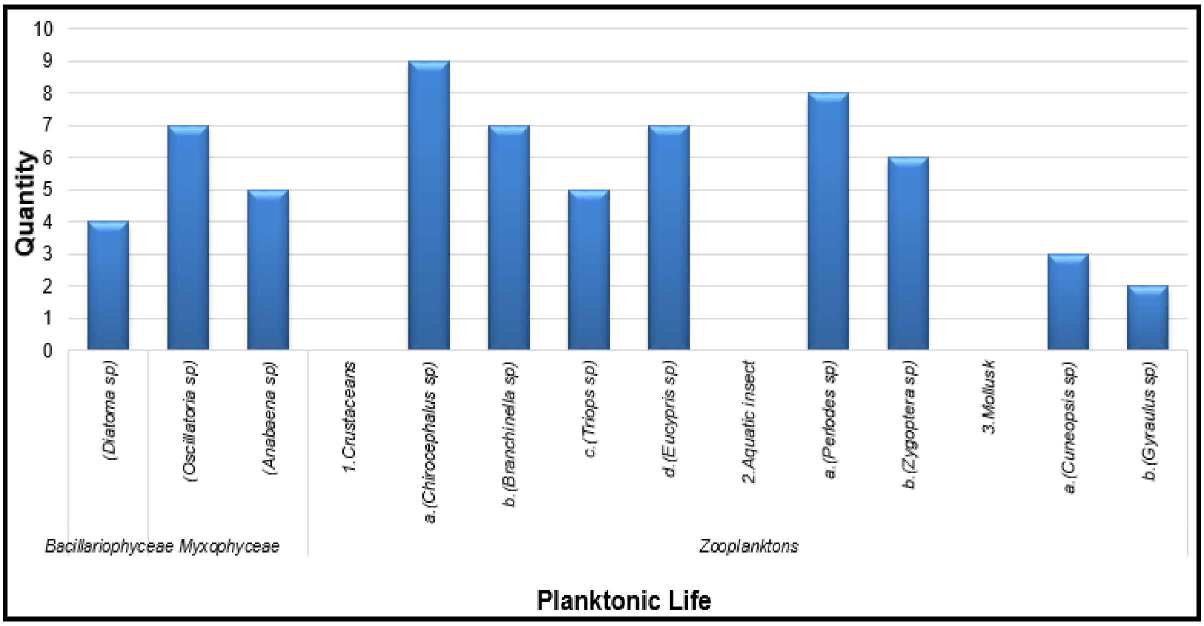


**Fig. 8.** Detailed Gut Content Results of *Cyprinus carpio* (Gulfam) River Sutlej.

The values of 15 N confirmed a strictly vegetal diet for *S. luridus* and *S. cretense* while *S. salpa* was significantly more enriched. The values of $\delta 13C$ matched the predicted ones for *S. luridus* and *S. salpa* while both species presented less enriched $\delta 15N$ values than expected. Grey *et al.,* 2002 investigated trophic relationships at the top of the Loch Ness food web. They used angling which provided samples of the top predator, the purely piscivorous ferox trout. Ferox trout exhibited a lower trophic level than predicted (4.3) by using $\delta 15N$ values. Charr displayed dietary specialization with increasing length. The isotope data also indicated that charr carbon was primarily autochthonous in origin.

## Conclusion

The results of this study concluded that *Labeo rohita* consumed the maximum food from chlorophyceae family in addition to other varieties while zooplankton were found absent in its gut contents. *Oreochromis niloticus* and *Cirrhinus mrigala* took its food from both phyto- and zooplanktonic families with almost same average values, however, Myxophyceae was dominant in case of the former specie while *Cyprinus carpio* mainly depended on zooplanktons for its diet. Therefore, through this research, it is revealed that *Labeo rohita* was found herbivorous while *Cyprinus carpio* was mainly found carnivorous; however, *Oreochromis niloticus* and *Cirrhinus mrigala* were found to be omnivorous presiding in the waters of river Sutlej.

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