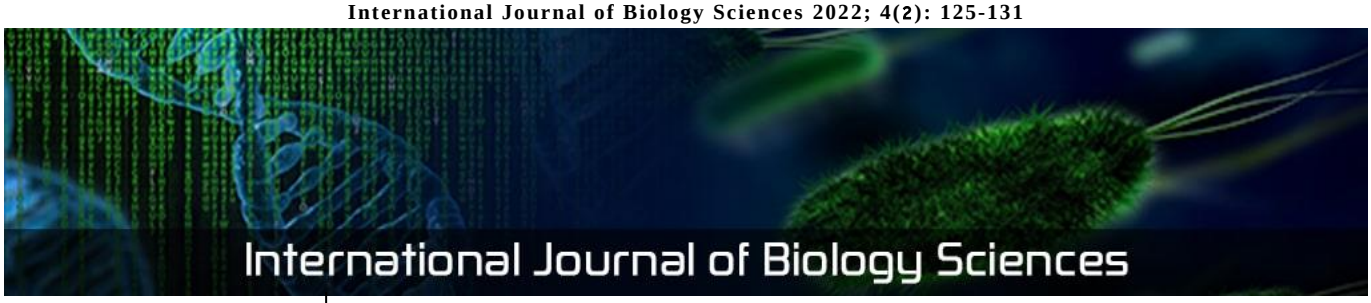

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Sehrish Ashraf
Department of Zoology, Ghazi University, D.G. Khan, Punjab, Pakistan

Khizar Samiullah
Department of Zoology, Ghazi University, D.G. Khan, Punjab, Pakistan

Riffat Yasin
Faculty of Veterinary Sciences, MNS, University of Agriculture, Multan, Punjab, Pakistan

Shah Zaib
Department of Zoology, Ghazi University, D.G. Khan, Punjab, Pakistan

Irish Atiq
Department of Zoology, Ghazi University, D.G. Khan, Punjab, Pakistan

Inayat Ullah Malik
Department of Zoology, Ghazi University, D.G. Khan, Punjab, Pakistan

Rana Mehroz Fazal
Department of Zoology, Ghazi University, D.G. Khan, Punjab, Pakistan

Muhammad Amjad Bashir
Department of Zoology, Ghazi University, D.G. Khan, Punjab, Pakistan

Kashif Hussain
Faculty of Veterinary Sciences, MNS, University of Agriculture, Multan, Punjab, Pakistan

Corresponding Author:
Khizar Samiullah
Department of Zoology, Ghazi University, D.G. Khan, Punjab, Pakistan

Morphometric characteristics, sexual dimorphic characters, length-weight relationship and feeding behavior of *Chitala chitala* from river Indus, Punjab, Pakistan

Sehrish Ashraf, Khizar Samiullah, Riffat Yasin, Shah Zaib, Irish Atiq, Inayat Ullah Malik, Rana Mehroz Fazal, Muhammad Amjad Bashir and Kashif Hussain

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Abstract
Chitala chitala commonly known as clown fish is an important fresh water fish found worldwide. A study on the morphometric characteristics, length-weight relationship and feeding habit from stomach and gut analysis of *C. chitala* was conducted on 32 specimens collected from different zones of River Indus, Pakistan during the month of January and April. These samples were examined in Fisheries lab, Zoology department, Ghazi University, Dera Ghazi Khan. Various external and internal morphometric characters were studied to know their correlation with reference to size (TL and W). Correlation coefficient (r) value (0.98) showed high significant relationship between total length and wet weight, while growth pattern was near to isometric as value of 'b' being 2.86. The interrelationship of total length to the eye diameter (0.63), length of dorsal fin (0.44), pectoral fin length (0.69), pre orbital length (0.59), IOD (0.36), DFB(0.53), PFB (0.47) show poor correlation to total length (p>0.05). All other external parameters are highly significant to the total length (p<0.05). In internal morphometry, relationship of GL, LW, StW were highly significant (p<0.05). The composition of food content revealed the major food items were small sized fishes, mollusk, insect, and crustaceans. This is the first study on the feeding habit of *C. chitala* and conclude that *C. chitala* is a carnivore species that is under pressure of food and its status is near threatened. It is recommended to conduct further studies on length-weight relationships and feeding behavior of threatened and commercially important fish *C. chitala* for management and conservation of populations in natural water bodies.

Keywords: *Chitala chitala*, Indus river, morphometric characters, carnivore

1. Introduction
Fish are most numerous vertebrate group on the planet, accounting for half of all vertebrate species (Rafique and Khan, 2012) [42]. Approximately 33,000 fish species have been discovered in marine, brackish and freshwater environments around the world (Di Pinto *et al*., 2015; Froese and Pauly, 2015) [14, 18]. In Pakistan, 531 fish species have been identified, with 233 being freshwater and the remaining 298 being marine (Ghouri *et al*., 2020) [19].
Chitala is a genus of fish of the family *Notopteridae.* Based on Fishbase (2019), there are 6 species of *Chitala*, namely *Chitala lopis, C. blanci, C. borneensis, C. chitala, C. hypselonotus* and *C. ornata*. They are native to freshwater in South and Southeast Asia, and commonly known as the Asian knifefishes or featherbacks (Anjarsari *et al*., 2021) [3]. *Chitala chitala* is one of the significant freshwater fish found worldwide in benthic environments is considered one of the most commercially important and high-priced delicacies (Haji Muhammad *et al*., 2017; Hussain *et al*., 2015) [33, 23]. This species can be found in deep rivers, reservoir, beels and ponds in Pakistan, Myanmar, India, Bangladesh, Thailand, Sri Lanka, Indonesia and Nepal (Mirza, 2004, Mitra *et al*., 2015) [30, 31]. This species is primarily carnivorous as well as insectivorous, but it also feed on planktons and crustaceans and can occasionally engage in cannibalism. Fish can attain maximum length up to 122 cm with maximum body weight of 14 kg (Sarkar *et al*., 2006a) [47].
Chitala chitala is classified as "Near Threatened" due to a lack of understanding of its biology, as well as falling stocks of this population in natural waters due to overfishing, over exploitation, pressure on their habitat and contamination (Khan, 2013; Sarkar *et al*., 2009)

[26, 49]. There are few studies on the length-weight relationship, morphometric indices and feeding habits of the Pakistani fish (*Chitala chitala*). (Hussain, *et al*., 2015; Mitra *et al*., 2017) [23, 32].

Length and weight relationship of fish has much significant and used commercially for fishery assessment (Haimovici and Velasco, 2000; Ali *et al*., 2000; Fafioye and Oluajo, 2005) [1, 16]. This relationship is applicable in defining a population. All fish in a certain length group are measured and assigned a predicted average weight. When a large number of live fish are sampled, this is typically faster and more appropriate than weighing them individually. Many authors have worked on significance of relationship between length and weight of various fish species (Sarkar *et al*., 2013, Chandran *et al*., 2020, Deka and Bura, 2015, Hussain *et al*., 2015) [50, 8, 11, 23]. The study on this relationship provide information about mortality rate, life duration, growth as well as production (Diaz *et al*., 2000; Fafioye and Oluajo, 2005) [16].

Fish morphology has traditionally been the principal source of data for taxonomy and evolutionary investigations (Rawat *et al*., 2017) [43]. Morphological measurements, meristic counts, shape and size provide information that can be used to determine taxonomic rank (Ihssen *et al*., 1981) [24]. Individual well-being and probable differences between independent unit populations of the same species can be assessed using morphometric relationships among various body sections of fish (King, 2007) [27]. Fish are extremely sensitive to changes in their environment and swiftly react by modifying their morphometrics (Hossain *et al*., 2010) [22]. Information for taxonomic studies, morphometric examinations of fish and the investigation of statistical relationships between them are required (Narejo, 2010; Brraich and Akhter, 2015) [38, 7].

Feeding is the most important activity in a fish's life cycle (Royce, 2013) [45]. Fishes have a more diverse diet than any other vertebrate, and they exhibit significant specializing in the architecture, function and food processing of the mouth and stomach behavior to optimize feeding behavior as part of their survival strategy. The diet and dietary patterns of fish are crucial and necessary for growth of fish (Dewan, 1979) [13]. The food item % composition contained in the stomach indicated the eating patterns of fish, as did the length of a fish's or any other animal's gut. The gastrointestinal system's stomach content gives nutrition information, dietary patterns and preferential feeding, in fish (Arthi *et al*., 2011) [4].

Fish populations in both fresh and marine habitats are steadily declining in many regions of the world due to a variety of factors. Furthermore, industrial pollution, illicit fishing, massive deforestation, global warming and the river's westward movement pose serious risks to the fish species of the Indus River. Fish variety and distribution patterns in the Indus River and its drainage system still need to pay attention (Muhammad *et al*., 2017) [33]. For success in maintaining and conserving the natural fauna to the desired levels, studies of threatened as well as commercially valuable animals require special attention (Hussain *et al*., 2015) [23].

The goal of present study was to determine the morphometrics, overall condition and growth pattern of *C. chitala* in their native home in order to conserve and appraise them. Major Morphometric proportions have been examined in this work to clarify taxonomic uncertainties in the case of *C. chitala.* Based on the contents of the fish stomach and the length of the alimentary canal as measured from the river Indus, Pakistan, the diet, feeding behavior an gut analysis of the fish were also determined. (Student) The research will aid taxonomists in the identification of suitable fish species with superior morphological characteristics and growth potential for excellent aquaculture output, and improve fisheries research management. It will important key in the systematic study of ichthyology.

Study Area

Different sites of Indus River near district Layyah of Punjab province (Pakistan) were selected as site of study. These sites of Indus river provides a huge and diverse macro-habitat in the form of off shoots, side streams, shallow waters, deep waters, clear stagnant water in hands, fast flowing water with high oxygen content, side water with low oxygen due to vegetation decomposition, shallow water with submerged vegetation, seepage water on sides to variety of fish fauna.

2. Material Method

A total of 32 samples of fish *Chitala chitala* ranging from 90 g to 752 g in body weight, 20 cm to 47.80 cm in total length were collected from January 2021 to end of April 2021. Sample were collected in early morning with the assistance of local fishermen who applied method of traditional fishing by using equipment such as cast nets with mesh sizes of 2 cm × 2 cm and 6 cm × 6 cm. 21 external morphometric characters were identified and measured samples were then dissected out in the laboratory from the abdominal cavity for internal morphometric measurement. Sartorius weighing balance was used to measure the weight of internal morphometric parameters. For the study of gut contents, the gut was removed intact from the esophagus to the anus and its length (GL) and weight (GW) were measured. The degree of stomach fullness and the degree of digestion were determined during the analysis. The gut section isolated was put in a Petri plate that contained distilled water and excised to take out the stomach contents with tweezers. The stomach contents were preserved in 70% alcohol for the assessment of prey species. Food items were identified and examined (magnifications: 6X to 400X) and counted to estimate their numerical involvement in the fish food. A log-log plot of data have been made for length-weight relationship (LWR), outliers were identified and eliminated and the regressions has done. Using the linear transformation as proposed by Wootton (1990) [52]. Dimorphic characters of male and female *Chitala chitala* are being summarized after Chonder (1999) [9] and Sarkar *et al.* (2006b) [48]. Statistical correlation between total length and body mass of the fish derived. To describe a fish's level of fitness or health following equation, given by Froese (2006) [17], was utilized in this work to estimate the condition factor (K);

$$K = W\ X\ 100/L^3$$

The data collected was analyzed by using percentage, mean and standard deviation. Regression analysis and descriptive analysis was done for morphometry and feeding habit analysis.

3. Results and Discussion

In fishery science, significance of length-weight relationships cannot be neglected as they are the basic indicators because they provide the knowledge about the seasonal changes in their specific environment, the physical well-being of the fish. It also determines the growth of the fish whether it is isometric or allometric because the information about the growth of the fish is considered to be an important aspect of the study of the fish population dynamics (Memon *et al*., 2021) [29]. Morphometric analysis is also standard approach for giving information about fish phenotypic traits, development, systemic variation, and population traits Because the length-weight connection of fish varies based on the state of life in an aquatic habitat, in fish bioecology the investigation of the length-weight association is important because it aids in a fish population, comprehending growth pattern and common good (Nagesh *et al*., 2004) [36]. Species of family Notopteridae are near threatened according to IUCN (Banik and Roy, 2014) [5] so its morphometric measurement and its length-weight relationship are most important to know its importance in fisheries.

A total of 32 samples of *Chitala chitala* were collected during study period from Indus River, Layyah, Pakistan. 21 external morphometric characteristics measured and their relations with wet total weight and with total length ranged 0.70-0.99 and 0.66 to 0.99 among the characters were compared respectively. The growth rate of different body regions in comparison to total length (TL) and wet mass of body (W) was studied by regression analysis, applying the formula: Y = a + bX and degree of interaction (r) between total length, was determined using the remainder of the body's characteristics by using SPSS 25 software. The connection among body weight (wet) and overall length was established by employing the formula: Y = a Xb, where Y and X are reliant variables and independence, respectively, and "b" and "a" are power and intercept, respectively. The logarithm form of the over formula suggested by Le Cren (1951) is as follows:

Logarithmic Weight = log a + b log TL

Total length of body and its body mass expressed as:

Weight = -1.92 TL= 2.86.

Log W = 1.92+2.86 Log TL (r = 0.96).

Degree of correlation value of 0.96 was immensely important (p<0.001) between weight and total length in both log-transformed data and untransformed. The value of b (regression coefficient) was 2.86. This did not correspond to the optimum slope value (b = 3); It indicates that both parameters do not increase and not in accordance with the cube legislation therefore, growth seems to be negatively allometric.

In *Chitala chitala* there were highly meaningful relationship (P=0.001) between overall length and the lengths of various components. However, the correlation coefficient for ED (0.63), DFL (0.44), PcFL (0.69), PrOL (0.59), IOD (0.36), DFB (0.53), PcFB (0.47) show very poor correlation to total length but other parameters such as SL (1.03), BD (1.01), BG (0.93), HL (0.87) all these are near to 1 shows isometric growth highly correlate with an overall length.

A significant association (P = 0.001) was found when wet body mass was compared to measures of other body metrics like standard length positive correlation (0.34), body girth (0.31), body depth (0.34) pelvic fin length (0.34), pre dorsal length (0.35) show a strong correlation. Other parameters show very poor correlation with body weight, DFB (0.18), PFB (0.15), PrPL (0.28), IOD (0.12), ED (0.22), PrOL (0.21), DFL (0.14).

Table 1: Average values, Range difference, Margins of error of different external morphometric characteristics and Length-Length relationship with total length and Length-weight relationship with Total weight in *Chitala chitala*

Sr.#	Body Measurements	Acronyms	Mean	S.D	Range MIN-Max	(Length-Length relationship)	(Length-weight relationship)
1.	Wet body weight	WW	444	173.11	90-752	2.86	-
2.	Total length	TL	37.6	5.60	20.90-47.80	-	0.339
3.	Inter Orbital Distance	IOD	1.37	0.125	1.20-1.50	0.36	0.12
4.	Standard length	SL	37.77	6.296	23-48	1.03	0.34
5.	Head length	HL	8.08	1.15	4.70-9.80	0.86	0.28
6.	Eye diameter	ED	1.07	0.158	0.90-1.40	0.63	0.22
7.	Body depth	BD	10.79	1.75	6.80-121.70	1.01	0.34
8.	Body girth	BG	21.82	3.31	14-25.40	0.93	0.31
9.	Dorsal fin length	DFL	3.35	0.320	2.50-4.20	0.44	0.14
10.	Pectoral fin length	PcFL	3.94	0.51	2.60-4.60	0.69	0.23
11.	Pelvic fin length	PvFL	28.18	4.3	17.20-36	1.01	0.34
12.	Anal fin length	AFL	2.75	0.2	1.7-3.4	0.64	0.21
13.	Upper jaw length	UJL	1.12	0.06	1.09-1.23	0.60	0.19
14.	Pectoral fin base	PcFB	1	0.13	0.70-1.30	0.47	0.15
15.	Dorsal fin base	DFB	0.99	0.11	0.70-1.20	0.53	0.18
16.	Condition factor	C.F	0.699	0.089	0.64-1.03	-0.25	-0.23
17.	Pre Pelvic length	PrPL	10.82	1.64	6.20-14	0.85	0.28
18.	Pre dorsal length	PrDL	22.39	3.72	12.70-28.10	1.04	0.35
19.	Post dorsal length	PsDL	16.51	2.09	10.50-22	0.90	0.29
20.	Pre orbital length	PrOL	1.01	0.19	0.70-1.30	0.59	0.21
21.	Mouth gap	MG	2.1	0.3	1-3	0.63	0.20

About 10 internal morphometric characters were analyzed and their weight were calculated. The mean weight with a standard deviation of Stomach length (StL), Stomach Weight StW), liver weight (LW), spleen weight (SW), Heart Weight (HW), Gut length (GL), Gill rakes weight (GrW), Gall bladder weight (GbW), Brain weight (BW) and Body Mass (BM) were calculated as 0.25+0.05, 6.72+0.970,

5.732+1.14, 8.07+1.9, 0.90+0.14, 3.69+0.8, 71+0.11, 68+0.29, 0.19+0.03 and 11.13+1.97 respectively.

Table 2: Average values, Range difference, Margins of error of different internal morphometric characteristics in *Chitala chitala*

Sr. No.	Weight	Acronyms	Mean	S.D	Range
1.	Stomach length	StL	0.25	0.05	0.1-2
2.	Stomach weight	StW	6.72	0.97	5-8
3.	Liver weight	LW	5.732	1.14	4-7
4.	Spleen weight	SW	0.90	0.14	0.67-1.03
5.	Heart weight	HW	0.71	0.11	0.50-0.90
6.	Gut length	GL	3.69	0.8	2.75-4.5
7.	Gill rakes weight	GrW	8.07	1.9	5.95-11
8.	Gall bladder weight	GbW	0.68	0.29	0.30-1
9.	Brain Weight	BW	0.19	0.03	0.1-0.25
10.	Body Mass	BM	11.13	1.97	10-16

Chitala chitala, establishing it as a strictly carnivorous species prioritized molluscs, insects and fish in its diet. In *Chitala chitala*, on the buccal cavity's dorsal wall, the presence of a sense organ aids in the identification of animal food, predatory behaviour is confirmed by the presence of a toothed jaw. *Chitala chitala's* greater relative mouth gap height and width aided it in engulfing larger prey species. The presence of molluscan remains and detritus in the gut suggested that C*hitala chitala* may be a benthic feeder. *Chitala chitala's* alimentary canals were revealed to be slightly less coiled, muscular, short, and bag-shaped. About 25% of the food was fish for *Chitala chitala*. Fish and prawns were the most popular foods for this fish. Small fishes about (2-3 mm) were found in semi-digested and entirely digested states and just the vertebral column was found in some stomach samples. After fish food mollusc's food were noticed about 18%. From gut analysis, this fish feed on insect about 15% of its food. Its food also includes crustaceans (10%), rotifer (4%) and chlorophyceae (8%). Other food preferences include protozoans (3%), sand and mud (4%), stone and other miscellaneous (4%), myxophyceae (4%) and annelids (5%).

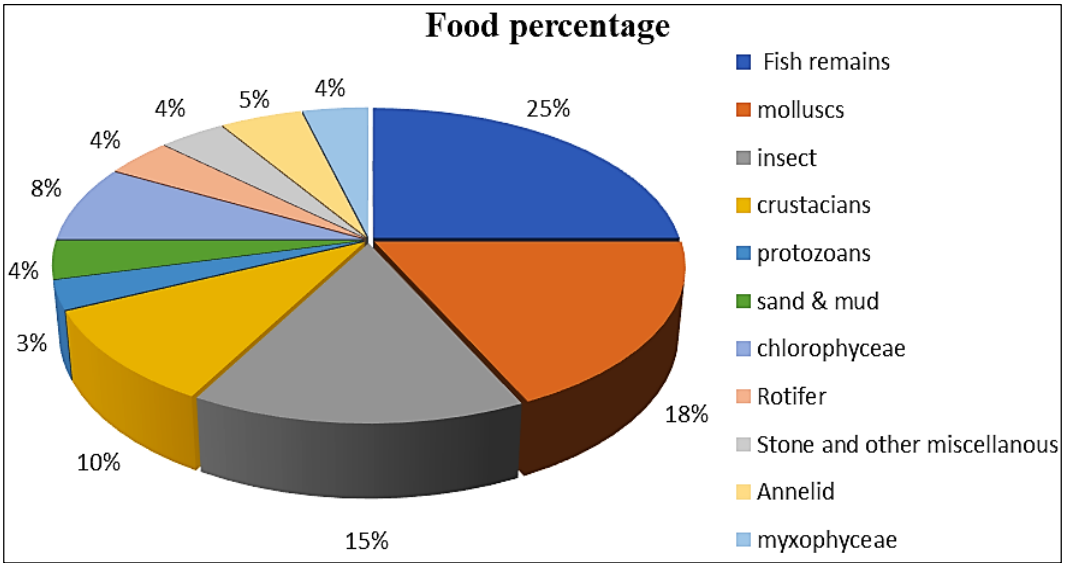

Fig 1: Analysis of feeding percentage of fish *Chitala chitala* from stomach and gut analysis

Sexual dimorphic characters as size and coloration of body, appearance of urinogenital papillae and gonads were distinguished. Males are generally smaller in size than females. The ground color of the body of males is usually silver white depending upon habitat. During the breeding season, the male *Chilata* shows bright red color at the base of paired and anal fins female does not show any marked coloration. The abdomen of female is bulged externally and could be seen prominently disposed as compared to male. The urinogenital papilla is conical shaped, reddish, thin, muscular, hard and pointed. They shows diffused vent. In females the urinogenital papilla is fleshy, thin walled, stouter, broad, less pointed than the males and may not be tipped with red color. Fully mature female shows freely oozing ova. The testis in male are single and unilateral in position. The ovary in female is oval shaped and single lobed lying in the body cavity.

In the regression analysis a total of 21 morphometric characters were included. A parallel study about morphometric and length-weight relation of *Notoptrus notoptrus* which is a member of the same family is presently conducted by Bano *et al*., (2015) [6]. The mean value of parameters in the present study is closely related to that morphometric study of *Chitala chitala* in India (Banik and Roy, 2014) [5]. It is pertinent to mention that only a few studies are available on the identification and length-weight relationship. The "b" value can be used to represent fish growth in statistical analysis. For most fishes, reported by Froese, (2006) [17] the values of b were inside the bounds 2.5-3.5. In the present study, the b value is within range. As a consequence, we regarded our findings has a highly significant calculation for the LWR. When the body mass and length of fish increase equally the growth will be isometric (Ricker, 1975). When the "b" value is larger than 3, it implies positive allometry and when it is less than 3, it shows negative allometry (Wootton, 1990) [52]. In the present study b value for *Chitala chitala* is b = 2.86. Our result is similar to Chandran *et al*., 2020 [8] who reported "b" values in various sites across various rivers at different time periods were significant (but not different from 3), indicating a stable isometric growth, over the years. This reflects the stability in biological behaviour of *Chilata chitala* in these rivers over varying conditions. Our result also coincides with (Hussain *et al*., 2015) [23] who reported the growth coefficient (b) value of length-weight relationships provides useful information on fish growth.

The study shows, b = 2.61 that indicated negative allometric growth pattern and specimens became lighter for their length. The length-weight regression's estimated a and b parameters were used to determine the anticipated standard weight for each observed length. The length-weight relationship indicates that populations of *Notoptrus notoptrus* examined by Naeem *et al*., (2010) [34] were 2.83. In the present study b value for *Chitala chitala* is b = 2.86 which is negative allometric and high than *Notoptrus notoptrus*. Our result is contradictory to the finding of Sarkar *et al*., 2009 [49] who reported the length-weight relationship of pooled data of males and females from 10 different geographical locations indicated that the value of b was almost 3.

When the fish's average weight doesn't rises proportionately to the length cube, the condition factor may fluctuate with length. Condition factor (K) is an indicator of favorable environmental supplies. According to the Nikos (2004) findings, The 'K' of well-fed fish was equal to or more than 1, but fish that are malnourished having a lower 'K' than 1. Another inquiry conducted by Naeem *et al*. (2011) [35], shows in *Oreochromis nilotica* condition factor showed a very really important relationship with mass of body and low significant relation with total length. The value of 'K' in this investigation is less than one, indicating that available fish feed may be insufficient. Its size does not expand in synchrony with its weight or length. Our study coincides with the study conucted by Hussain *et al*., (2015) [23] who reported the K value of the sampled specimens from the polluted site ranged from 0.65 to 0.78 g/cm3 and may indicate disturbances in fish physiology, biochemistry and reproduction also reported by Lohner *et al*. (2001) [28] and Hedayati and Safahieh (2012) [21]. These changes in k value in different fish species are due to different factors like due to a transition in the cycle of spawning (Narejo *et al*., 2002) changes in environmental factors (Doddamani *et al*., 2001) [15] or maybe due to pollution (Devi *et al*., 2008) [12]. So at the river Indus due to inadequate food availability or pollution this species experiences difficulty in their ideal growth. Due to these factors, these fish are categorized as near threatened species by IUCN and serious attention are required towards this for its conservation.

Few studies are available regarding *C. chitala* fish food studying its gut contents and stomach analysis (Alikunhi, 1957; Das and Moitra, 1963; Sharma, 1964) [2, 10, 51]. Due to its relatively large mouth gap and small gut length relative to total length indicates that *C. chitala* is a carnivore fish. Its gut length is less than 1.5cm in relative proportion to the total length of fish. The current findings revealed that highest feeding intensity occurred during the premonsoon and lowest during the monsoon, which might be linked to a drop in food supply. *C. chitala's* food preferences, as observed in this study, were comparable to those of catfishes in general. Gupta *et al*., (2021) [20] also confirm its carnivore nature and their studies shows that *C. chitala* gut length is relatively small to the total length. Similar work was done by Indian researchers (Gupta *et al*., 2021; Sarkar and Deepak, 2009) [20, 49] at different sites and identifies the feeding habit of that fish. Similar work on feeding habits on many carnivores and omnivore fish species in Pakistan done by many researchers (Narejo *et al*., 2005; Iqbal and Waseem, 2008; Narejo, 2010; Pervaiz *et al*., 2012; Sandhu and Lone, 2017) [37, 25, 38, 41] None of them worked on feeding habit of *C. chitala* in Pakistan in the river Indus, Pakistan. This is the first study on the feeding habit of *C. chitala* and conclude that *C. chitala* is a carnivore specie that is under pressure of food an its status is near threatened.

4. Conclusion

The present study is the first to report Indus *Chitala chitala* fish with complete morphometric description and length-weight relationship, feeding habit, sexual dimorphic characters from river Indus, Layyah, Pakistan. Degree of correlation was significant (p<0.001) between weight and total length but value of b (regression coefficient) did not correspond to the optimum slope value which is negative allometric. Relationship between overall length and the lengths of various components shows isometric growth with an overall length. Feeing habit analysis revealed this specie is carnivorous with male being smaller in size as compared to female. The value of 'K' in this investigation is less than one indicating due to inadequate food availability or pollution at the site of River Indus this specie experiences difficulty in their ideal growth. Due to these factors, these fish are categorized as near threatened species By IUCN and serious attention are required towards this for its conservation.

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