A comparative morphologic and craniometric analysis of grass carp (*Ctenopharyngodon idella*) population from Punjab, Pakistan

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HIGHLIGHTS

1. Collection of fish samples (*Ctenopharyngodon idella)* from head Balloki (river Ravi) and head Trimmu (river Chenab), Pakistan.
2. Morphometric and craniometric analysis of *Ctenopharyngodon idella*.
3. Length-weight relationship of *Ctenopharyngodon idella*
4. Fish growth is more negatively allometric at head Balloki as compared to head Trimmu

ABSTRACT

Morpho-craniometric analysis of a fish plays a very important role to determine intraspecific variations. The intraspecific variations of *Ctenopharyngodon idella* were investigated in the current study through morphological and craniometric analysis. These variations are due to the sensitivity of fish in altering aquatic habitat. A total of twenty samples of *C. idella* were collected from two different sampling sites (ten from each) named as head Balloki (River Ravi) and head Trimmu (River Chenab). The results of the present study demonstrated the clear-cut variations among the morpho and craniometric variables of different *C. idella* populations. Moreover, the populations at both sites showed negative allometric growth but it was more prominent at site head Balloki.

Keywords: Morphometric analysis, Craniometry, Phenotypic plasticity, Morphological variations, intraspecific variations

1. INTRODUCTION

Morphometric analysis is a basic tool to understand fish health in a particular environment in addition to identification and taxonomy. Significant information about the fish identification key is provided by the morphological characters of an individual (Dhanya et al., 2004). Morphology is specie specific and the variations in morphological characteristics are due to particular habitat (Cavalcanti et al., 1999). Fish are very sensitive to their environment and bring the necessary morphological variations in its body due to phenotypic plasticity to adapt the altered aquatic environment (Hossain et al., 2010). The health of a fish species in an environment can be accessed by the length-weight relationship (Gulland, 1983; Richardasin, 2000).

C. idella is herbivorous and is native to the Russian Far East and China where it is found in Pearl, Min, Yangtze, and Yellow River (NACA, 1989). It has been introduced in many countries including the USA, Asia, Western Europe, and Hungary (Shireman and Smith, 1983) mostly for aquatic weed control (Van Dyke et al., 1984; Wiley et al., 1987, Klussman et al., 1988). Freshwater habitats enriched with submerged aquatic vegetation (like ponds, lakes, and rivers) are its preferred habitat (Bain et al., 1990; Page and Burr, 1991) where it feeds on algae, vertebrates, invertebrates, and aquatic macrophytes (generalist feeder) (Colle et al., 1978; Van Dyke et al., 1984; Leslie et al., 1987).

Fish are the vertebrates with highly complex skeletons. (Ferry-Graham and Lauder, 2001). The adult teleostean cranium consists of 60 interconnected bones (Aerts, 1991; Akmal et al., 2020). The study of skeleton morphology is very important to analyze the phylogenetic relationship among various fish species. (Mafakheri et al., 2014; Eagderi and Adriaens, 2014). Craniometry is very important for fish classification, and for determining the genetic relationship between different fish species (Diogo and Bills, 2006). Many important phenomena like speciation and diversification can be studied by analyzing the variations in skeletal morphology. Population divergence and speciation may significantly be influenced by resource polymorphism (Smith and Skulason, 1996). The aim of this study is to observe the intraspecific variations in the populations of *C. idella* by using morpho and craniometric data. A baseline data will be provided to land managers, taxonomists, and aqua culturists by this study that will help them to analyze the morphometric variations and growth patterns of grass carp in different areas of Punjab, Pakistan. These findings may be used by conservation biologists to conserve economically significant species of fish.

2. MATERIALS AND METHODS

2.1. *Sampling collection and processing*:

A total of 20 samples of *C. idella* were collected from head Balloki (HB) (31.2260° N, 73.8698° E) and head Trimmu (HT) (31.1448° N, 72.1465°E) (10 from each site) located on river Ravi and river Chenab of Punjab, Pakistan respectively with the help of local fisherman in December 2021 (Figure 1).

C. idella was selected for morphometric and craniometric analysis. By using morphometric data, we also found a length-weight relationship of grass carp. The samples of *C. idella* from head Balloki and head Trimmu were preserved in 10% formalin and transferred to the Ecology and Evolutionary biology laboratory, Institute of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore. *C. idella* was identified by using an identification key. Following accurate identification, the fish was photographed and using tps-dig, landmarks were applied to these images. The morphometric variables of *C. idella* were measured after the landmarks had been applied.

For craniometric analysis heads of all the specimens of *C. idella* were separated and boiled in hot water for only seven minutes. After boiling the heads were kept in cool water for 12 minutes. For the extraction of skulls, extra muscles and tissues were removed. The extracted skulls were kept in 10% formalin solution for seven days. After seven days the skulls were washed with water and placed in a 70% solution of ethyl alcohol for seven days. After one week the skulls were brought out from ethyl alcohol solutions and dried at room temperature. After drying the craniometric analysis was done.

2.2. *Morphometric and craniometric analysis*:

Fourteen morphometric characteristics were selected for morphometric analysis of different *C. idella* populations including fork length (FL), head length (HL), body depth (BD), total length (TL), standard length (SL), eye diameter (ED), pre-pectoral length (PrPecL), pre-dorsal length (PrDL), pre-pelvic length (PrPL), pre-anal length (PrAL), anal fin base length (AfBL), pelvic fin base length (PfBL), dorsal fin base length (DfBL), and caudal depth (CD) (Table 1, Figure 2). All the morphometric characteristics were measured by the measuring scale in centimeters. The weight of each fish was measured in kilograms.

Seven craniometric characteristics were selected for craniometric analysis including eye socket length (EHL), eye socket depth (EHW), skull width (SW), skull height (SH), skull length (SH), pre-orbital (PrOL), and interorbital length (IOL) (Table 2, Figure 3a, 3b, 3c). These craniometric characteristics were measured by using a vernier caliper.

3. RESULTS

The morphological and craniometric characteristics of *C. idella* were measured by using tps-dig software and vernier caliper respectively. The r-value (correlation coefficient) ranges from −1 to +1. The r- values from 0 to 1 characterize a positive correlation; from −1 to 0 represent a negative correlation while "0" represents no correlation amongst independent and dependent variables.

The samples collected from HT showed a good correlation between TL and eight morphometric characters including FL (0.923), SL (0.919), PrDL (0.820), PrPecL (0.812), BD (0.820), PrPL (0.851), CD (0.951) and PrAL (0.815)) while only six morphometric characters (FL (0.984), SL (0.949), HL (0.760), PrPL (0.836), BD (0.836), and AfBL (0.827)) showed a good correlation with TL from the samples collected from HB. Only 4 morphometric characters (from HT samples) including HL (0.667), DfBL (0.598), PfBL (0.537), and AfBL (0.717) represented a moderate correlation with total length while 5 morphometric characters (DfBL (0.740), ED (0.731), PrDL (0.734), PrAL (0.611) and PrPecL (0.684) represented a moderate correlation with total length from HB samples. ED (0.342) of *C. idella* from HT site and CD (0.295) from the HB site revealed a weak correlation with TL. The samples harvested from HB represented no correlation between total length and PfBL (0.016) but a moderate correlation was analyzed between total length and PfBL (0.537) of grass carp from HT samples. CD (0.951) represented a good correlation with TL at HT while it showed a weak correlation (0.295) with TL at HB (Table 4).

The allometric growth is represented by the value of b. Positive allometric growth is represented by a b-value larger than 3, whereas negative allometric growth is shown by a b-value less than 3. All the morphometric characteristics from HB and HT for both fish populations represented negative allometric growth ($b<3$). Negative allometric growth was analyzed in grass carp from both sampling sites when the relationship between total length and weight was examined (Table 4).

The results of the craniometric analysis revealed a significant correlation between SL and SW of *C. idella* from head Balloki and head Trimmu while EHL, IOL, EHW, and SH revealed a nonsignificant correlation (Table 6).

4. DISCUSSION

In fisheries biology, morphological traits are frequently utilized to assess connections and discreteness among the diverse taxonomic category. Numerous morphometric investigations with extensive documentation support stock discreteness (Corti et al., 1988, Villaluz and Maccrimmam, 1988; Shepherd, 1991; Avsar, 1994; Haddon and Willis, 1995; Bembo et al., 1996 and Turan, 1997). Phenotypic plasticity allows the fishes to adaptively respond to environmental variations by modifying their behavior and physiology which leads to variations in their reproduction and morphology that lessens the effects of environmental changes (Stearns, 1983 and Meyer, 1987). However, phenotypic variations induced by environmental changes may be advantageous in identifying stocks, particularly when there is not enough time for significant genetic differences to develop among populations. Fish are highly plastic in terms of their phenotypes. They quickly adjust to environmental changes by changing their physiology and behavior. Their morphology is ultimately altered by these modifications (Stearns, 1983).

The results of the present study represented the morphometric and craniometric variations among two different populations of grass carp from head Trimmu and head Balloki. Intraspecific variations in morphological characters were also analyzed by Hossain et al. (2010), Zamani-Faradonbe et al. (2020), Bagherial and Rahmani (2007), Mir et al. (2013), Razzaq et al. (2015), and Rahman et al. (2014) on various fish species like *Labeo calbasu*, *Garra rufa*, *Chalcalburnus chalcoides*, *Labeo rohita*, *Mugil incilis*, and *Heteropnuestes fossilis*, respectively. The findings of the present study are supported by Swain (1991) and Allendorf and Phelps (1988) who also found that fish morphological characteristics are affected by altering environmental parameters like habitat, water current, food availability, and temperature range while the craniometric characteristics are affected by gene, water quality and nature of food Cooper et al, (2009).

Morphological characteristics are very helpful in analyzing the allometric growth that was analyzed in both of the grass carp populations collected from head Balloki and head Trimmu. The negative allometric growth was more prominent at the head Balloki site as compared to head Trimmu.

The results of the present study are in line with, Imam et al. (2021) who verified the negative allometric growth ($b<3$) in four fish species including *Clarias gariepinus*, *Hemichromis bimaculatus*, *Oreochromis niloticus*, and *Tilapia zilli*. Similarly, the negative allometric growth (1.18 to 2.71) in the species of Schizothorax (*S. niger*, *S. progastus*, *S. esocinus*, and *S. plagiostomus*) was confirmed by Akhtar et al. (2021) at the sites of river Neelum and Jhelum of Azad Jammu and Kashmir, Pakistan.

CONCLUSION

Craniometric and morphometric characteristics are very essential for the classification and identification of fish species. Fish exhibits the ability of plasticity (phenotypic) and bring some necessary variations in their morphology to adapt to a changing environment. Aquatic habitat is the major factor behind these variations. The results of the present studies revealed the variations in morpho and craniometric characteristics of two populations of grass carp from head Balloki and head Trimmu. These variations are very helpful in analyzing the phenomenon of speciation.

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Table 1: Landmarks indication on the sample (*C. idella)*

Landmarks	Description	Landmarks	Description
1-9	Total length (TL)	1-8	Standard length (SL)
1-10	Fork length (FL)	1-6	Pre-dorsal length (PrDL)
1-4	Head length (HL)	6-7	Dorsal fin base length (DfBL)
2-3	Eye diameter (ED)	1-5	Pre-pectoral length (PrPecL)
1-15	Pre-pelvic length (PrPL)	15-14	Pelvic fin base length (PfBL)
1-13	Pre-anal length (PAL)	13-12	Anal fin base length (AfBL)
8-11	Caudal depth (CD)		

Table 2: Craniometric landmarks indication on *C. Idella*

Landmarks	Description
1-8	Skull length (SL)
6-7	Skull width (SW)
2-5	Eye socket length (EHL)
3-4	Inter-orbital length (IOL)
1-2	Pre-orbital length (PrOL)
9-10	Eye socket depth (EHW)
11-12	Skull height (SH)

Table 3: Morphometric characteristics of *C. idella* from HB (River Ravi) and HT (River Chenab)

Morphometric variables	Head Balloki (HB)			Head Trimmu (HT)		
	N=10			N=10		
	Range (cm)		Mean ± SD	Range (cm)		Mean ± SD
	Min	Max		Min	Max	
TL	34.95	50.82	44.47±5.90	45.90	51	48.21±1.94
SL	28.80	44	37.45±4.96	37.20	42.50	40.23±1.52
FL	31.22	47.99	40.67±5.79	43	48	45.07±1.71
PrDL	13.25	23	18.23±2.85	19.90	22	20.89±0.72
DfBL	2.650	8	4.797±2.17	3.40	5	4.17±0.56
HL	5.50	11.32	8.23±1.75	8.20	10.80	9.12±0.88
BD	6.20	13	10.08±2.52	10	12.60	11.63±0.78
ED	1.20	1.50	1.40±0.12	1.30	1.50	1.43±0.08
PrPecL	5.70	11.35	8.24±1.78	8.60	10.60	9.76±0.65
PrPL	14.02	23.01	18.53±2.66	20.20	23	21.15±0.82
PfBL	1.4	5.88	2.91±1.51	1.50	3.50	2.63±0.64
PrAL	24.10	32	28.73±2.37	30	33	31.35±1.0
AfBL	3	3.59	3.30±0.24	3.30	4.80	3.76±0.43
CD	4.50	6.80	5.67±0.65	4.80	7.50	6.02±0.98
W (kg)	0.63	1.17	0.98±0.24	1.30	1.60	1.45±0.16

Table 4: Regression analysis of morphometric characteristics of *C. idella* from HB and HT

Morphometric variables	*C. idella* (Head Balloki)			*C. idella* (Head Trimmu)		
	Y= a+ bX	R	p-value	Y= a+ bX	R	p-value
SL	1.997+0.797X	0.949	0.000	5.43+0.722 X	0.919	0.000
FL	−2.257+0.965X	0.984	0.000	5.69+0.817 X	0.923	0.000
PrDL	2.482+0.354X	0.734	0.016	6.17+0.305 X	0.820	0.004
DfBL	−7.274+0.271X	0.740	0.014	−4.214+0.174 X	0.598	0.068
HL	−1.795+0.225X	0.760	0.011	−5.470+0.303X	0.667	0.035
BD	−5.795+0.357X	0.836	0.003	−4.199+0.328X	0.820	0.004
ED	0.820+0.013X	0.731	0.016	2.129+(−0.014) X	0.342	0.333
PrPecL	−0.948+0.207X	0.684	0.029	−3.442+0.274 X	0.812	0.004
PrPL	1.779+0.377X	0.836	0.003	3.70+0.362X	0.851	0.002
PfBL	3.092+(−0.04) X	0.016	0.966	−5.973+0.178X	0.537	0.110
PrAL	17.835+0.245X	0.611	0.061	11.078+0.420X	0.815	0.004
AfBL	1.806+0.034X	0.827	0.003	−3.856+0.158X	0.717	0.020
CD	4.221+0.033X	0.295	0.407	−17.22+0.482X	0.951	0.000
W	−0.731+0.038X	0.934	0.000	−0.962+0.05X	0.624	0.05

Table 5: Craniometric measurements of *C. idella* from HB (River Ravi) and HT (River Chenab)

Craniometric characteristics	*C. idella* (Head Balloki)			*C. idella* (Head Trimmu)		
	N=10			**N=10**		
	Range (mm)		**Mean ± SD**	**Range (mm)**		**Mean ± SD**
	Min	**Max**		**Min**	**Max**	
SL	79.30	84.50	80.88±1.81	76.30	84.50	81.09±2.92

SW	50.02	55.70	52.87±1.94	50.02	55.60	53.09±2.10
EHL	25.90	28.60	27.07±0.95	25.80	26.55	26.19±0.26
IOL	32.55	33.78	32.93±0.42	32.55	34.20	33.27±0.61
PrOL	18.55	20.27	19.37±0.63	19.10	20.25	19.63±0.41
EHW	18.30	20.65	19.39±0.74	17.20	19.45	18.95±0.80
SH	27.65	28.90	28.45±0.47	28.30	29.60	28.66±0.46

Table 6: Correlation between the craniometric characteristics of *C. idella* from HT and HB

Application		**SL**	**SW**	**EHL**	**IOL**	**EHW**	**SH**
SL	Pearson Correlation	0.678*	0.796**	0.046	0.745*	0.338	0.574
	Sig. (2-tailed)	0.031	0.006	0.899	0.013	0.339	0.083
SW	Pearson Correlation	0.464	0.638*	0.026	0.413	0.031	0.289
	Sig. (2-tailed)	0.177	0.047	0.942	0.235	0.933	0.418
EHL	Pearson Correlation	0.684*	0.598	−0.120	0.407	0.171	0.445
	Sig. (2-tailed)	0.029	0.068	0.742	0.244	0.637	0.198
IOL	Pearson Correlation	−0.048	−0.226	−0.198	−0.430	−0.292	−0.238
	Sig. (2-tailed)	0.895	0.531	0.584	0.215	0.412	0.507
EHW	Pearson Correlation	0.394	0.473	0.133	0.473	0.473	0.571
	Sig. (2-tailed)	0.260	0.168	0.756	0.168	0.168	0.085
SH	Pearson Correlation	−0.399	−0.477	−0.197	−0.416	−0.491	−0.474
	Sig. (2-tailed)	0.253	0.163	0.585	0.232	0.150	0.166

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

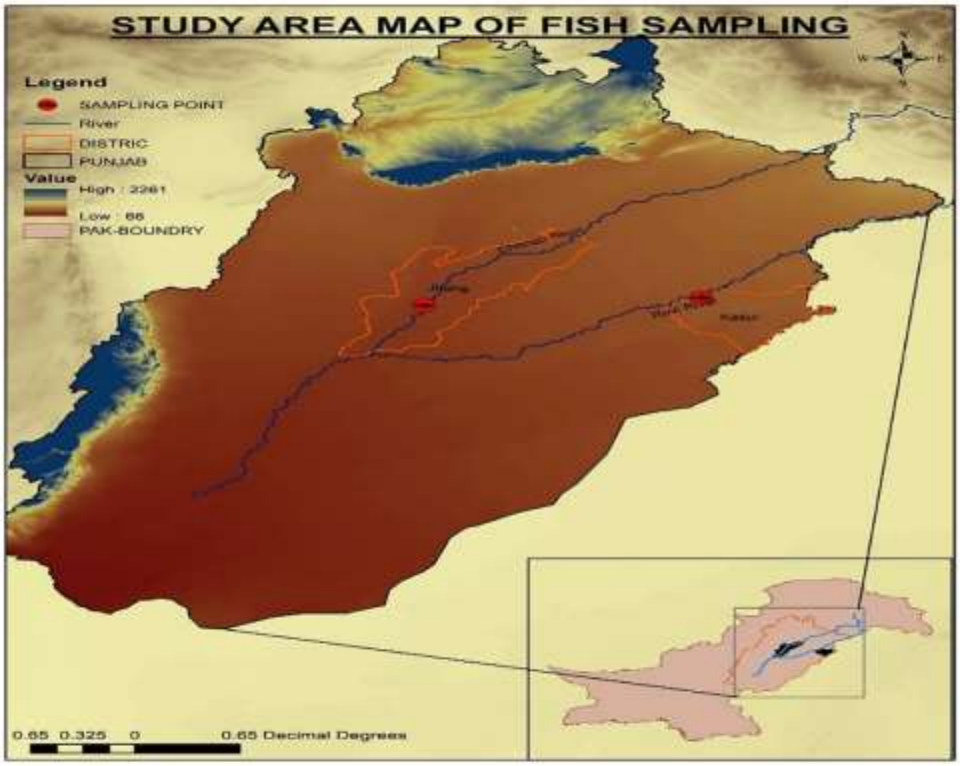

Figure 1: Pictographic presentation of sampling sites

Figure 2: Specimen (*C. idella*) demonstrating morphometric landmarks

Figure 3(a): Dorsal View *of C. Idella*'s skull showing Landmarks

Figure 3(b Figure 3(a): Ventral View *of C. Idella*'s skull showing Landmarks

Figure 3(c): Lateral View *of C. Idella*'s skull showing Landmarks