



## STOCK IDENTIFICATION OF ENDANGERED CLOWN KNIFE FISH *CHITALA CHITALA* (HAMILTON-BUCHANAN, 1822) FROM INDIAN RIVERS INFERRED BY MORPHOLOGICAL ATTRIBUTES

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**Abstract:** Clown knife fish *Chitala chitala* (Hamilton Buchanan) belongs to the family Notopteridae, is an endangered species geographically distributed in India, Pakistan, Bangladesh, Nepal, Thailand and Indonesia. The population of fish has been declined more than 50% over the last 10 years and the species is categorized as endangered (EN) in India. The species is highly priced, cultivable and have high conservation significance and due to its high demand it has been declared as a “State Fish” of Uttar Pradesh, India. The purpose of the study was to determine the stock identification of fish using morphometric and meristic methods. Stocks of *C. chitala* from twelve different geographical locations were collected and studied well. It was observed that the stocks were differed in the body structure, pectoral fins and mouth shape from most of the population but meristic character was not much varied from each other. The ratio of measurements of various body parts are computed and described systematically in a comparative form. To obtain a reliable result on raciation of population of twelve different stocks of featherbacks, comparison of regression coefficient of correlation, analysis of variance were carried out for the first time. This study also provides a first report into the distinct morphological variation including descriptors in their early life stages.

**Keywords:** *C. chitala*, morphometrics, stock identification, conservation, India,

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### Introduction

Information on stock structure of an endangered fish is essential, as each stock must be managed separately to optimize its exploitation. Various methods have proven to be powerful tools for studying stock structure, such as morphometric comparison, protein electrophoresis and nucleotide sequence analysis (Ryman et al. 1984, Ferguson et al. 1995). Morphometric variability between different geographical populations can be attributed to distinct genetic structure and environmental conditions (Waldman et al. 1988, Mahindra et al. 2007). The variation between stocks can pro-

vide a basis for stock structure and might be more applicable for studying a short-term environmentally induced variation. The morphological attributes of an organism are not autonomous, and changes in variation aspects of morphology are coordinated (Zelditch et al. 1992). This morphological variation depends upon the anatomical, functional, ecological, behavioral and evolutionary factors. Morphology of fish is regularly interpreted as a set of physical attributes linked to the use of habitat and food resources. When morphology and resources use have been investigated morphometric measurements were typically lim-

ited to selected body structure such as fins and body shape. These measurements tend to concentrate on specific regions of the body, such as head and caudal peduncle, causing analyses to be biased towards specific body regions. Morphological variations have indicated a relationship between habitat use and morphometry.

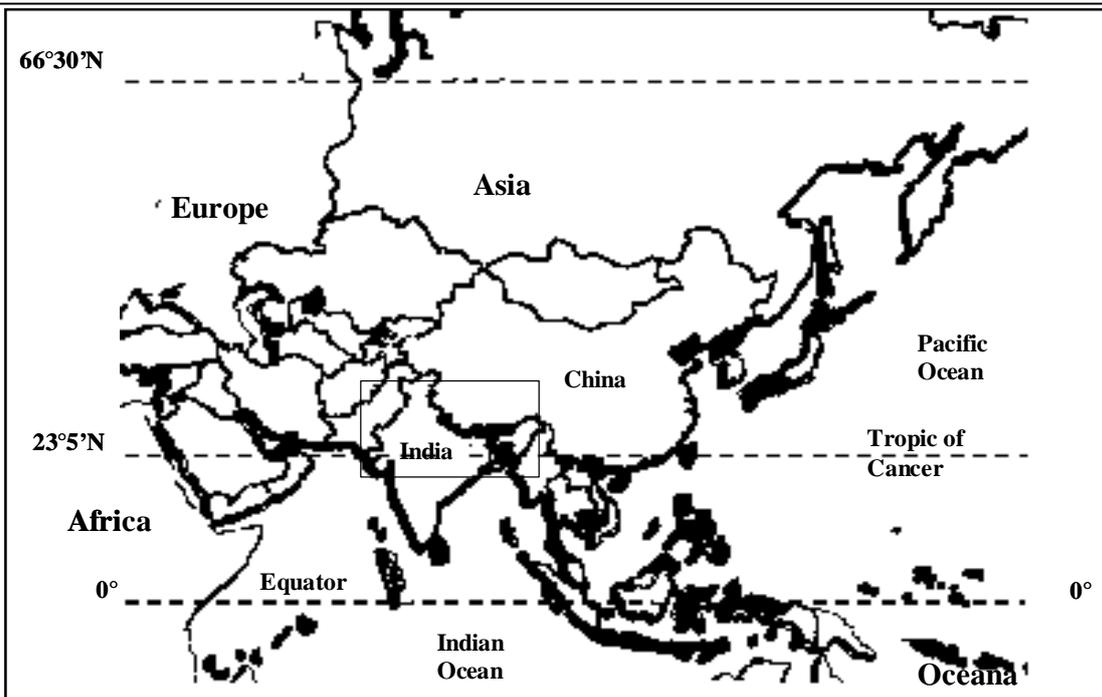
The analysis of morphometric and meristic characters has been widely used by ichthyologists to differentiate among different species and among different populations within a species (Ihsen et al. 1981, Vidalis et al. 1997, Tudela 1999, Murta 2000) and continue to be used successfully (Tzeng 2004, Ognjanovic et al. 2008). Studies of morphologic variation among populations continue to have an important role to play in stock identification, despite the advent of biochemical and molecular genetic techniques which accumulate neutral genetic differences between groups.

*C. chitala*, which is commonly known as clown “knife fish”, identify easily with a very long anal fins. The back is a small slender dorsal fin, which the fish derives the name “featherback”. It is distributed in tropical Africa and South-east Asian countries. The flesh is of good flavor and commercially important and has been prioritized as food, sport, aquarium, and highly priced cultivable fish (Sarkar et al. 2006). Out of total landings of freshwater fishes feather back contributes only 5%. Based on the CAMP report, (1998) the population of fish has been declined more than 50% over the last 10 years and the species is categorized as endangered (EN) as per International Union for Nature and Natural Resources (IUCN) criterion. Though some of the biological parameters (age profile and reproduction, captive breeding) of this fish have been investigated (Sarkar et al. 2006, Sarkar et al. 2008), no worthwhile attempt towards current status of stock structure as well as pattern of morphological characters during its early

life stage(ELS) from different populations has been made yet. Hence, the objective of the present study are to assess morphological and meristic variables of different Indian wild stocks in order to see the heterogeneity within the different locations and to establish different stocks which will be helpful in conservation and management for their sustainable utilization. The morphological information of the ELS of fish was also studied from natural and captive bred population with the aim to document various attributes which could be useful for stock identification.

## Material and Methods

The specimens of *C. chitala* were sampled from twelve different populations using various fishing methods, drag nets, cast nets, gill nets of different mesh size and the collections were made during 2000 to 2005. The location of sample collection sites of *C. chitala* is shown in figure 1. The abbreviations used for different body parameters etc. is shown in appendix I. The locations were Samaspur Bird Sanctuary (SBS, 25. 97<sup>0</sup>N, 81.670E. n=24), Katraniaghat Wildlife Sanctuary (KWS, 25. 97<sup>0</sup>N, 25. 97<sup>0</sup>E; n=32), river Ghagra (26<sup>0</sup>01'N, 83<sup>0</sup> 22'; n= 14), Ganga (Kanpur, 26<sup>0</sup> 19'N, 78<sup>0</sup> 04'E; N=38), Gomti (Lucknow, 25.90<sup>0</sup>N 82.56<sup>0</sup>E n=14), Bhagirathi(24<sup>0</sup>05'N, 88<sup>0</sup>06'E; N= 43), Farakka (24.53<sup>0</sup>N, 88.10<sup>0</sup>E; n = 15), Saryu (26.12<sup>0</sup>N, 82.450E; n=2), Satluj (31<sup>0</sup> 09N, 74<sup>0</sup>56' E; n=13), farm (24.92<sup>0</sup>N, 88.14<sup>0</sup>E; n = 21) Ganga (Rajmahal, 24. 80<sup>0</sup>N, 87.93<sup>0</sup> E; n= 22), Yamuna (25.41<sup>0</sup>N,81.91<sup>0</sup>E, n=2). SBS was the open lake while in KWS river Gerua flows through the Sanctuary area. The Riverine locations were chosen to cover geographically distant populations. Altogether 250 specimens ranging from 32 cm to 100 cm in length and 750g to 20,000 g in weight. were collected and studied.



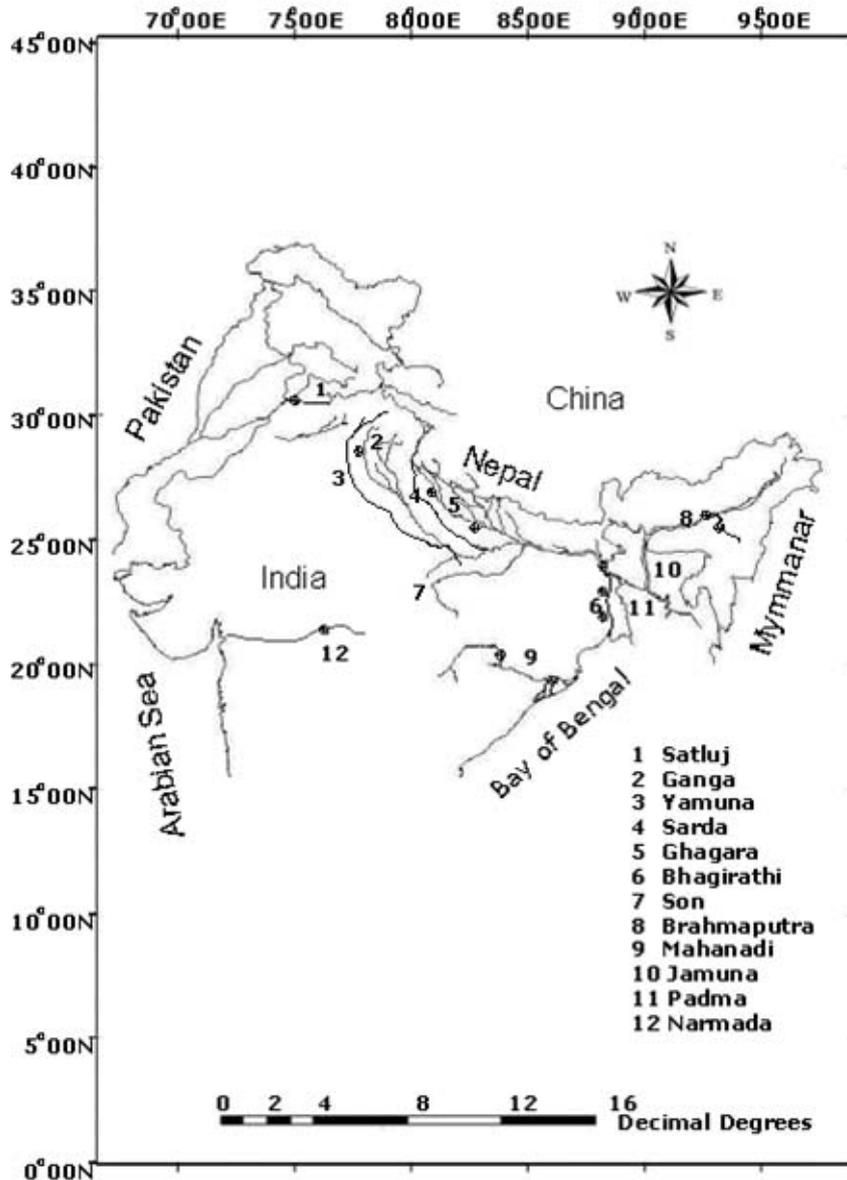
**Figure 1 (a).** General map of the region, study area is located within the box.

Collections were made at random intervals because of their low population density and periodic arrivals. Due to its low availability the number of samples was limited and they were collected depending on the availability. Representative fish samples were preserved in 10% formaldehyde kept in plastic fish carrier and transferred to laboratory for further use. Fish samplings were carried out by gillnet and cast net in protected water bodies. While for riverine collection fish were directly purchased from landing site in fresh condition. Sometime help from local fishermen were taken to directly net the fish.

Samples of ELS were collected from captive bred population developed by the authors (Sarkar et al. 2006) and collected from a fish farm and the riverine population was collected from river Bhagirathi, West Bengal. Five set of nets (8 – 10' having circular opening diameter of 1.5 – 2') were fixed side by side,

towed with bamboo poles in the shoreline area of main river where water flow was low and average depth was 1.5 m. The total number of larvae per net was ranged from 1000 – 4000 per 5 – 10 hours. Fresh samples were measured for the all the morphometric characters and meristic character upto nearest millimeter (mm) and weighed to the nearest 0.01 gram (g) by the help of digital caliper and electronic balance. The morphometric characters and other details during ELS were recorded up to 65 days.

A total 20 morphometric variables of fish from different wild population were collected. For all the major morphometric characters, means were estimated along with the corresponding standard deviation and comparison of each character was made by using multiple analyses of variance to determine the significance variation within the different locations. Comparison tests were performed using



**Figure 1 (b).** Locations of sampling station ( ) across different river basins for stock structure study of *C. chitala*. Out of the 12 rivers listed in the figure, 8 rivers were sampled, with multiple locations within some rivers.

t test. Mean values of TL, SL, BD, TW were computed. The corresponding ratio between the major morphometric variables was worked out in order to determine the variations between different wild populations. The different abbreviations used are as follows;

Total length (TL)- distance in a straight line between the anterior most parts of the body to the tip of the tail.

Standard length (SL) – Tip of snout to the end of the last caudal vertebra.

Body depth (BD)- Insertion of first dorsal fin to the insertion of pelvic fin

Head depth (HD)- Measured perpendicular to the main axis of the body from the top of the head through the center of the eye to the lower jaw.

Head length (HL) - Tip of the snout to posterior tip of the operculum.

Length of snout (LOS): Distance from the anterior most part of the body to the anterior margin of the orbit.

Eye diameter (ED) - Measured through the center of the eye parallel to the main axis of the body.

Predorsal length (PDL): Distance from anterior most part of the body to the first dorsal fin rays.

Interorbital distance (IOD): The distance between the upper margin of the right orbit to the upper margin of the left orbit as measured from the dorsal surface. This measurement is also called as the least distance between the two orbital.

Length of dorsal fin (LDF): Distance between the base of the dorsal fin and the tip of the largest fin ray.

Length of pectoral fin (LPF): Length of the longest fin rays from the base of the pectoral fin.

Length of anal fin (LAF): Length of the longest ray from the anterior insertion of the anal fin.

Length of ventral fin (LPVF): Length of the longest ray from the anterior insertion of the ventral fin.

Hump distance (HUMD): Measured through the maximum elevation from head to the lower jaw of the fish.

Width of mouth (MW): Measured maximum opening of the mouth

ELS : Early life stage, LLS: later life stages, KWS : Katraniaghat Wildlife Sanctuary, SBS : Samaspur Bird Sanctuary, d : day

The regression values of dependent variable such as SL, HL, BD, ED, LDF, LPF, HD, LOS, IOD and MW with respect to TL and HL of all the population were calculated. Statistical analysis of data was done using statistical software SPSS Base 10.0 user's guide.

## Results

### Morphological variables and development of ELS

Unfertilized eggs were white in colour and opaque in appearance. The data of the morphological attributes of the ELS is presented in table 1. Fertilized eggs were milky pale yellow in colour, transparent in appearance and spherical in shape with 4.01 to 5.05 mm in diameter and were adhesive in nature. The eggs collected from natural waters, was milky white in colour and egg diameter was ranged from 0.045 – 0.129 mm with a mean of 0.097 mm. The average egg diameter of fertilized eggs was 4.10mm. The first cleavage was observed about 50 h of fertilization resulting in the formation of two uniform blastomeres. The second cleavage followed approximately four hours later and third cleavage was after 54 h and the third after 68 hrs of fertilization. The sixteen-celled stage was observed in about 75 h and thirty-two celled stage followed in next 80 h of fertilization. The blastoderm assumes a dome shaped and gradually spreads over the yolk mass attaining the morula stage in about 85 h after fertilization. At about 90 h, blastoderm cells cover about one half of the yolk mass and the yolk plug stage were noticeable after 95h.

**Table 1. Morphometric characters (mean  $\pm$  Sd ) of early life stages of *C. chitala*.**

Characters	Days							
	5-6	7-8	9-10	12-13	18-20	24-25	40-45	60-65
Total length (mm)	12.5 $\pm$ 1.9	12.57 $\pm$ 0.97	12.80 $\pm$ 1.14	13.34 $\pm$ 1.08	22.28 $\pm$ 1.06	37.09 $\pm$ 1.9	39.73 $\pm$ 1.86	50.50 $\pm$ 0.09
Total weight (gm)	0.03 $\pm$ 0.005	0.09 $\pm$ 0.007	0.09 $\pm$ 0.002	0.092 $\pm$ 0.006	0.094 $\pm$ 0.008	0.15 $\pm$ 0.009	0.57 $\pm$ 0.006	0.80 $\pm$ 0.008
Standard length (mm)				10.6 $\pm$ 1.20	20.3 $\pm$ 1.16	35.2 $\pm$ 1.12	37.52 $\pm$ 1.50	44.74 $\pm$ 1.1
Head length (mm)				3.86 $\pm$ 0.35	5.50 $\pm$ 0.38	7.68 $\pm$ 0.09	10.9 $\pm$ 0.08	12.41 $\pm$ 0.45
Body depth (mm)				4.29 $\pm$ 0.17	4.94 $\pm$ 0.21	7.90 $\pm$ 0.30	8.77 $\pm$ 0.51	12.57 $\pm$ 0.36
Head width (mm)					5.68 $\pm$ 0.89	6.95 $\pm$ 0.9	7.10 $\pm$ 0.79	8.06 $\pm$ 0.92
Eye diameter					1.44 $\pm$ 0.51	2.14 $\pm$ 0.6	2.56 $\pm$ 0.65	2.62 $\pm$ 0.71
Inter orbital distance					3.21 $\pm$ 0.07	3.86 $\pm$ 0.081	4.09 $\pm$ 0.081	4.48 $\pm$ 0.08
Mouth gape					1.321 $\pm$ 1.02	1.389 $\pm$ 1.05	2.58 $\pm$ 1.06	2.82 $\pm$ 1.07
Length of dorsal fin					2.90 $\pm$ 0.08	3.95 $\pm$ 0.091	4.38 $\pm$ 0.098	5.78 $\pm$ 0.68
Length of pectoral fin					3.12 $\pm$ 0.04	4.14 $\pm$ 0.05	5.28 $\pm$ 0.06	7.35 $\pm$ 1.2
Length of anal fin						22.36 $\pm$ 1.52	25.26 $\pm$ 1.41	33.79 $\pm$ 0.5
Snout length						2.84 $\pm$ 0.56	3.85 $\pm$ 0.57	3.46 $\pm$ 0.36
Insertion of dorsal fin					10.91 $\pm$ 0.09	12.32 $\pm$ 0.08	20.99 $\pm$ 0.07	28.95 $\pm$ 0.92
Number of dorsal bands							12 $\pm$ 2	13 $\pm$ 1
Diameter of yolk sac	4.07	3.74	3.82	absorbed	absorbed	absorbed	absorbed	absorbed
Appearance of scale							Appeared	

The yolk sacs get compressed at about 100 h and the embryonic rudiments were noticeable approximately 105 h after fertilization. Cephalic and caudal ends were faintly discernable after about 110 h of fertilization. When the embryo gets 115 h old the cephalic region becomes prominent and rudiments of notochord and arteries appeared. Yolk mass showed elongation and slow twitching movement was noticeable when the embryo was 120 h old. The yolk showed gradual elongation as the development progressed and the embryo executed vigorous twitching movement. Water temperature at the range of 27 – 30°C, the period of incubation was 5-6 days. However, it took about 7 days for the whole brood of egg to hatch out. At this stage, just before hatchling, comma shaped notochord can be seen.

**Newly hatched larva (1-3 d):** Just hatched out larva was measured to be 5.00 mm in length and 0.03g in weight. It gets attached with hard substratum and possesses conspicuous yolk sac beset with a network of blood capillaries. The diameter of the yolk sac was 4.05 mm.

**5-6 d :** The total length and weight of the larvae was 12.5mm  $\pm$  1.9 and 0.08g  $\pm$  0.05 respectively. The diameter of yolk sac was 3.85mm. The eye diameter was 0.40 mm. Rudimentary head with deep yellow coloration appeared on the yolk surface. The colour of body was dark pink and adhesive on the hard surface. The mouth appeared, upper and lower jaw were clearly visible. They feed on the mucous released by the parent fish during the parental care.

**7-8 d:** The larvae were 12.57 mm  $\pm$  0.97 in length and 0.092 g  $\pm$  0.007 in weight. The diameter of the yolk sac was 3.64 mm at this stage. The head length and eye diameter was 2.8 and 0.55 mm respectively. The mouth was prominently developed, eye was clearly visible and notochord rudimentary. The alimentary canal became thicker and convoluted. Head still remain adhere with yolk sac.

**9-10 d:** The length and weight at this stage

was 12.34 mm  $\pm$  1.08 and 0.097 g  $\pm$  0.05 respectively. The head length and eye diameter was 2.73 and 0.75 mm respectively. The diameter of yolk sac was 3.42 mm and deep yellow in colour. Yolk sac became smaller and oval in shape. Head well developed, notochord increased and anal fin slightly visible. At this stage larvae started to get detached from the hard substratum.

**12 -14 d:** Average length and weight at this stage was 13.8 mm  $\pm$  1.14 and 0.0985 g  $\pm$  0.06 respectively. Yolk sac was absorbed by most of the larvae. In some cases it appeared very small in size and oval in shape. Body colour of the larvae was light brown with clearly visible notochord. All the fins like dorsal, pectoral and anal were clearly visible but rays were not cleared and ventral fins were rudimentary developed. Abdominal portion was more segmented than earlier stage. The larva has four gill arches in the opercular cavity. Air bladder had got developed. The eyes were shining, mouth was terminal with nearly straight large cleft reaching mid ventral orbit. Lateral line appeared on the lateral side of the body with some dark pigmentation.

**18 - 20 d:** Dorsal, pectoral and anal fins got well developed with rudimentary rays. The length and weight recorded at this stage was 22.28 mm  $\pm$  1.06 and 0.099 g  $\pm$  0.08 respectively. The body depth increased with 4.79 mm  $\pm$  0.21 in length. Gills were well developed and post larvae started free swimming. They showed shoaling behavior and preferred to settle in dark. Yolk sac completely absorbed and they accept exogenous feed. Scales did not appear at this stage. Several dark and light bands were present through out the body surface. Early fry moved very fast and in laboratory condition performed up and down movement, engulfing atmospheric air, while during night the early fry moves round the wall of the plastic pool. It exhibited cannibalistic attitude if they hungry. The early fry were reared successfully in 100 liter of plastic pool with about 4 - 5 thousand stocking density.

**25 - 30 d:** At this stage, fry was 22.68 mm

$\pm 1.30$  in length and  $0.12 \text{ g} \pm 0.03$  in weight. The body depth was  $4.44 \text{ mm} \pm 0.017$ . The pectoral, pelvic, dorsal and anal fins developed with 7 - 8, 4 - 5, 5 - 7, and 30 - 60 fin rays. Dorsal profile was more humped than the larval stage. Transverse bar along the dorsal ridge, relatively prominent than earlier stage. Dark spots also appeared 2 - 12 in numbers near the tail and along the anal fin base.

We also observed the morphology of the ELS of *C. chitala* collected from river Bhagirathi. The unfertilized eggs collected from rivers, appeared milky white in colour and their diameter was ranged from 0.045 - 0.129 mm with a mean of 0.097 mm. The mean diameter of fertilized egg was  $4.10 \pm 0.06 \text{ mm}$  and it was yellow in colour. They were sticky in nature and mean length and weight of 25 days old stage was  $22.6 \text{ mm} \pm 1.29 / 0.11 \text{ g} \pm 0.01$  and  $22.28 \text{ mm} \pm 1.25 / 0.093 \text{ g} \pm 0.01$  for two separate collections. The external yolk sac was fully absorbed at this stage and colour of the fry was light brown with small black spots on the anterior part of the body. The dorsal fin originated from middle of the body. Dorsal, anal and pectoral fins were also fully developed with rudimentary rays. A short alimentary canal was visible with small appearance of stomach. Gills were fully developed with four-gill slits. Dorsal profile was slightly arched near the head region and some dark pigmentation was present on the anterior portion of the body and operculum of the fry.

#### **Morphological variables of the later life stages (LLS)**

The body of *C. chitala* is oblong and strongly compressed and finely scaled. Head compressed, its length was about 4.2 times in standard length. Head carernous with the large membranous operculum flap. Mouth wide, teeth small on both the jaws, also on palate and tongue. Eyes large and dorso-lateral in position. Dorsal fin small, placed in the center of back, anal fin long confluent with small caudal fin. Anal fin razor sharp with 100-130 rays, while pelvic fin rudimentary. Vent placed far

forward, abdomen edge serrated with about 25-30scutes. Maxilla extends considerably beyond posterior edge of eye pre orbital smooth, a row of about 15-19 silvery bars on the back. Colour in life coppery brown, flanks silvery fins with dark blotches. This species can be distinguished from its congener. *Notopterus notopterus* by its strongly humped dorsal profile, which declines gradually from the front to the tail, marking along the dorsal ridge and black spots near the caudal fin, are the characteristics features of *C. chitala*. Number of characteristic black spot on the anal fin as well as posterior part of the body was also counted. There was no fixed number of black spots. In most of the cases this spot was either present or absent on both side of the anal fin and posterior region of the body. The number of black spots in the present study was varied from 0 - 16 on the left side and 1 - 19 on the right side. Similarly, the number of silvery bands present on the body decreased, as the fish grew adult. In adult the number of transverse bands were recorded in the range of 16 -18.

#### **Ratio of body parameters and their relationship**

The ratio of dependent variables of fish such as standard length (SL), head length (LOH), body depth (BD), eye diameter (ED), length of dorsal fin (LDF), length of pectoral fin (LOPF), head depth (HD), length of snout (LOS), interorbital distance (IOD), mouth width (MW), with respect to total length (TL) have been presented in Table 2. There were significant variations in the ratios of the TL: SL, TL: TW, TL: LOPV, HD: ED, HD: MW and HD: HUMD. It was observed that the values of different morphometric ratios in relation to HL from the captive bred samples of farm were not different from riverine populations, while other parameters were significantly differed. The analysis showed that there were significant differences in river Bhagirathi , river Saryu and river Satluj . Similarly, a major difference in the ratio of TL and length of

dorsal fin in the samples of river Bhagirathi, river Gomti and SBS was recorded. Significant differences ( $p < 0.05$ ) were observed in TL and length of pectoral fins for river Gomti, and SBS and in the ratio TL: LAF for KWS and river Bhagirathi. Among other body proportions significant differences of the ratio of head depth and eye diameter (HD: ED) ( $p < 0.05$ ) in KWS, river Ganga (Kanpur), river Saryu, SBS and river Bhagirathi, in the ratio of HD: HUMD of all except river Gomti. The ratio of HD: LOS for river Bhagirathi, river Ganga (Farakka) and river Gomti also showed significant variation. The regression analysis of dependent variables such as SL, BD with respect to TL and other dependent variables such as head depth, eye diameter, length of snout, interorbital distance and width of snout in relation to HL i.e. independent variables are shown in Table 3 and 4. The values between TL and SL, showed more differences in the sample of river Satluj ( $r = 0.54$ ) while the values between TL and BD, showed little differences between the river Gomti (0.71) and river Ghagra (0.86). While comparing the regression value with length of head and the head depth, there was difference in the values of river Satluj (0.73) and river Ganga at Kanpur (0.77). While comparing the regression value with eye diameter it was observed that there were differences from river Ganga, Kanpur (0.75) and river Satluj (0.71). Likewise, regression of length of snout from river Satluj (0.74) differs from other population. While comparing the regression value of interorbital distances, there were differences in the in KWS (0.72), river Saryu (0.73) and river Kosi (0.75). The width of mouth varies from other populations in KWS (0.71), river Saryu (0.67) and river Kosi (0.65). It was also observed that the regression value of different morphometric ratio in relation to length of head from the farm samples were not different from other populations (Table 4). The regression value shows negative differences for the population

of Bhagirathi and Gomti for HL: HD. There is much difference in regression value of HL: ED for the population of Satluj and Koshi and HL: LOS between all the populations. Negative regression value of HL: IOD of the location Bhagirathi and Koshi and no much difference were recorded in the regression value of HL: MW from all the population except samples from River Ghagra at Mahmoodabad. In some cases (Malda population) there is negative  $r$  value between TL and LDF followed by TL and SL. Regression value of TL and BD was maximum in KWS (0.986) followed by Koshi (0.974), Kanpur (0.968), Saryu (0.964), Farakka (0.963), Bhagirathi (0.962), SBS (0.959), Malda (0.937), Satluj (0.909), Ghagra (0.800) and lowest in Gomti (0.715). Regression values between TL and SL was maximum in KWS (0.998) followed by Farakka (0.997), Farm (0.997), Kanpur (0.992), Bhagirathi (0.991), Saryu (0.979), Ghagra (0.973) SBS (0.970), Gomti (0.955), Satluj (0.545) and lowest in Koshi (-0.004)

Analysis of variance (ANOVA) was tested for the different body parameters of *C. chitala* like to find out significant difference. Significant differences were observed between Bhagirathi, Saryu, Satluj and farm population, Farakka is significantly different from the farm population and Satluj. Population of Gomti is significantly differs from Ghagra, Satluj and farm samples. Population of Saryu is significantly different from Ghagra, SBS, farm and Bhagirathi while Ghagra is differing only from Koshi. There is a significant difference in population of SBS to Satluj and farm. The ratio of TL and SL of river Satluj is significantly different ( $p < 0.05$ ) with all the population except SBS. There is significant difference in the ratio of TL and LDF between all the populations. However, there is no significant difference between total length and length of pectoral fin except population of Gomti and SBS.

**Table 2: Comparative results of morphological variation (mean  $\pm$ SD) in *Chitala chitala* a, b, c, d, e indicate significant difference ( $p < 0.05$ ).**

River and Locations	TL: BD	TL: SL	TL: LDF	TL: LPF	TL: LPVF
Bhagirathi (WB)	3.60 $\pm$ 0.19 <sup>b</sup>	1.08 $\pm$ 0.05 <sup>c</sup>	12.28 $\pm$ 1.13 <sup>d</sup>	9.40 $\pm$ 2.00	71.49 $\pm$ 24.50
Ganga (Farakka)	3.61 $\pm$ 0.28	1.10 $\pm$ 0.08	11.37 $\pm$ 1.53	9.46 $\pm$ 1.87	73.63 $\pm$ 21.3
Gomti	3.48 $\pm$ 0.32	1.09 $\pm$ 0.03	10.79 $\pm$ 0.63 <sup>d</sup>	9.85 $\pm$ 0.79 <sup>e</sup>	65.86 $\pm$ 11.8
Gerua (KWS)	3.39 $\pm$ 0.07 <sup>b</sup>	1.07 $\pm$ 0.03	11.98 $\pm$ 2.29	9.87 $\pm$ 1.62	70.35 $\pm$ 10.5
Ganga (Kanpur)	3.57 $\pm$ 0.44	1.09 $\pm$ 0.07	3.57 $\pm$ 0.44	9.34 $\pm$ 1.83	64.50 $\pm$ 18.0
Saryu	3.40 $\pm$ 0.27 <sup>b</sup>	1.14 $\pm$ 0.07 <sup>c</sup>	9.48 $\pm$ 1.10	9.10 $\pm$ 2.39	64.96 $\pm$ 16.0
Ghagra	3.79 $\pm$ 0.27 <sup>b</sup>	1.09 $\pm$ 0.04	7.8 $\pm$ 1.0	8.5 $\pm$ 1.0	65.2 $\pm$ 10.2
SBS	3.69 $\pm$ 0.39	1.14 $\pm$ 0.10	9.23 $\pm$ 2.69 <sup>d</sup>	8.63 $\pm$ 1.82 <sup>e</sup>	79.12 $\pm$ 11.1
Satluj	3.29 $\pm$ 0.16	1.21 $\pm$ 0.14 <sup>c</sup>	10.2 $\pm$ 1.2	9.1 $\pm$ 1.0	69.3 $\pm$ 12.3
Koshi	3.48 $\pm$ 0.25 <sup>b</sup>	1.07 $\pm$ 0.03	10.3 $\pm$ 1.3	8.9 $\pm$ 1.1	66.2 $\pm$ 11.6
Farm	3.22 $\pm$ 0.09	1.07 $\pm$ 0.02	10.1 $\pm$ 1.5	9.1 $\pm$ 1.0	65.3 $\pm$ 10.6

No significance difference observed between the ratio of TL and length of pelvic fin except Bhagirathi to Gomti, KWS, Kanpur and Saryu and SBS. No significant differences of HD: ED was observed in Bhagirathi, Farakka, and Gomti from KWS, Ganga (Kanpur), Saryu and SBS. In case of HD: MW significant difference recorded for Bhagirathi and Farakka only and between Farakka to KWS while no significant difference was noticed between Gomti, Kanpur, Saryu and SBS. Significant differences ( $P < 0.05$ ) of ratios (HD: HUMD) from the population of Bhagirathi vs. Gomti, Farakka vs Gomti and Gomti vs. Saryu and SBS. There is significant difference in the ratio of TL with HL for river Bhagirathi and Gomti, river Gomti and Ganga, (Kanpur), Gomti and SBS. Significant difference between the ratio of HL and hump distance in KWS to Farakka and KWS to Saryu was also observed. Among other ratios significant difference between the ratio of HL and length of snout with all the locations was observed.

#### Meristic characters

The details of the meristic counts from different populations are presented in table 5. The general fin formula of *C. chitala* reported by Day (1889) was D 9 - 10 (1-2/7-9), P 16, V6, A 110-125 (135), C 12-14, LI 180. However, samples from different sampling locations showed little deviations from the general fin formula. Dorsal fin varies from 5 - 10 (1-2/4-8). The dorsal rays were generally 5-8 from all the locations except river Bhagirathi (7 - 10). There was no variation in pectoral fin and ventral fins. Number of pectoral rays was 8 - 16, and ventral rays varied from 3 - 6 from all locations. Anal rays varied from 122 - 131 from all the locations. Considerable differences were noticed in the lateral line scale from river Ganga (Kanpur) and Koshi, while the samples from other locations showed no differences. Number of dorsal spine and dorsal rays showed no differences like wise pectoral spine and fin rays also showed no any differences.

**Table 3. Comparative regression for different morphometric ratios in relation to total length(TL) of *C. chitala***

Location/River	TLX SL			TLX BD			TLX TW		
	a	b	r	a	b	r	a	b	r
River Bhagirathi	-0.04	1.00	0.99	-0.37	0.93	0.96	-5.35	3.06	0.99
River Ganga (Farakka)	-0.05	1.00	0.99	-0.34	0.92	0.96	-4.90	2.92	0.98
River Gomti	0.45	0.82	0.95	0.48	0.62	0.71	-3.38	2.37	0.88
Gerua(KWS)	-0.07	1.01	0.99	-0.58	1.01	0.98	-5.28	3.05	0.99
River Ganga (Kan- pur)	0.01	0.98	0.99	-0.67	1.04	0.96	-6.04	3.32	0.98
River Saryu	-0.31	1.09	0.97	-0.79	1.09	0.96	-6.09	3.33	0.98
River Ghagra	-0.24	1.07	0.97	0.36	0.65	0.86	-5.26	3.02	0.98
SBS	-0.64	1.21	0.97	-1.10	1.19	0.95	-6.51	3.49	0.99
River Satluj	0.72	0.71	0.54	-0.26	0.90	0.90	-8.27	4.10	0.92
River Koshi	-0.65	1.06	0.98	-0.58	0.47	0.94	-5.14	3.01	0.99
Farm	-0.11	1.02	0.99	-0.58	1.02	0.93	-5.54	3.15	0.97

**Table 4: Regression analysis for different morphometric ratios in relation to length of head (HL) of *C. chitala***

Locations/River	HD			ED			LOS			IOD			MW		
	a	b	r	a	b	r	a	b	r	a	b	r	a	b	r
River Bhagirathi	2.03	0.05	0.91	- 0.15	0.61	0.88	0.31	0.39	0.81	2.59	0.57	0.85	0.67	0.42	0.83
River Ganga (Farakka)	0.01	0.93	0.87	0.71	0.20	0.95	- 0.07	0.61	0.91	- 1.43	1.31	0.84	0.73	0.31	0.75
River Gomti	2.08	0.18	0.82	0.58	0.23	0.92	- 0.93	0.99	0.98	0.74	0.23	0.92	0.13	0.51	0.85
Gerua(KWS)	0.69	0.49	0.86	0.36	0.34	0.77	0.41	0.32	0.84	0.26	0.84	0.72	0.73	0.11	0.71
River Ganga (Kanpur)	1.37	0.24	0.77	0.46	0.28	0.75	0.99	0.04	0.90	1.16	0.06	0.74	0.54	0.36	0.85
River Saryu	-0.88	1.36	0.96	- 0.14	0.61	0.81	- 0.19	0.67	0.91	- 0.71	0.95	0.73	- 0.67	1.01	0.67
SBS	-1.11	1.45	0.98	0.33	0.35	0.83	- 1.28	1.17	0.96	0.29	0.57	0.83	- 1.61	1.47	0.82
River Ghagra	-0.26	1.06	0.92	0.69	0.18	0.79	0.92	0.09	0.84	0.89	0.27	0.90	1.43	0.04	0.84
River Satluj	0.56	0.66	0.73	1.26	0.09	0.71	- 0.02	0.59	0.74	0.10	0.63	0.86	0.52	0.48	0.77
River Koshi	1.60	0.17	0.97	0.53	0.26	0.88	0.62	0.25	0.83	0.78	0.30	0.75	1.04	0.21	0.65
Farm	0.15	0.87	0.93	2.00	0.03	0.90	0.67	0.12	0.85	1.38	0.02	0.83	1.31	0.05	0.82

Table 5. Meristic counts in *C. chitala* from different locations

Locations/River	No. of Dorsal Spine	No. of Dorsal Rays	No. of Pectoral Spine	No. of Pectoral Rays	No. of Ventral Spine	No. of Ventral Rays	No. of Anal Spine	No. of Anal Rays	Lateral Line Scales	No. of Black Spots	No. of Dorsal Bands
Gerua(KWS)	(1-2)	(4-6)	(1--2)	(12-14)	0	(3-5)	(1-3)	124.5 ± 3.9	181 ± 11.24	2-4L/3-9R	17.29 ± 0.75
SBS	(1-1)	(4-5)	(1-1)	(11-14)	0	(4-6)	(1-2)	124.66 ± 2.7	170.8 ± 9.12	1-3L/0-5R	16.80 ± 1.7
Saryu	(1-2)	(5-7)	(1-2)	(10-12)	0	(4-5)	0	126.91 ± 1.7	162.16 ± 7.96	0-4L/2-5R	16.75 ± 0.62
Bhagirathi	(1-2)	(6-8)	(1-1)	(08-15)	0	(3-5)	(1-3)	127.06 ± 5.6	158.36 ± 10.36	0-6L/3-6R	17.66 ± 1.12
Ganga (Farakka)	(1-2)	(5-6)	(1-2)	(11-14)	0	(3-5)	(2-3)	121.75 ± 2.4	147.54 ± 15.66	0-4L/-6R	17.00 ± 1.0
Ganga (Kanpur)	(1-2)	(4-5)	(1-1)	(12-13)	0	(4-5)	(1-2)	122.16 ± 7	149.55 ± 8.37	0-4L/0-4R	18.16 ± 1.0
Gomti	(1-2)	(5-7)	(0-2)	(10-16)	0	(4-6)	0	131.4 ± 4.8	166.2 ± 7.11	1-16L/1-19R	18.06 ± 1.66
Koshi	(1-1)	(4-4)	(1-1)	(12-13)	0	(4-5)	(2-3)	126.64 ± 6.8	141.92 ± 3.64	1-4L/0-5R	17.71 ± 0.82
River Satluj	(1-1)	(4-4)	(1-1)	(11-12)	0	(4-5)	(2-3)	122.64 ± 6.6	142.92 ± 2.64	1-4L/0-5R	16.71 ± 0.82
River Ghagra	(1-2)	(5-7)	(1-2)	(10-12)	0	(4-5)	0	126.91 ± 1.7	162.16 ± 7.96	0-4L/2-5R	16.75 ± 0.62
Farm	(1-2)	(5-7)	(1-2)	(8-10)	0	(4-5)	0	136.91 ± 1.7	164.16 ± 6.88	0-4L/2-5R	17.75 ± 1.62

There was no anal spine in the sample of river Saryu and Gomti while number of anal rays was varied from 121 – 132 for all the population.

## Discussion

This is the first report on the stock structure and pattern of phenotypic plasticity of *C. chitala* from different river of India. The ELS has been useful in identifying stocks in the context of genotypic, phenotypic and contingent stock concepts. In our study the morphological differences of the ELS (25 - 30 d) of fish collected from river Bhagirathi and captive bred population showed certain differences. The length of dorsal fin, pectoral fin, insertion of dorsal fin and body depth in riverine population was higher than that of captive bred which might be due to several factors (genetic or phenotypic?). Larval morphometrics have been used in stock identification similar to adult morphometrics. Harding et al. (1993) used seven morphometric measurements of American lobster larvae from the northwestern Atlantic Ocean and identified three spatial groups, inferring three phenotypic stocks. They also found a significant effect of temperature on the morphometric variables and extended phenotypic analysis closer to genotype stock identification. Olsen and Vollestad (2001) reported significant differences in ELS between the two populations of brown trout from the same stream, living either above or below an impassable waterfall and most of the ELS had an additive genetic component.

Morphometric and meristic characters of *C. chitala* of the later life stages showed significant phenotypic plasticity among the different samples indicating that migration among different wild stocks is limited. Considering the geographical distance and the different environmental condition among Katarniaghat sanctuary samples (KWS), Samaspur Bird Sanctuary (SBS), river Bhagirathi and captive bred fish farm is likely to be distinct however,

the body shape variation were in accordance with our expectative. Differences in the morphometric measurements and counts might be due to interference of various factors (racial, habitat specific, genetic). In this study, out of twenty one character eleven characters showed significant variation ( $p < 0.05$ ) includes total length (TL), standard length (SL) length of dorsal fin (LODF), length of pelvic fins (LOPV) height of anal fin (HAL), length of anal fin (LAF), head depth (HD), eye diameter (ED), mouth width (MW), hump distance (HUMD) and length of snout (LOS). On the basis of the calculated value, derived by regression equation, it can be inferred that Bhagirathi stock is morphologically differed from rest of the stocks studied which might be due to geographical isolation between the locations. The shape of the fish mouth has been small to influence its ability to detect and engulf the small prey items. The main feature associated with swimming of featherback from SBS was reduction in pectoral fin width. The general trend of variation of SBS fit the profile of population adapted to life in standing water habitat with abundant vegetation (Miguel Hermida *et al* 2005). The differences in characters may be attributed to the environmental conditions prevailing at different locations since fish exhibit a large component of environmentally induced variations, which might reflect different feeding or developmental environment. Therefore, environmental factors such as temperature, food availability, prolonged swimming movement, habitat structure may determine the phenotypic plasticity in featherbacks. The pattern of intersample variation may indicate reproductive isolation between the populations that would confirm the genetic basis of observed differentiation among the samples. Recently, in a another study related to age pattern of *C. chitala*, Sarkar et al (2007) reported maximum 6+ ages of *C. chitala* from four river basins namely river Bhagirathi, Koshi, Saryu and Ganga ( Kanpur ) while only 3+ age classes were observed in the other locations (river Ganga, Ra-

jmahal), river Gomti, river Satluj) indicating wide variation of the distribution of population structure across geographical range as has been reflected in age composition which might be due habitat alterations or overfishing etc. The variation of number of black spots and transverse bands in *C. chitala* might be due to differences in environmental and habitat attributes and needs further research on pigmentation mechanism in *C. chitala* to make any conclusion.

The findings of Savvaitova (1963) indicated that the Chars dwelling in the river differed from those of the lake in a number of morphometric characters in the feeding habits. He did not consider this character as reliable for classifying them into different natural stocks. Singh (1972), while conducting racial studies on *Rhinomugil corsula* from different environments found that only a few characters significant while most of them may not significant thus he considered, different stocks as a part of a single population. Similarly *C. chitala* stocks of different locations are found statistically significant and some of the characters found non significant. Therefore, based on the views of Savvaitova (1963), the population of *C. chitala* may be considered to comprise a homogeneous stock. Similarly, Wood and Bain (1995) reported morphological variation between microhabitat generalist and specialist species and suggested that all species may vary in morphology relative to their environment. Turan (2004) observed significant phenotypic plasticity of the morphometric and meristic counts among Mediterranean horse mackerel samples and suggested relationship between the extent of phenotypic divergence and geographic distance. They also stated that significant morphological differences do not necessarily demonstrate restrictions of gene flow among populations.

### Meristic characters

Meristic counts of *C. chitala*, in populations collected from different locations also slightly differed. The meristic characters are known to vary under the influence of environmental factors especially temperature during early life stage (Barlow, 1961). Natrajan *et al.* (1977) reported three-intra species population of *C. catla* from Rihand reservoir on the basis of size of the pectoral fins. Kristjansson (2005) opined that most of the freshwater population has apparently evolved after isolation of marine sticklebacks in freshwater. After colonization of freshwater habitats, they show rapid morphological changes and associated genetic isolation within few generations.

### Conclusions

Our study generated information on diversity in stock structure pattern from the rivers which is new to basic science and has implications in conservation biology of rapidly declining fish species like *C. chitala*. Morphological attributes are affected by genetic and environmental factors (Murta 2000). Therefore, morphological diversity can reflect genetic variations between stock and / or environmental/ habitat differences between geographical localities. Morphometric data can be used to identify samples from different rivers, even if in close proximity to each other, whereas, differences in meristic characters are less pronounced, but can be used successfully to discriminate samples from broad geographic regions. The consistency between the results indicates that they should be considered as complimentary and not alternative approaches to the same problem. In general traits associated with feeding and habitat structure were the most variable across the stocks, hinting at the actions of different selective regimes as well as input from environmental factors. A variety of methods should be used for reliable identification of fish stocks, since different methods may produce different results (Fourneir *et al.* 1984). The

results from this study should be compared with data obtained from other phenotypic and genetic studies using molecular tools to confirm stock identity of this important fish. Otolith chemistry is also increasingly used as a natural tag of chemical signature that reflects differences in the composition of the individual's habitat, and assess relative contribution of the potential nursery areas to mixed adult stocks (Campana and Thorrold 2001). Overall, *C. chitala* stock could be differentiated at certain level upon the morphological traits from population living in India provided additional support for the decision to list the fish as endangered.

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