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**RESEARCH ARTICLE**

# Establishment of base population for selective breeding of catla (*Catla catla*) depending on phenotypic and microsatellite marker information

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**Abstract.** The phenotypic and microsatellite marker information of nine strains of catla (*Catla catla*) for growth trait was used to infer relationship within and between strains. This information helped in optimizing the proportion of individuals to be used from each strain while creating a base population for selective breeding. For this purpose, nine strains were collected from different sources and places of India namely West Bengal, Bihar, Odisha, Andhra Pradesh and Uttar Pradesh. Two riverine sources i.e. Ganga and Subarnarekha were also represented among the nine strains collected for base population. They were brought to Indian Council of Agricultural Research-Central Institute of Freshwater Aquaculture (ICAR-CIFA) at fry stage and reared separately till fingerlings. After passive integrated transponder tagging fingerlings were stocked in three communal ponds for one year culture. Live body weights were then measured and least square means were obtained after pond effect correction. A wide range of variation was observed among and between strains. Microsatellite markers were used to estimate genetic differences of different strains of catla using pair wise $F_{ST}$ estimates. Overall multi locus $F_{ST}$, including all loci was estimated to be 0.4137 ($P < 0.05$), indicating genetic heterogeneity among them. Analysis of molecular variance revealed that, 58.63% of variation was due to within individual variation, 3.45% of variation was due to among individuals within strain and 37.92% was due to among strain variations. Both phenotypic as well as microsatellite data will be used to form a base population of catla with individuals from the stock having broad genetic variation for selective breeding programme.

**Keywords.** base population; selective breeding; growth trait; microsatellite markers; *Catla catla*.

## Introduction

Carps have emerged as the largest contributor in world freshwater fish farming, 95% of which is contributed by South and Southeast Asia. Presently, India ranks second in the world after China in being the major producer of fish and second largest aquaculture nation in the world. The total fish production during 2015–2016 (provisional) is at 10.79 million metric tonnes (MMT) with a contribution of 7.21 MMT from the inland sector and 3.58 MMT from the marine sector. The fish production has increased from 3.84 MMT in 1990–1991 to 10.79 MMT in 2015–2016 (DAHDF 2016). The freshwater aquaculture production of the country has been dominated by carps and the three major Indian carps namely catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) contribute significantly to the aquaculture productivity. Carps are affordable protein source for poor consumers and sustain both inland capture and aquaculture. It can be cultured with integrated systems to reduce the financial risks and maximize food security in the country.

Catla is the second most important Indian major carp after rohu and gaining popularity due to certain characteristics such as fast growth, taste and market demand. Catla is indigenous to major river systems of India, Pakistan and Bangladesh and is of high worth for aquaculture

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production in India and the whole Indian subcontinent. The induced breeding technology for this important species has been standardized. At the same time, seed from the natural sources is decreasing day by day. This stimulated establishment of large number of hatcheries producing large amount of seeds. With the rapid expansion of aquaculture, entire seeds of catla used for culture are produced at public and private rearing facilities and fry are subsequently distributed to fish farms. The profit driven private hatchery owners have focussed mainly on quantity of spawn produced than the quality. Usually, less number of relatively small-sized broodfish is maintained to minimize production cost resulting in poor quality fish seed with the characteristic poor survival and slow growth as well as disease susceptibility. Effective population size of hatchery reared catla through demographic method was observed to be as low as 3.8–16.0 in different hatcheries of India (Eknath and Doyle 1990).

Seed is the primary input for the aquaculture production system, and therefore, utmost importance is given to seed production. Although large quantities of seeds of catla are produced in India by public and private hatcheries, most hatcheries do not follow any genetic norms. Brood stocks in these hatcheries are kept over years for repeated use and occasionally brood stock from the wild is included to replenish induced bred stock. Consequently, these hatcheries in India are facing decline in seed quality due to accumulation of inbreeding (Eknath and Doyle 1990). At this juncture, a better procedure for production of quality catla seed is needed. Keeping this in mind recently, a selective breeding programme of *C. catla* has been initiated at ICAR-Central Institute of Freshwater Aquaculture (CIFA) to cater to the needs of the farming community.

After the success and wide adaptability of genetically improved rohu, Jayanti, an attempt was made by ICAR-CIFA to develop base population of *C. catla* intended for selective breeding focussing on the enhanced growth rate. Assessment of genetic variability observed in the base population breeders is critical for the success of a selective breeding programme. The success of the selective programme depends on the genetic variation observed in the base population and on other factors as to how the population is maintained from one generation to the next. The role of selecting brooders and establishing base population will determine the long-term genetic variance and genetic gain observed in the selective breeding programme (Holtsmark *et al.* 2006).

Broad genetic variation is important in establishing base population as the selection response depends on the additive genetic variation observed in between the stocks. One way of ensuring the presence of abundant genetic variation in the base population is by aggregating a 'synthetic' or 'composite' population, using breeders from different sources. Riverine (wild populations) contribute positively to a synthetic base population when there is only moderate genetic variation between wild and domesticated strains. Often, the knowledge of the genetic information of these populations is limited when starting any breeding programme. The genetic characterization by differentiation between stocks and genetic variation observed within stocks will have consequences on the genetic progress and genetic gain observed in the breeding programme.

The use of molecular markers in population genetic studies to determine genetic variability within and among populations is well established (Brown and Epifanio 2003; Liu and Cordes 2004). This would bring about assembly of the highest genetic variability in the founder breeding populations (Eding and Meuwissen 2001; Caballero and Toro 2002; Eding *et al.* 2002; Fernandez *et al.* 2014) and captures the populations with the highest genetic diversity contributing to maximum genetic gain in the subsequent generations. Microsatellite markers are widely used in aquaculture and are the markers of choice for selective breeding studies related to parentage assignment, pedigree analysis and identification of genetic variability between and within stocks in many fish species (Sekino *et al.* 2002; Mojekwu and Anumudu 2013).

The objective of this work was to optimize the base population breeders participation from different sources based on phenotypic records and genetic variability data analysis obtained through microsatellite marker information. For the present study, nine strains of *C. catla* from different hatcheries were collected and reared at the ICAR-CIFA farm. The phenotypic information of nine strains of catla (*C. catla*) for growth trait and microsatellite marker information was used to infer relationship within and between strains. Further, this information will be used to optimize proportion of individuals to be used from each strain while creating a synthetic base population for selective breeding. The present study reports the results of phenotypic and genotypic information generated from nine strains of catla for optimizing their contribution to the base population.

## Materials and methods

### *Collection of C. catla from different sources*

For establishment of base population of catla, nine strains were collected from different sources and places of India namely West Bengal, Bihar, Odisha, Andhra Pradesh and Uttar Pradesh (figure 1). Two riverine strains i.e. Ganga and Subarnarekha were included among the nine strains collected for base population. The nine strains are (i) Ganga river strain from Patna (C01), (ii) Subarnarekha river, strain Balasore (C02), (iii) Awalsidhi hatchery, West Bengal (C03), (iv) State Fisheries hatchery, Odisha (C04), (v) Kumar Swamy hatchery, Andhra Pradesh (C05), (vi) Nilu Ghosh hatchery, Kolkata, West Bengal (C06), (vii) Sai Ram hatchery, Andhra Pradesh (C07), (viii) ICAR-CIFA farm, Kausalyaganga, Bhubaneswar (C08) and

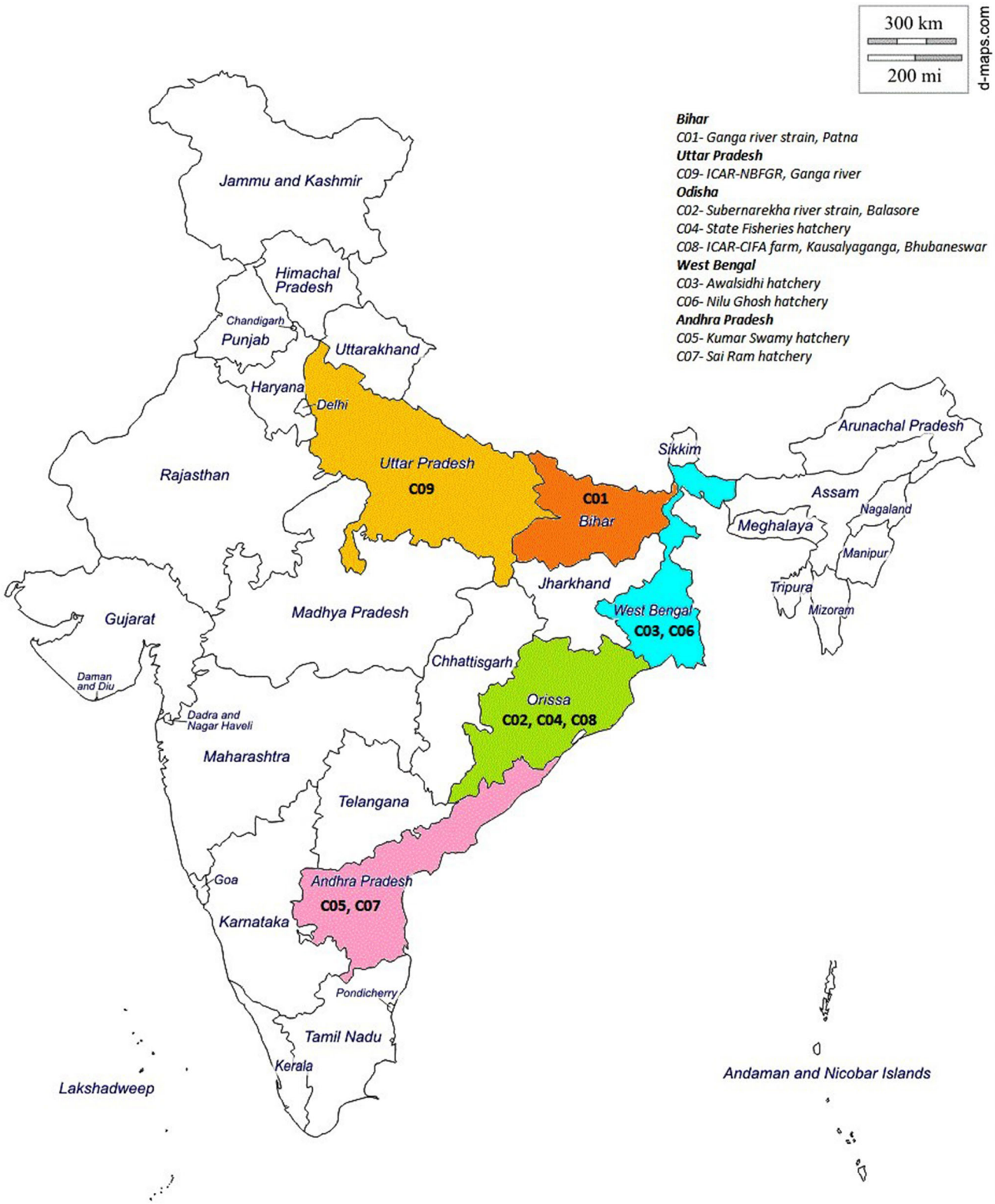


**Figure 1.** Map showing the source of different catla stocks.

(ix) ICAR-National Bureau of Fish Genetic Resources (ICAR-NBFGR), Ganga river, Uttar Pradesh (C09). They were brought to ICAR-CIFA at fry stage. Collected fry were under quarantine for about two weeks in cement cisterns and then reared separately in 100 $m^2$ nursery ponds till they attained taggable size.

#### *Tagging and communal rearing*

After attaining desired taggable size of about 20 g, a random sample of 60 fish from each of the nine strains were individually tagged with passive integrated transponder tags. Before implantation of tags, the fingerlings were collected and kept overnight in well aerated water without giving any feed so that the digestive tract will be clear, thus creating more space in the body cavity of the fish to implant the tag without any injury to the visceral mass. During the tagging operation, each fingerling is anaesthetized individually with a 0.3% MS 222 solution similar to the procedure followed for rohu tagging (Das Mahapatra *et al.* 2001). Pretagging sampling data pertaining to length and weight were recorded before tagging. The tags were implanted in the abdominal cavity of the fingerlings intraperitoneally through a 12-gauge needle attached to a spring-loaded syringe or an implanter. Utmost care was taken to load the tag in the ventral area of the abdominal cavity. In comparison to rohu, special care was taken in case of catla as the space for implantation is small.

After tagging, the fingerlings were kept in aerated water with additional aeration being provided through aerators. The tagged fishes were kept overnight under observation for any possible mortality. After 24-h observation, the tagged fingerlings were stocked in their respective well-prepared grow out ponds for communal rearing, in triplicate. Rearing was done in polyculture systems along with rohu at a stocking density of about 6000 fingerlings/ha and catla contribution was 30%. Besides fertilization, fish were also provided with supplementary feed at the rate 3–4% of body weight daily.

#### *Recording of data*

After a period of one year, data pertaining to growth (length and weight) were collected and analysed to evaluate the growth performance of each strain after pond correction, pond being the fixed effect. Ranking of strains was done for all nine strains as per their corrected body weight.

#### *Length–weight relationship (LWR)*

Total length was measured from the tip of the snout to the end of the caudal fin and the measurement was done on a measuring board expressed in centimetres (cm) and weighed using a weighing balance expressed in grams (g). The statistical relationship between these parameters of fish was established by using the parabolic equation by Froese (2006):

$$W = aL^b$$

where $W$ is the weight of fish (g), $L$ is the length of fish (cm), '$a$' is the intercept and '$b$' represents the slope of the line (growth exponent). The relationship ($W = aL^b$) when converted into the logarithmic form gives a straight line relationship graphically with a high correlation coefficient (Le Cren 1951):

$$\log W = \log a + b \log L$$

All statistical analysis including regression and calculation of correlation were done with Microsoft Office Excel 2007 and PAST software (PAlentologial STatistics, Hammer *et al.* 2001).

***Determination of condition factor (K):*** The condition factor $K$ (Fulton 1904) was estimated as:

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

where $K$, coefficient of condition; $W$, weight of the fish in grams (g); $L$, length of the fish in millimetres (mm).

In the case of carps, the length was measured from the tip of snout to the longest end of the caudal fin. All statistical analysis were performed using Microsoft Office Excel (v. 2007).

#### *Statistical analysis for body trait*

Statistical analysis for body trait i.e. growth in g was carried out for 540 animals of nine strains at final harvest. Preliminary analysis using a general linear model procedure in SAS (SAS 9.1) was performed using the following effect:

$$y_{klm} = \mu + P_k + S_l + e_{klm},$$

where $y_{klm}$ is the observed body weight on $m$th individual of $l$th (1, 2, 3…9) strains, $\mu$ is a constant, $P_k$ is the fixed effect of $k$th pond (1, 2, 3) and $e_{klm}$ is the random residual.

## Molecular characterization

#### *Sample collection and DNA isolation*

In total, 359 live specimens were collected from six hatcheries and two riverine sources. Samples from upper stretch of Ganga, Uttar Pradesh (C09) were not included in the present molecular characterization study due to a low sample size. Fin clips were taken and stored in 95% alcohol. Total DNA was isolated from fin tissue by proteinase-K digestion followed by standard phenol and chloroform extraction (Sambrook *et al.* 1989). The DNA samples were then resuspended in TE buffer. The concentration and purity of isolated DNA was estimated at wavelength 260/280 nm using a spectrophotometer.

***Microsatellite analysis***

A set of nine microsatellite loci isolated and characterized in our laboratory (Das *et al.* 2005; Patel *et al.* 2009) and elsewhere for *L. rohita* were selected for genetic diversity analysis based on high polymorphic information content (PIC) values and good cross species amplification (table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/). These loci were used in four multiplexes for genotyping of 359 catla individuals. Primers were procured from Applied Biosystems (Foster City, USA) with forward primer labelled with FAM, HEX or NED fluorescent dye. Polymerase chain reactions (PCR) were carried out in 15 $\mu$L volumes containing 1× *Taq* DNA polymerase reaction buffer with 1.5 mM $MgCl_2$, 200 $\mu$M dNTPs, 5 pmol of both labelled and unlabelled primers, 0.25 U of *Taq* DNA polymerase (Bangalore Genei, India) and 20 ng of template DNA.

The PCR conditions were optimized for each multiplex. The thermal cycle consisted of initial denaturation of 4 min at 94°C followed by 35 cycles of 45 s at 94°C, 1 min at the annealing temperature of the corresponding primer and 2 min at 72°C and finally a 15 min extension at 72°C. One $\mu$L PCR product and 0.1 $\mu$L GeneScan 500 LIZ size standards (ABI) were added to 9.9 $\mu$L of Hi Di formamide, denatured and electrophoresed on an ABI 3730 (Applied Biosystems) sequencer. Fragment size was measured according to the GeneScan 500 LIZ size standards using GeneMapper v. 3.7 110 software (ABI). CONVERT 1.31 (Glaubitz 2004) was used to obtain data formats suitable for the statistical analysis software. The program Micro-Checker (Oosterhout *et al.* 2004) was used to detect and identify genotyping errors resulting from null alleles, allele dropout and stuttering. Descriptive statistics such as expected heterozygosity, observed heterozygosity, inbreeding coefficient, Hardy–Weinberg equilibrium (HWE), linkage disequilibrium etc., were estimated using GDA. The program FSTAT was used to estimate the allelic richness in the present study. A Bonferroni correction (0.05/number of tests) was used to correct for multiple testing. Pairwise $F_{ST}$ values and analysis of molecular variance (AMOVA) were calculated using ARLEQUIN v. 3.1. The program STRUCTURE v. 2.3 (Pritchard *et al.* 2000) was used to investigate the patterns of genetic clusters in catla populations. By using admixture model and correlated allele frequency parameters, 20 replicates of each run from $K = 2$ to 9 ($K$ is number of genetic clusters) were performed with 20,000 iterations (burn-in) and 20,000 Markov chain Monte Carlo generations. STRUCTURE HARVESTOR (Earl 2012) was used to apply the Evanno method (Evanno *et al.* 2005) to detect the value of $K$ that best fit the data. A test for recent bottleneck in the catla populations was performed using the program BOTTLENECK v. 1.2.02. All applicable international, national and/or institutional guidelines for the care and use of animals

**Table 1.** Strain ranking of catla according to average body length and weight ± SD.

| Strain | | Source | Number of fish tagged/number harvested | Average body length (cm) mean±SD At tagging | Final sampling after pond correction | Average body weight (g) mean±SD At tagging | Final sampling after pond correction | CV | Rank |
|---|---|---|---|---|---|---|---|---|---|
| 1 | C01 | River Ganga lower stretch, Bihar | 60/54 | 21.29 ± 2.32 | 58.44 ± 2.95 | 55.50* ± 12.5 | 3342 ± 624.09 | 18.68 | 1 |
| 2 | C02 | Subarnarekha river, Balasore | 60/55 | 12.49 ± 2.57 | 48.92 ± 3.12 | 22.40 ± 10.2 | 1960 ± 393.37 | 19.97 | 9 |
| 3 | C03 | Awalsidhi hatchery, Kolkata | 60/50 | 17.71 ± 2.71 | 49.86 ± 2.61 | 31.90 ± 9.8 | 2175 ± 477.15 | 14.43 | 5 |
| 4 | C04 | State Fisheries hatchery, Odisha | 60/54 | 18.94 ± 2.24 | 51.85 ± 4.73 | 36.98 ± 10.5 | 2325 ± 313.91 | 22.24 | 3 |
| 5 | C05 | Kumar Swamy hatchery, Andhra Pradesh | 60/52 | 11.16 ± 1.48 | 49.92 ± 3.52 | 15.98 ± 5.2 | 2216 ± 517.20 | 21.54 | 4 |
| 6 | C06 | Nilu Ghosh hatchery, Kolkata | 60/50 | 10.76 ± 1.45 | 48.81 ± 3.54 | 16.50 ± 5.8 | 2129 ± 238.19 | 23.38 | 7 |
| 7 | C07 | Sai Ram hatchery, Andhra Pradesh | 60/55 | 11.91 ± 2.17 | 48.91 ± 2.57 | 32.22 ± 9.5 | 2361 ± 391.44 | 10.09 | 2 |
| 8 | C08 | ICAR-CIFA Farm, Bhubaneswar | 60/52 | 17.68 ± 1.53 | 49.13 ± 4.38 | 37.49 ± 7.91 | 2168 ± 497.58 | 28.93 | 6 |
| 9 | C09 | ICAR- NBFGR, Ganga upper stretch, Uttar Pradesh | 58/50 | 13.75 ± 1.67 | 48.91 ± 2.21 | 28.80 ± 10.5 | 2069 ± 627.24 | 19.01 | 8 |

*$P < 0.01$.

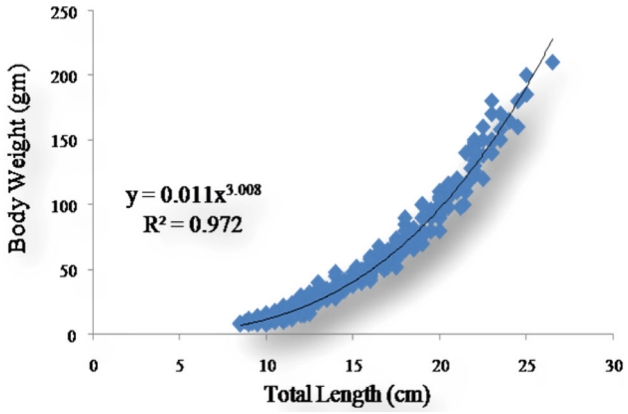


**Figure 2.** Initial LWR of catla base population.

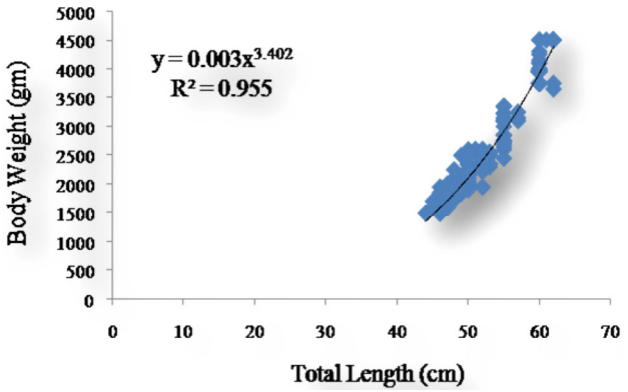


**Figure 3.** Final LWR of catla base population.

were followed. The research undertaken complies with the current animal welfare laws in India. The care and treatment of animals used in this study were in accordance with the ethical guidelines of ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, India. As the experimental animal *C. catla* is not an endangered finfish, the provisions of the Govt. of India's Wildlife Protection Act of 1972 are not applicable for experiments on this finfish.

## Results

### *Growth in nursery ponds*

During tagging, fish were about three months old and the initial average weight of each strain is shown in table 1. Although stocking density in nursery ponds was the same for all the strains i.e. 5000 numbers/100 $m^2$ pond, significant variations were observed among the different strains. Variation observed in Ganga strain collected from Patna, Bihar (C01) was significantly higher than the other strains.

### *Growth in grow-out ponds*

After one year of culture, live body weight was measured and least square means was obtained after pond effect correction (table 1). The correction was done considering the pond as the fixed effect. Sixty individuals from each strain were stocked in three communal ponds (20 numbers in each pond) except for C09 where a total 58 individuals were stocked. A wide range of variation was observed among and between strains. The Ganga riverine strain collected from Patna, Bihar (C01) showed a significantly higher growth performance than the other strains. A wide range of variation was observed within the strain also. The coefficient of variation (CV) ranges from 10.09 to 28.93 among different strains. The different strains of catla were ranked according to their average body weight ± SD (table 1).

### *LWR and Fulton's condition factor 'K'*

The statistical relationship of total body length with body weight of *C. catla* indicated a positive significant correlation (figures 2 and 3). The results depicted the increase of '*b*' from 3.008 (initial sampling phase) to 3.402 (final sampling phase). Isometric growth ($b = 3$) was recorded in the initial sampling phase while positive allometric growth ($b > 3$) was recorded in the final sampling phase indicating an increase in relative body thickness or plumpness.

**Table 2.** Descriptive statistics and estimated length weight relationship (LWR) parameters in the initial and final sampling phase.

| Parameters | Initial sampling phase | Final sampling phase |
|---|---|---|
| Length range (cm) | 8.5–26.5 | 44.0–62.0 |
| Weight range (g) | 8–210 | 1500–4500 |
| *a* | 0.011 | 0.003 |
| *b* | 3.008 | 3.402 |
| $r^2$ | 0.972 | 0.955 |
| *K* | 1.523 | 1.785 |

*a*, *b*, regression coefficients; $r^2$, correlation coefficient and *K*, condition factor.

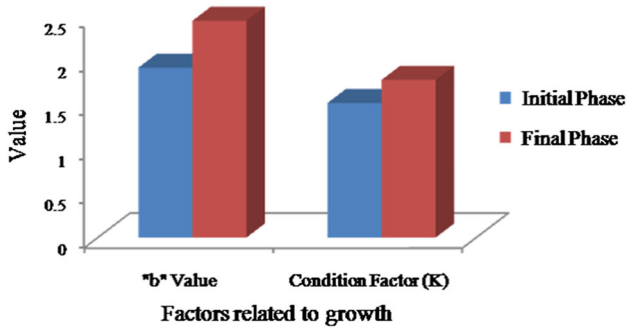


**Figure 4.** Comparison of '*b*' value and Fulton's condition factor (*K*) in the initial and final sampling phases.

**Table 3.** Total number of alleles observed per locus.

| | Locus | Total number of alleles |
|---|---|---|
| 1 | Lr-162 | 28 |
| 2 | Lr-432 | 8 |
| 3 | Lr-463 | 6 |
| 4 | Lr-416 | 6 |
| 5 | Lr-384 | 14 |
| 6 | Lr-158 | 28 |
| 7 | Lr-388 | 9 |
| 8 | Lr-41 | 18 |
| 9 | Lr-190a | 5 |

The value of '*a*' was also found to be 0.011 in the initial sampling phase and 0.003 in the final sampling phase. Correlation coefficient ($r^2$) too varied from 0.955 (final sampling phase) to 0.972 (initial sampling phase) (table 2). There was a marked increase in Fulton's condition factor (*K*) in the final sampling phase from initial 1.523 to 1.785. A comparison of *b* and *K* value was done for the initial and final sampling phase (figure 4).

### *Microsatellite information*

In total, 359 individuals were screened for population genetic variation at nine microsatellite loci originally developed for rohu, *L. rohita*. All nine microsatellite loci were found to be polymorphic in all populations of catla. Analysis of the data with Micro-Checker revealed that there was no evidence of scoring error due to stuttering and no evidence of large allele drop out. In total 116 alleles were observed. Allele number per locus ranged from 5 to 28 (table 3). Mean expected heterozygosity ($H_e$), observed heterozygosity ($H_o$) and inbreeding coefficient ($f$) ranged from −0.321 to 0.834 (tables 2 and 3 in electronic supplementary material. Deviations from HWE were observed in all populations except for two populations (C06 and C07). At least one locus is not in HWE after an adjustment of *P* values across nine loci using the Bonferroni correction method for multiple observations. The deviations from equilibrium were observed at loci: Lr-162, Lr-432, Lr-416, Lr-384 and Lr-388 for different catla populations. These deviations from HWE resulted due to excess of homozygosity in either population at these loci. No significant linkage disequilibrium was observed among the microsatellite loci after Bonferroni correction in either of the population. The observed heterozygosity of different strains of catla is shown in table 4.

Microsatellite markers were used to estimate genetic differences of different strains of catla using pair wise $F_{ST}$ estimates (table 5). Overall multi locus $F_{ST}$, including all loci was estimated to be 0.4137 ($P < 0.05$), indicating genetic heterogeneity among them. AMOVA revealed that 58.63% of variation was due to within individual variation, 3.45% of variation was due to among individuals within strains and 37.92% was due to among strain variations. The highest genetic differentiation was observed between strains from Awalsidhi hatchery, West Bengal (C03) and Kumar Swamy hatchery, Andhra Pradesh (C05). The lowest genetic differentiation was observed between strains from State Fisheries hatchery, Odisha (C04) and ICAR-CIFA farm, Bhubaneswar (C08). The results of structure analysis supported the above observations in the present study (figure 5). Out of eight populations, four catla populations experienced a recent population bottleneck (table 6).

## Discussion

The present study was initiated during 2013–2014. Information about the performance of farmed and wild strains of catla is not well documented. Such information is not only important for farmers who want to purchase the seed from best performing strains but also important for establishing high merit based base population optimization for selective breeding. The information collected will be of importance for establishing base population leading to selective breeding of *C. catla* in India. The initial difference in performance between good and poor strains might be equal to the response of selection across several generations (Reddy *et al.* 2002). In fish breeding, the base population can be made up of fish either originating from wild strains, as in the Norwegian breeding programme for Atlantic salmon (*Salmo salar* L.) (Gjedrem *et al.* 1991), from domestic strains, or a combination of both wild and domestic strains, as in the Genetic Improvement of Farmed Tilapia (GIFT) experiment (Eknath *et al.* 1993).

In the present study, three riverine strains (two strains from the same river Ganga at different places) (C01 and C09) and one Subarnarekha river strain (C02) were evaluated. With them, six farm reared strains were also evaluated for their growth performance. The wild strain of Ganga collected from Patna, Bihar (C01) showed a

**Table 4.** Observed heterozygosity of different strains of catla.

| | Strain | Source | Observed heterozygosity |
|---|---|---|---|
| 1 | C01 | River Ganga lower stretch, Patna, Bihar | 0.758 |
| 2 | C02 | Subarnarekha river strain, Balasore | 0.741 |
| 3 | C03 | Awalsidhi hatchery, Kolkata | 0.727 |
| 4 | C04 | State Fisheries hatchery, Odisha | 0.700 |
| 5 | C05 | Kumar Swamy hatchery, Andhra Pradesh | 0.875 |
| 6 | C06 | Nilu Ghosh hatchery, Kolkata | 0.934 |
| 7 | C07 | Sai Ram hatchery, Andhra Pradesh | 0.842 |
| 8 | C08 | ICAR-CIFA Farm, Kausalyaganga, Bhubaneswar | 0.783 |
| 9 | C09 | ICAR-NBFGR, Ganga upper, Uttar Pradesh | – |

**Table 5.** $F_{ST}$ values among all pairs of *C. catla* populations.

| | C01 | C03 | C02 | C04 | C05 | C06 | C07 | C08 |
|---|---|---|---|---|---|---|---|---|
| C01 | 0 | * | – | * | – | – | * | – |
| C03 | 0.095 | 0 | * | – | * | * | * | * |
| C02 | 0.009 | 0.132 | 0 | * | * | – | * | – |
| C04 | 0.092 | 0.004 | 0.110 | 0 | * | * | * | * |
| C05 | 0.038 | 0.056 | 0.037 | 0.052 | 0 | * | * | – |
| C06 | −0.011 | 0.079 | 0.020 | 0.087 | 0.045 | 0 | * | – |
| C07 | 0.061 | 0.263 | 0.039 | 0.249 | 0.112 | 0.072 | 0 | * |
| C08 | −0.014 | 0.076 | 0.009 | 0.067 | 0.013 | −0.017 | 0.089 | 0 |

C01, River Ganga lower stretch, Bihar; C02, Subarnarekha river, Balasore; C03, Awalsidhi hatchery, Kolkata; C04, State Fisheries hatchery, Odisha; C05, Kumar Swamy hatchery, Andhra Pradesh; C06, Nilu Ghosh hatchery, Kolkata; C07, Sai Ram hatchery, Andhra Pradesh; C08, ICAR-CIFA Farm, Bhubaneswar; C09, ICAR- NBFGR, Ganga upper stretch, Uttar Pradesh. *Significant ($P < 0.05$); –, nonsignificant ($P > 0.05$).

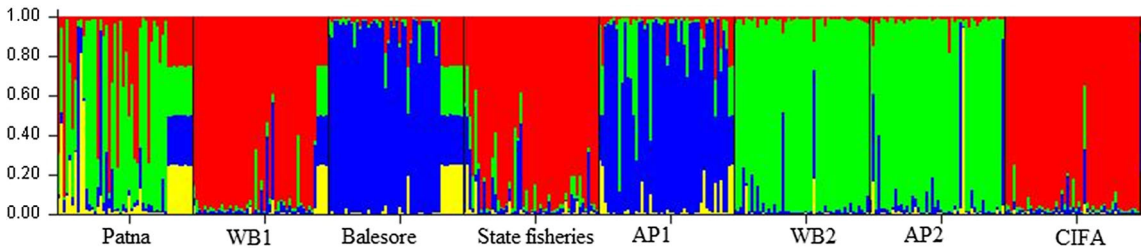


**Figure 5.** Genetic structure analysis of catla populations.

**Table 6.** Bottleneck effect of catla populations.

| | Population | Bottleneck effect |
|---|---|---|
| 1 | Patna | Mode shift (experienced bottleneck) |
| 2 | Awalsidhi | No bottleneck |
| 3 | Balasore | Mode shift (experienced bottleneck) |
| 4 | State Fisheries | No bottleneck |
| 5 | Kumar Swamy | Mode shift (experienced bottleneck) |
| 6 | Nilu Ghosh | No bottleneck |
| 7 | Sai Ram | No bottleneck |
| 8 | CIFA | Mode shift (experienced bottleneck) |

significantly higher growth performance than other strains and even the strain collected from another place of the same river Ganga, Uttar Pradesh (C09). All six strains collected from hatcheries showed variable growth performance and better performance than rivers Subarnarekha (C02) and Ganga from Uttar Pradesh strain (C09). This finding indicated that, hatchery owners are now conscious of broodstock management and, changes have been observed in many hatcheries for quality seed production. Also, rivers are polluted and also receive hatchery stocks due to flood and other inundations. For firm conclusions, strains should also perform under different agro-climatic conditions in order to evaluate the magnitude of possible genotype due to the environment interaction effect.

The presence of variability among the populations and between individuals within a population is essential for their ability to survive and successfully respond to environmental changes (Chauhan and Rajiv 2010). Molecular

genetic diversity of fish is shaped by several factors and is associated with life history traits. The population genetic structure of a stock is dynamic. It is variable and the rate of variation depends on the degree of interventions. Possibility of population extirpation or population fitness reduction might be a result of low level genetic diversity observed through inbreeding depression (Saccheri *et al.* 1998; Puurtinen *et al.* 2004). Therefore, examination of genetic diversity is an important component for management, conservation and sustainable production of commercially important aquaculture species. In commercially important aquaculture species such as catla, information on genetic diversity of population is important, because it is the source of variation for economical important traits like growth rate, feed efficiency and disease resistance. Further, harmful effects on fitness traits are exerted through change of allele frequencies due to artificial production. Microsatellite analysis of catla revealed a high level of genetic diversity in catla populations as evident form allelic richness, expected heterozygosity and observed heterozygosity. Similar results were observed by Rahman *et al.* (2009) in Bangladesh using random amplification of polymorphic DNA (RAPD) markers in *C. catla*. No significant departure from HWE was observed except at a few loci in each population. This may imply that all the populations are stable in genotype frequency and gene frequency (Ma *et al.* 2012). Deviation of HWE is population specific rather than loci specific. Deviations from HWE have been reported in several studies (Karlsson and Mork 2005; Bunje *et al.* 2007; Langen *et al.* 2011). This could be due to several factors like the presence of null alleles, natural selection acting on the genetic marker, heterozygote deficiency and the presence of population sub structures or a combination of these factors (An *et al.* 2013). AMOVA analysis revealed that, 37.92% of strain differentiation was observed due to variation among groups, 3.45% of variation was due to among strain within groups and 58.63% variation was due to among individuals within strains. The average fixation index ($F_{ST}$ = 0.4137, $P = 0.00000 \pm 0.00000$) revealed significant level of strain differentiation. Pair wise $F_{ST}$ estimates ranged from 0.038 to 0.541 indicating low to high level of genetic differentiation among the strains. Similar levels of population differentiation were observed while examining genetic divergence in wild catla populations on employing the Cyt *b* gene (Das *et al.* 2012). Significant genetic differentiation was observed among the catla populations. This could be due to factors such as habitat fragmentation, reduction in the effective number of contributing parents and the effect of artificial selection on hatchery progeny. A similar phenomenon was observed by Rana *et al.* (2004) using biochemical markers. However, no significant genetic differentiation was observed between a few pairs of catla strains (C01–C02, C01–C06, C01–C08, C02–C06, C08–C02, C08–C05 and C08–C06). The results of structure analysis supported the above observations. This could be due to intermixing samples between hatcheries through human intervention. Therefore, a possible explanation for this phenomenon could be that *C. catla* strains sampled from different river basins in the present study might have a common ancestral gene pool for which at least a historical connectivity existed between different rivers. The results of the molecular study supported the phenotypic observations.

It has been shown that strains harbouring low level genetic diversity should contribute less individuals in order to maximize the genetic diversity of the base population. Beyond that we have to also consider that, profitability of a breeding programme depends on actual mean level of phenotypic value. A compromise solution between diversity and performance of different strains was considered while creating base population for catla giving equal weightage to both the information.

Knowledge of morphometric studies such as the LWR and condition factor is important in studying fish biology, determining the condition of the fish and assessment of growth rate in the fish (Sarang *et al.* 2015). The LWR is ideal for taxonomic studies, relationship changes associated with various developmental stages such as metamorphosis, growth and onset of maturity (Thomas *et al.* 2003) and yield calculations (Beverton and Holt 1957). The condition factor is a quantitative parameter that indicates the well-being of a specific fish in a water body and its value fluctuates influenced by both biotic and abiotic environmental conditions (Abowei 2010). Comparative studies on the growth parameters confirmed significant increase in $a$, $b$, $r^2$ and $K$ values. The present study is on par with the range of b lying between 2.5 and 3.5 in the case of Indian Major carps (Froese 1998; Das *et al.* 2015). The increased values depict that the fish were in good health during the study period with optimal feeding and management practices conducive for their optimum growth potential. These results showed notably that the catla population collected from different stocks received proper feeding and environmental conditions for its optimal growth. This will definitely contribute in proper management and optimization of the base population.

In conclusion, phenotypic and microsatellite information together would facilitate enhanced knowledge of genetic variation of quantitative traits within and between strains of different species. If differences between strains are not large, it may be useful to have a minimum level of representation of each of the wild strain and of each domesticated strain to ensure a broad genetic base in the base population at the beginning of a selection programme. Such inclusion of molecular marker details would help in understanding the molecular underplay associated with phenotypic selection and changes in associated traits. In the catla breeding programme one compromising strategy will be taken for long-term effect. For establishment of base population, both growth as well as heterozygosity information will be taken into consideration for

full-sib family production. This would contribute in the validation of the broad genetic variance attributed by the diversity of the base population for a particular desirable trait eventually making the selective breeding programme a profitable and successful one in the subsequent generations.

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