



Assessing the Impact of Genotype and Environment Interaction on Growth Performance of Hatchery Produced *Catla catla*

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ABSTRACT

The culture conditions influence the magnitude of genetic expression in fish. This study was undertaken to assess the impact of Genotype (G) × Environment (E) interaction on the growth performance of hatchery produced *Catla catla*. The different hatchery stocks including Farooqabad (FQB), Lahore (LHR), Faisalabad (FSD), Bhalwal (BHL) and Qadirabad (QBD) assumed as five distinct genotypes were compared for genetic variability in relation to growth performance. Appraisal fish fingerlings were reared in glass aquaria (twenty fish per population) for 60 days under similar environmental conditions. Condition factor (K) and growth parameters (wet weight, fork and total length) were measured fortnightly. The morphometric data were analyzed using SPSS. Genetics characterization was performed by employing five species specific polymorphic microsatellite markers. Overall, the results of growth trial confirmed that FQB genotype performed maximum while QBD evinced lowest performance among all the genotypes based on wet weight (g) and total length (mm). Genetic analysis revealed significant differences based on allelic frequencies, heterozygosity, inbreeding and genetic distance among hatchery genotypes. Impact of G×E interaction is illustrated by results where genetically diverse FQB genotype performed maximum under similar cultural environment among all genotypes.

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Authors' Contribution

KA and TA proposed the experimental design and helped in collecting data. SR performed laboratory work and analysis. HN assisted in laboratory work.

Key words

DNA markers, Farm cultured, Growth performance, Population genetics, Freshwater fish

INTRODUCTION

Genotype-environment interaction is a situation in which various genotypes respond differently to environmental conditions. Features of the environment affect differently on genotypes (Davies *et al.*, 2012). These interactions specify the phenotypic uniqueness to the individuals most importantly growth, reproductive success and continuing survival of species. Determination of G×E interactions has significance in breeding programs (Macbeth and Wang, 2014).

Protected environment in fish culture permits them to use surplus energy for better growth rate, spawning and maturity in early age, increased survival, improved tolerance to elevated temperatures and disease resistance (Thorpe, 2004). Most of the seed originates from hatcheries (Alam and Islam, 2005) where fry production is intensified but genetic quality of seed is deteriorated. Lack of management skills, inbreeding and impoverished fauna with poor genetic characterization, inadvertent selection like choosing a few brooders, large and more fertile females, males with high quantity of milt and non-random selection etc. result into genetic decline in hatcheries (Brown *et al.*, 2005). Escape or stocking of this seed to wild on an immense scale can create serious issues. Both natural and farmed fishes are facing drastic genetic problems which are relatively distinctive in fisheries (Sultana *et al.*, 2015). It is crucial to examine the genetic structure of fishery stocks after several generations for proper management and conservation. Selective breeding programs, stock identification and improvement along with stock augmentation is imperative for a diverse gene

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pool (Ciftci and Okumus, 2002).

Better growth and maximum yield rely on genetic diversity in any aquaculture species. It is the key to *protect* a species to become extinct (Ashley *et al.*, 2003), as it presents the base for adaptation in continuous varying environment. Lack of knowledge has led to genetic variability loss and declined commercial traits like growth rate, yield, immunity and health, might result into gene fixation and ultimately extinction of species. Unintentional hybridization of *C. catla* with *L. rohita* and *C. mrigala* might be a reason of poor growth performance (Simonsen *et al.*, 2004). Consequently, immensity of genetic diversity is needed to manage and conserve the depleting populations (Sultana *et al.*, 2015).

The *C. catla*, is a freshwater fish belonging to family Cyprinidae. It has highest growth rate among the Indian major carps and contributes extensively to the entire inland fisheries (Belton and Azad, 2012). In freshwater environment, the total production of *C. catla* was 157,340 t in 2017 (FAO, 2018). However, its gene pool is dwindling due to lack of rigorous management in captive conditions.

Among various DNA marker systems, microsatellites have emerged as most compliant neutral markers (Abbas *et al.*, 2017). As they are polymorphic (Romana-Eguia *et al.*, 2004), have high mutation rate and occur in both coding and non-coding regions and easily detectable (Sultana *et al.*, 2015). These markers are variable in nature, inherit in Mendel's co-dominant fashion and provide contemporary estimates in population genetics like gene inheritance, genetic fingerprinting, gene mapping, genetic diversity estimates and genotype environment interactions etc. (Chistiakov *et al.*, 2006).

In face of ongoing genetic deterioration due to artificial propagation programs, the current study endeavored to assess the impact of $G \times E$ interaction by studying growth performance of various hatchery stocks.

MATERIALS AND METHODS

Fish sampling

A total of one hundred individuals (twenty individuals from each site) of three months old *C. catla* (weight ranged from 8-8.5 g) were collected from five hatcheries located in Punjab, Pakistan. The sampling populations localities included: Fish Seed Hatchery, Faisalabad (FSD), Fish Seed Nursing Farm, Farooqabad (FQB), Fish Seed Hatchery Bhalwal (BHL), Central Fish Seed Hatchery, Lahore (LHR) and Qadirabad Fish Seed Hatchery (QBD). Initial letters of the sampling locations were used to name the populations. Experiment was conducted in two phases. Growth trial of fish was accomplished in the first phase followed by DNA markers based genetic analyses of the

respective hatchery stocks.

Growth performance

The fish were acclimatized in laboratory environmental conditions for a week in cemented tanks at Fisheries research farms, University of Agriculture, Faisalabad. Initial average weight and lengths were noted, and fish was reared under intensive conditions in glass aquaria, given ISO-caloric standard fish feed (fed @ 3% body weight twice/day on daily basis) for sixty days. Continuous aeration was provided, and the remnants of feed and fecal matter were removed by siphoning off the water from aquaria. Fish was analyzed fortnightly for morphometric characteristics viz. wet weight, lengths (fork and total length) and condition factor was also calculated.

Genetic analyses of the stocks

In second phase of experiment, the same stocks were subjected to genetic analyses.

Each individual of representative genotype was sacrificed, and the dorsal muscle tissues were removed to store at -20°C. Following the Yue and Orban (2005) procedure with minor changes were used to extract total genomic DNA. Agarose gel electrophoresis techniques were used to assess the quality of extracted DNA while quantity was tested through Nanodrop.

A total of five species specific microsatellite loci (*Cc-6*, *Cc-7*, *Cc-8*, *Cc-9* and *Cc-10*) reported by McConnell *et al.* (2001) were obtained from Gene-link, USA and amplified by PCR (Table I). The PCR reaction was performed in a 20 µL reaction mixture, which contained 0.8 µL of each primer set, 0.4 µL of dNTPs, 1.5 µL MgCl₂, 2.0 µL of 10×PCR buffer, 0.4 µL Taq polymerase, and template DNA (about 50 ng) using gradient thermal cycler. The PCR cycles were as follows: 5 min at 94°C, 32 cycles of one min for denaturation at 94°C, 30 seconds for annealing, 1 min at 72°C, and again 4 min at 72°C for elongation.

After amplifying the microsatellite loci, PCR products (5 µL) were mixed with loading dye (1 µL). This mixture was loaded along with DNA ladder on 8% polyacrylamide gels (non-denaturing) for resolution at standard conditions. Silver staining of the gel produced bands which were visualized in gel documentation system. The bands were counted manually, and 1 KB gene ruler was used to estimate the sizes of alleles.

Data analyses

For each growth trait, means ± SD were calculated. Data on fish growth was compiled and analysis was done by ANOVA and correlation coefficients were computed to discover relationships among variables. The analysis was done by using Statistical package SPSS 21.0.

Table I. Characteristics of *C. catla* specific microsatellite loci.

S. no.	Locus	Repeat sequence	GenBank accession No.	Primer sequence (5'-3')	T _a °C	Allele size (bp)
1	Cc 6	(TG) ₁₃ (ATGT) ₃ (ATT) ₉	AJ294954	ATTTGAGGTTAAAGGTTAAAAAG AAGAACTCTAAATGATGCCAG	48	180-121 (180)
2	Cc 7	(GT) ₂₁	AJ294955	CACTCTGTGCCTAGACCTCG CTGGAGTTTAAGCCCTGTTC	55	137-159 (157)
3	Cc 8	(CA) ₁₇	AJ294956	GAGTGACATTTTCATTTATT ACACTCAGGAATGAGCAG	48	101-111 (105)
4	Cc 9	(AC) ₁₃	AJ294957	F-TCACATGGGAACAACAAACC R-CCGCCGCTTACCCATCAC	55	207-203 (211)
5	Cc 10	(GTTT) ₅	AJ294958	GTGAGCAGAAGAGACTG AGTTTTTGAACAGTGAGTG	48	63-71 (71)

Where; F and R represents forward and reverse; N is # of alleles; Ta, annealing temperature.

FSTAT ver. 2.9.3.2 (Goudet, 2002) was used to find the genetic constitution of stocks i.e. allele frequency and richness (A_r), observed and expected heterozygosity. The departure from *HWE* across every locus was found out by implementing the Markov-chain random by GENEPOP ver. 4.2 (Raymond and Rousset, 1995). The significance of *HWE* test was assessed by an adjusted alpha using the sequential Bonferroni correction (Rice, 1989).

F_{IS} was estimated for each hatchery stock at every locus by applying F-statistics (Weir and Cockerham, 1984) using FSTAT ver. 2.9.3.2 (Goudet, 2002). Differentiation (F_{ST}) was described by Weir and Cockerham (1984) for the comparisons between stocks. UPGMA dendrogram based on Nei's (1972) unbiased distance was made using TFGA ver. 1.3 software.

RESULTS

Growth performance

Mean ± SD of body wet weight (g), fork and total lengths (mm) and condition factor at harvest are depicted in Table II. Body weight and lengths of FQB were significantly higher than other genotypes at $P < 0.05$. Growth performance measured in terms of wet weight, fork and total length increase was in the following order: FQB > LHR > BHL > FSD > QBD (Table II) (Fig. 1). These results on growth evaluation indicated that fish of FQB performed maximum while that of QBD performed minimum.

Data on condition factor (K) of fish was computed after every fortnight. The values of K varied significantly among five populations for this study period. Analysis of variance exhibited statistically highly significant differences among different genotypes at $P < 0.01$ for fish weight, fork and total length increase and condition factor. The survival rate was 100% and it was not significantly different at $P > 0.05$ for all genotypes.

Genetic diversity

Among five selected genotypes, all the observed alleles present at five microsatellite loci, were found polymorphic. The value of N_a ranged from 2 to 5, with an average allelic value of 3.2. The observed allelic diversity values were lowest (2.8) in QBD genotype whereas the values were highest for FQB (3.6) (Table III). Differences in N_a and allelic size were observed at a locus in the present work and the data described earlier for *C. catla* (Table III). The allelic size fluctuated from 140-250 bp at all loci in every genotype. H_o varied from 0.4887 at locus (Cc-6) to 0.7537 at Cc-9. At all the loci, the average H_o and H_e was 0.6536 and 0.6684, respectively. The most heterozygote deficient genotype was QBD while the patterns of heterozygosity were highly pronounced in FQB genotype. Heterozygosity level was moderate at every locus, in the studied genotypes of *C. catla*, (Table III). The inbreeding coefficient (F_{IS}) executed for every locus revealed a loss of heterozygosity. The average values of F_{IS} ranged from 0.1183 to 0.1288.

Table II. Growth parameters of *C. catla* measured at final harvest.

Populations	Body weight (g)	Fork length (mm)	Total length (mm)	Condition factor (K)
LHR	18.4±0.10	97.0±0.3	107.1±0.10	2.01±0.01
FQB	21.4±0.20	103.2±0.20	113.8±0.10	1.94±0.03
FSD	18.0±0.20	99.0±0.20	109.0±0.10	1.85±0.01
BHL	18.0±0.20	101.2±0.10	111.0±0.10	1.92±0.01
QBD	16.2±0.10	96.0±0.20	102.0±0.20	1.83±0.01

LHR, Central Fish Seed Hatchery, Lahore; FQB, Fish Seed Nursing Farm, Farooqabad; FSD, Fish Seed Hatchery, Faisalabad; BHL, Fish Seed Hatchery Bhalwal; QBD, Qadirabad Fish Seed Hatchery.

Population genetic structure

Pair-wise comparison of F_{ST} values confirmed that all the populations (genotypes) were not homogenous. The minimum differentiation was observed between LHR-FQB genotype pair (0.0137) while much higher divergence was found for FQB-QBD (0.0788). The higher differentiation in FQB-QBD genotype pair indicated minimum gene flow. On the other hand, LHR-FQB pair confirmed an ongoing gene flow between them. Genetic distance calculated among pair of genotypes was significant ($P < 0.05$) but exhibited variability in magnitude (Table IV).

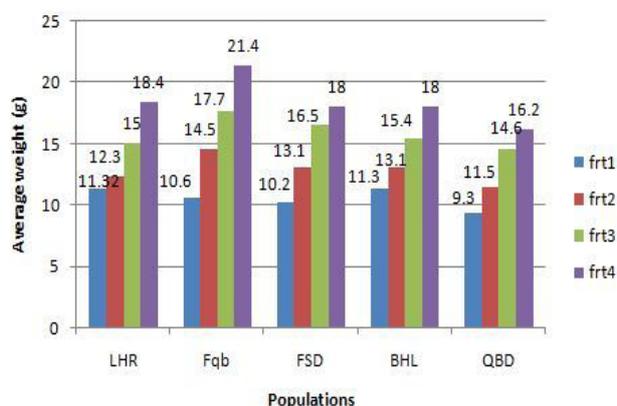


Fig. 1. Average weight gain among different hatchery populations of *C. catla*, observed fortnightly.

A total of 15 out of 25 tests significantly deviated from *HWE*. The locus *Cc-6* in FSD, *Cc-7* in QBD and *Cc-8* in FQB and *Cc-10* at BHL showed highly significant deviation ($P < 0.05$) from *HWE*. The genotypes of LHR and BHL departed from *HWE* by four and two microsatellite loci, respectively. On the other hand, FSD, QBD and FQB deviated from *HWE* by three microsatellite loci.

UPGMA dendrogram based on Nei's Genetic Distance (1972), illustrated the underlying differentiation of genotypes. The analysis revealed two major clusters containing LHR, FQB, FSD and BHL in one cluster while genotype QBD in second cluster and further divides into sub-clusters (Fig. 2). The clustering pattern clearly demonstrates that genotypes clustered according to their present geographical configuration.

Genotype-environment interaction

Genetically diverse fish is more productive, adaptable and vigorous. In the given circumstances, fish genotypes of different origin express their phenotypic potential with respect to their genetic makeup. It was revealed that individuals from FQB genotype were genetically more diverse (lowest F_{IS} and highest H_o) expressed maximum

growth in terms of weight gain (13.2 g). While organisms of QBD genotype were genetically least diverse (highest F_{IS} and lowest H_o) showing lowest growth in terms of weight gain (8 g) (Fig. 3).

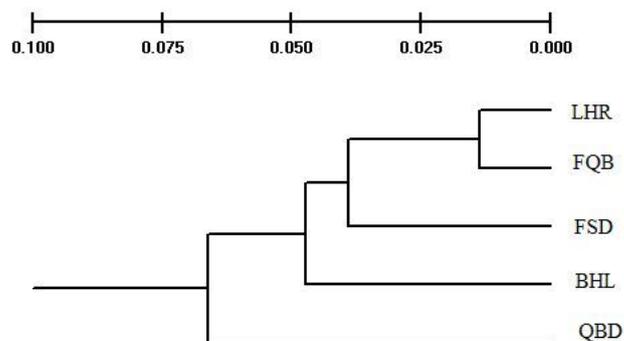


Fig. 2. The UPGMA dendrogram representing genetic relatedness between five *C. catla* genotypes.

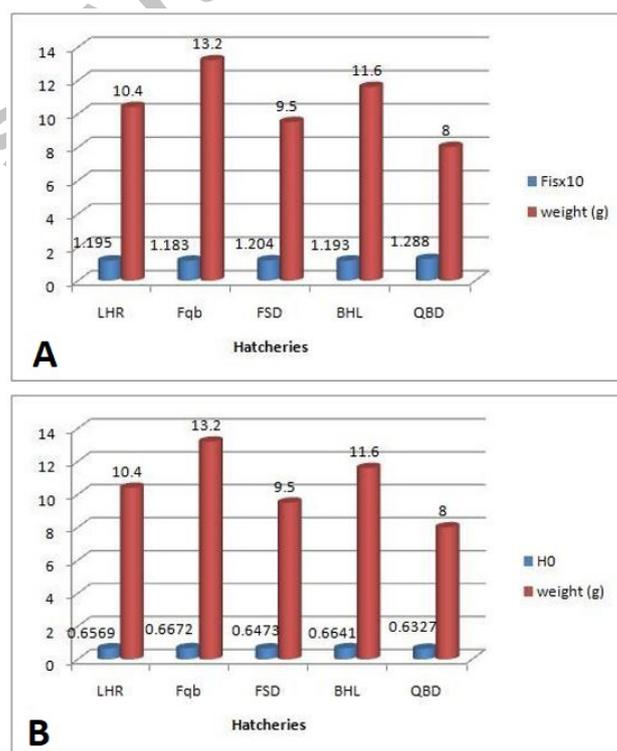


Fig. 3. Genetic diversity in terms of F_{IS} (A) and H_o (B) and growth in terms of weight gain (g) revealed impact of G×E interaction.

DISCUSSION

Several studies on growth performance and genetic status of *Catla catla* populations has been undertaken.

Yet meticulous evaluation of growth performance of Catla carp in relation to its genetic structure has not been studied. Fisheries sector has suffered a dramatic turn-down in Pakistan due to poor culture conditions and low quality of fish seed. Lack of resources and technical knowledge on genetic status of fish stocks has caused genetic degradation in numerous fish hatcheries. In face of shrinking fish yield, the impact of G × E on growth performance of hatchery produced *C. catla* was assessed in the present study.

Table III. Genetic diversity parameters for *C. catla* populations at five microsatellite loci.

Populations	Parameters	Cc-6	Cc-7	Cc-8	Cc-9	Cc-10	Average
LHR	N_a	2	2	4	4	5	3.4
	A_r	2.00	2.00	4.00	4.00	5.00	3.40
	H_o	0.4950	0.6350	0.7258	0.7537	0.6750	0.6569
	H_e	0.5077	0.6513	0.7454	0.7731	0.6923	0.6739
	F_{IS}	0.2877	0.1408	0.0388	0.0493	0.0811	0.1195
FQB	N_a	2	3	4	4	5	3.6
	A_r	2.00	3.00	4.00	4.00	5.00	3.60
	H_o	0.4887	0.6400	0.7313	0.7413	0.7350	0.6672
	H_e	0.5013	0.6564	0.7511	0.7603	0.7538	0.6845
	F_{IS}	0.2812	0.1400	0.0373	0.0499	0.0833	0.1183
FSD	N_a	2	2	3	4	5	3.2
	A_r	2.00	2.00	3.00	4.00	5.00	3.60
	H_o	0.4950	0.6287	0.6939	0.6862	0.6788	0.6473
	H_e	0.5077	0.6449	0.7127	0.7038	0.6962	0.6530
	F_{IS}	0.2901	0.1399	0.0380	0.0511	0.0832	0.1204
BHL	N_a	2	3	4	3	5	3.4
	A_r	2.00	3.00	4.00	3.00	5.00	3.40
	H_o	0.4988	0.6537	0.7382	0.7312	0.6988	0.6641
	H_e	0.5115	0.6714	0.7582	0.7500	0.7167	0.6815
	F_{IS}	0.2881	0.1410	0.0378	0.0515	0.0799	0.1193
QBD	N_a	2	3	4	3	2	2.8
	A_r	2.00	3.00	4.00	3.00	2.00	2.80
	H_o	0.5000	0.6607	0.6938	0.5942	0.7150	0.6327
	H_e	0.5128	0.6785	0.7115	0.6102	0.7333	0.6492
	F_{IS}	0.3301	0.1414	0.0400	0.0500	0.0825	0.1288

For population abbreviation, see Table II.

Genetic diversity

All the five selected loci were found to be polymorphic in all the five genotypes. Moderate level of allelic diversity was found in all the *C. catla* genotypes. The reasons behind limited allelic diversity are the founders and bottleneck effect because managers in hatcheries have limited stock of brooders with poor genetic management (Wang *et al.*,

2008). Loss of allelic diversity has also been reported by Alam and Islam (2005); Ahmed and Abbas (2018) in hatchery stocks of *C. catla* and Nazish *et al.* (2018) in hatchery populations of Silver Carp. The lower values of H_o in this study might result of inbreeding depression and negative selection in the process of hatchery propagation plans (Zhou *et al.*, 2011). The maximum value of F_{IS} was of the QBD, followed by FSD, BHL, LHR and FQB stocks respectively. Various studies on Cyprinids confirmed inbreeding depression in hatcheries (Sultana *et al.*, 2015; Nazish *et al.*, 2018; Ahmed and Abbas, 2018). The influence of geographical isolation with no exchange of brooders in relatively small populations might results into genetic diversity loss which in turn enhances the rate of inbreeding depression leading to increased risk of population's extinction (Hayer *et al.*, 2014).

Table IV. Measures of genetic distance (D) (above diagonal) and geographic distance (Km) (below diagonal) between five populations of *C. catla*.

Population	LHR	FQB	FSD	BHL	QBD
LHR	—	0.0137	0.0345	0.0392	0.0772
FQB	73.9	—	0.0427	0.0451	0.0788
FSD	184	93.4	—	0.0598	0.0586
BHL	194.4	134.0	141	—	0.0498
QBD	198.0	143.0	147.2	66.5	—

For population abbreviation, see Table II.

Genetic structure

Significant genetic differentiation was evinced by screening microsatellite loci in examined genotypes of *C. catla*. Generally, the F_{ST} values ranging from 0-0.05 indicates low genetic differentiation (Wright, 1978). Pairwise comparison (60%) showed moderate genetic differentiation ($P < 0.001$) indicating ongoing gene flow due to fisheries management mediation. However, the genotypes pairs (40%) of F_{ST} values specifies significant genetic differentiation ($P < 0.005$). LHR and FQB genotypes showed minimum genetic differentiation that might be due to same origin of parental brood stock exchange. Maximum genetic differentiation was demonstrated by QBD genotype particularly from FQB and LHR. These results can be justified by demographic configuration of the samples. Alam and Islam (2005) found significant F_{ST} values between Halda river and a hatchery population. Most of the examined loci in this study exhibited significant departure from HWE. The heterozygote deficit may emerge through increased levels of inbreeding, non-random sampling, selection against heterozygotes, fishing pressure and the occurrence of null alleles (Hansen *et al.*

al., 2006; Abbas *et al.*, 2010). Pairwise genetic distances (D) were pronounced between genotypes which were geologically distant and vice versa. This is in accordance with population genetics theory which states that limited gene flow might result into divergence of populations (Zattara and Premoli, 2005). The UPGMA dendrogram clustering pattern reveals the genetic relationship. Two major clusters evidently followed geographical patterns of distribution: QBD in one cluster while remaining all the genotypes (LHR, FQB, FSD and BHL) in second cluster. Clustering within group can be explained because of common origin of brood stock or gene flow through human mediation while divergence between distantly related genotypes is due to restricted gene flow.

G × *E* interaction

$G \times E$ interaction is when two different genotypes respond differently to environmental parameters in different ways (Davies *et al.*, 2012). In this experiment, different hatchery stocks (genotypes) were reared under same culture condition; the studied traits of growth performance corresponded to their genetic structure.

The genetic variability quantified in this experiment (high heterozygosity and lowest inbreeding) is in the following order FQB>LHR>BHL>FSD>QBD. Growth performance (increase in weight and lengths) of the populations is also in aforementioned order. These results came up to confirmation that individuals of FQB genotype represented maximum growth owing to the genetic superiority of FQB (highest value of H_o and lowest value of F_{IS}) as compared to other populations. On the other hand, individuals of QBD genotype emerged as lowest growth performers due to least genetic variability. The results previously reported by Muiocha *et al.*, (2017) on growth performance and genetic variability of two populations of *Clarias macrocephalus*, reinforce the present findings. The varied performance of the genotypes is due to difference in genetic background (Besnier *et al.*, 2011; Bicskei *et al.*, 2014).

CONCLUSION

The results of this study deduced that genetic diversity is essential to get maximum production from any aquaculture species. In face of reduction in catla yield, management programmes are the need of hour to avoid genetic deterioration of hatchery-produced seed. The escalating environmental apprehension, demands growth performance monitoring and divulgence of genetic structure on an immense geographical scale by employing polymorphic markers in order to assess the impact of $G \times E$ on growth performance of *C. catla*.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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