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Body weight at harvest and its heritability estimate in *Clarias magur* (Hamilton, 1822) reared under mono and polyculture systems

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ABSTRACT

The present study was aimed to identify the effect of non-genetic factors on harvest body weight of *Clarias magur* (Hamilton, 1822) reared under mono and polyculture systems and to estimate its heritability. Magur fishes from three different geographical regions *viz.,* Andhra Pradesh, Assam and West Bengal were procured and stocked at Fresh Water Fish Farm (FWFF) of ICAR-Central Institute of Fisheries Education, Balbhadarpuram, Kakinada, Andhra Pradesh, India. A total of 139 pairs of fishes were mated to produce full-sib families of which only 42 families had either 15 or more offsprings or survived till tagging and of these a total of 1373 fishes were PIT (Passive Integrated Transponder) tagged. Tagged fishes were reared in mono and polyculture systems. In polyculture system, magur were stocked along with rohu *Lobeo rohita* fishes. The average harvest body weight of magur reared in monoculture was 146.27±1.36 g and was significantly higher than the average body weight of fishes reared in polyculture (137.33±2.28 g) ($p<0.05$). Male fishes were significantly heavier than females at harvest. The heritability for harvest body weight from animal model was 0.55±0.76 and from single pair mating it was 0.63±0.10. The heritability for harvest body weight in mono and polyculture systems differed from one another.

Keywords: Harvest body weight, Heritability, Magur, Monoculture, Polyculture

Introduction

Indian aquaculture is mainly based on three species of Indian major carps (IMCs) and common carp. There is need for diversification of fish species for aquaculture. However, no major success in this direction has been achieved so far, with the exception of introduction of Pangasius in the recent past. Pangasius is an exotic species which requires high investment and is not suitable for polyculture and hence not favoured by majority of Indian fish farmers. Polyculture of IMCs is a common practice in the country. A limited number of farmers also make use of *Clarias magur* (Hamilton, 1822) for polyculture along with the IMCs as it is a bottom dwelling fish and combines well with any surface or column feeder. *C. magur* is a highly sought after fish species owing to its flavour and excellent nutritional profile (Hossain *et al*., 2006). Polyculture of magur with suitable species was found to be more remunerative.

C. magur also commonly known as magur is an air breathing catfish and is endemic to India (Khan *et al*., 2000). This species is adapted to a wide range of climatic conditions. It has good potential for aquaculture as it matures in pond conditions and controlled reproduction is possible, does not require specialised feed, accepts artificial feed, tolerates poor water quality and is generally considered as a hardy species. However, magur culture in India is not well established, mainly due to the non-availability of quality seed on demand, low fecundity, difficulty in collecting milt for large scale seed production, low hatchability and non-existence of genetic selection programmes to improve the performance. It is also facing competition from *Clarias gariepinus* and its hybrids as they grow much faster (Brummett, 2008).

Majority of the fish farmers depend on natural seed of magur for culture, leading to the depletion of the natural stocks in many areas. Natural seeds also exhibit a wide range of variability in their performance and many times harbour pathogens which are difficult to detect. Even though controlled breeding of magur has been demonstrated, with low reproductive rate, there is hardly any hatchery which produces magur seed on a large scale. A few hatcheries that produce magur seed use farm raised brooders for seed production. As with other cultured fish species, captive holding of fishes in farms has resulted in inbreeding and deterioration in the performance of the species.

As has been demonstrated in other fish species, it is also possible to enhance the growth and reproductive performance of magur by adopting selective breeding programmes (Dunham and Smitherman, 1983; Hershberger *et al.,* 1990; Padi, 1995; Gjedrem and Thodesen, 2005). Genetic selection for several generations helps to improve the economic traits in desired directions, it ensures the availability of quality seed on demand, optimises the production efficiency and most importantly it reduces the pressure on natural stocks. To establish a genetic selection programme for any species, understanding the magnitude of genetic parameters such as heritability of important economic traits and genetic correlations among them, is a prerequisite (Falconer and Makay, 1996; Robinson and Jerry, 2009).

Genetic parameters are properties of the population and environment in which they are cultured and therefore it is also important to know the magnitude of these parameters in the respective culture conditions. Increase in the growth rate due to genetic selection has been reported in channel catfish *Ictalurus punctatus* and blue catfish *I. furcatus* (Rezk *et al*., 2003; Small, 2006) with realised heritability ranging from 0.16±0.02 to 0.23±0.02 for body weight at marketable size, respectively. Bondari (1983) reported a much higher heritability (0.58±0.29) for 40 week body weight in channel catfish. Genetic variation in body and morphometric traits in *C. gariepinus* was reported by Rezk (2008). To the best of our knowledge, to date no information is available on genetic parameter estimates of growth traits of *C. magur*. Therefore the present study was aimed to identify the effect of non-genetic factors on harvest body weight of *C. magur* reared under mono and polyculture systems and to estimate its heritability.

Materials and methods

Parent fish stock

Magur fishes from three different geographical regions *viz.,* Andhra Pradesh, Assam and West Bengal, India were procured and stocked at the freshwater fish farm (FWFF) of ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Balbhadarpuram, Kakinada, Andhra Pradesh, India. The Andhra Pradesh stock comprising of 240 fishes weighing 130-230 g was collected during May-June, 2013 from various farms, hatcheries and natural sources from different places in Andhra Pradesh and assembled at Kakinada Research Centre of ICAR-CIFE, Bhalbhdrapuram. The Assam stock (69 fishes) was collected from Pabhoi Fish Farm, Pabhoi, Sonitpur, Assam on 10th June 2013. These fishes were transported by road up to Guwahati and were airlifted to Bhalbhdrapuram, Kakinada. The West Bengal stock consisting of 194 magur fishes with body weight ranging from 100 to 190 g. was collected from various fish markets at Kolkata and other places during May-June, 2013 and first assembled at Kolkata Centre of ICAR-CIFE and then transported to ICAR-CIFE Centre at Bhalbhdrapuram by road in June, 2013. Initially all the fishes were quarantined for two weeks and observed for any disease and deformities. Then fishes were PIT tagged and released in to 200 m^2 earthen ponds. They were reared and conditioned for breeding in these ponds for about one year. Initially fishes were fed at least twice a day with feed containing a minimum of 25% protein at a rate of 5% of body weight. Feeding rate was then reduced to 3% of the body weight a month before breeding.

Induced breeding

Breeding was conducted between July and September 2014. Female and male brooders were chosen by external examination of genitals prior to spawning. Females were identified with their short round genital papillae and the readiness to spawn was confirmed by observing the bulged belly. Males with reddish pointed genital papilla were considered to have mature testes. Spawning was induced in females by administering the commercially available inducing agent 'Ovatide®', a synthetic analogue of gonadotropin releasing hormone (GnRH) at a rate of 1 ml kg body weight^{-1}. The same agent was injected at the rate of 0.5 ml kg body weight^{-1} in males. Sixteen hours post-administration, eggs were collected in a plastic tub by manually stripping the females. To collect milt, males were anesthetised and both the testes were removed by incising the abdomen. These testes were then mashed with a mortar and pestle and immediately diluted in 0.9% normal saline and this solution was poured on to the stripped eggs and normal freshwater was added to initiate motility and fertilisation. The egg sperm solution was gently mixed by swirling the tub for about two minutes after which these eggs were washed with freshwater. Thoroughly cleaned roots of *Eichornia* plants were gently dipped into the tub containing the eggs, to which the sticky eggs attached. These roots were then transferred to hatching tanks with running water. Fertilised eggs hatched out 24-26 h post-fertilisation.

A single pair mating design was employed wherein milt suspension of one male was mixed only with the eggs stripped from a single female. A total of 139 pair of fishes were mated to produce full-sib families, of which only 42 families had 15 or more offspring and survived till tagging. The families were produced over a period of three months' time as the breeding was scheduled as per the availability of brooders with fully mature eggs and the hatchery capacity.

Nursery rearing and tagging

Two days old larvae were fed with newly hatched *Artemia* nauplii, 4 times a day (08:00, 12:00, 16:00 and 20:00 hrs) at a rate of minimum 5 nauplii per larvae. Ten days old fry were shifted to one ton FRP tanks and were then fed using mixed zooplankton, tubifex and *Artemia* flakes once in a day. After another 10 days, advanced fry were shifted to cement tanks and were fed with mixed zooplankton and tubifex. Weaning of the advanced fry to artificial diet started by 20 days post-hatching. In order to identify the full-sibs, each family was reared in individual cement tanks till they were tagged. After about six months from hatching, the body weight of majority of the fishes was above 15 g. and were considered fit for PIT (Passive Integrated Transponder) tagging. PIT tags were inserted in the abdominal region. Fishes weighing about 15 g were chosen randomly for tagging. A maximum of 30 fishes or all available fishes from each full-sib families were tagged.

Culture systems

In the present study, both mono and polyculture systems were followed. In polyculture system, along with magur, rohu (*Labeo rohita*) were also stocked. Monoculture was undertaken in three earthen ponds while polyculture was carried in two ponds. These ponds were of 200 m^2 each and the sides of the ponds were pitched with granite stones. While stocking the fishes, care was taken that all families were distributed in all ponds and in both culture systems. Magur fishes were stocked at a density of 1.5 and 0.6 fishes m^{-2} in monoculture and polyculture ponds respectively. Advanced fingerlings (70-80 g) of rohu were stocked with magur in polyculture ponds at a rate of 0.75 fishes m^{-2}. A total of 1373 magur fishes belonging to 42 full-sib families were tagged and stocked for culture in grow-out ponds. For the entire grow-out period, the fishes were fed twice a day with sinking pellet feed with approximate 30% protein at a rate of 5% body weight.

Fish sampling and data collection

All tagged fishes were stocked in grow-out ponds on the same day. Data on body weight, total length and body depth of all fishes were collected before stocking. After one year of culture period, the ponds were drained completely and the fishes were harvested by hand picking. Data on body weight, total length and body depth of all harvested fishes were collected. Body weight was assessed using a digital weighing balance with precision of 0.1 g.

Statistical analysis

Statistical analysis of phenotypic data was performed using SAS 9.3 (SAS, 2017). Data were checked for normality by employing *Proc Univariate* procedure of SAS and wherever required the data were brought to normality either by removing the outliers and/or employing the appropriate scale transformation procedure. The effect of non-genetic factors on the harvest body weight was identified through analysis of variance employing the Proc GLM of SAS® (SAS, 2017) assuming the following fixed effect model. (Model 1):

$$Y_{ijklm} = \mu + \text{Gender}_i + \text{Culture-system}_j + \text{Pond (culture type)}_k + \text{Age class}_l + e_{ijklm} \text{ ------------ Model 1}$$

where, Y_{ijklm}= observation on m^{th} individual belonging to i^{th} gender reared in j^{th} culture system in k^{th} pond with l^{th} tagging age class. Duncan test was used to find the significant difference between the means of groups within a class. The traits were considered significant at $p<0.05$.

Initially all possible interaction effects were incorporated in Model1 and after the analysis, when found non-significant, were removed from the model and analysis was repeated without those interaction effects.

The heritability of the harvest body weight was estimated by fitting a linear mixed model employing Proc Mixed of SAS (Model 2). Restricted maximum likelihood (REML) was used to partition the phenotypic variance into genetic and environmental components by fitting an animal model (Henderson 1984; Saxton *et al*., 2004). The following model was used to estimate the heritability by animal model:

$$y = X\beta + Zu + e \text{ ------------ Model 2}$$

where, y=vector of observations on harvest bodyweight, β=vector of fixed effects, u = vector of the random animal additive genetic effect, e = vector of random residual effects and X and Z are incidence matrices that relate observations to the respective effects. Heritability of the trait was estimated as $\sigma^2A/(\sigma^2A + \sigma^2e)$,where σ^2A and σ^2e are the variances attributed to additive genetic and residual error effects, respectively.

Heritability was also estimated by employing the single pair mating design as per Becker (1992). Heritability of the trait was estimated as $(2*\sigma^2A)/(\sigma^2A + \sigma^2e)$ and its standard error as follows:

$$\text{S.E. } h^2 = \sqrt{\frac{2(n.-1)\,(1-t)^2\,[1+(k1-1)t]^2}{k_1^2\,(n.-s)\,(s-1)}}$$

where, n = total no. of individuals; k1 = weighted average of the progeny per family; t = intra class correlation and S = number of families.

Results

Effect of non-genetic factors

After one year of culture period, 952 fishes out of the 1373 fishes stocked were recovered. Details on the number of fishes harvested, overall mean of harvest body weight and its standard error are provided in Table 1. Means and their standard errors of non-genetic factors are also provided in Table 1. Analysis of variance (ANOVA) to identify the significant effect of non-genetic factors on the recorded trait is presented in Table 2. The interaction effects between culture type and gender, culture type and age group as well as gender and age group were all non-significant and hence were removed from the analysis.

Table 1. Overall and different class least squares means of harvest body weight and standard error

Source	N	Mean body weight±SE. (g)
Overall	948	143.71 ± 1.17
Tagged age class		
Age class 1(upto 6 months)	45	136.66^{b} ± 5.38
Age class 2 (6-7 Months)	429	147.55^{a} ± 1.74
Age class 3 (above 7 months)	474	140.90^{a} ± 1.65
Culture type		
Monoculture	699	145.87^{a} ± 1.36
Polyculture	249	137.59^{b} ± 2.28
Pond no. and culture type		
14; Monoculture	232	137.31^{b} ± 2.37
19; Monoculture	231	152.59^{a} ± 2.37
22; Monoculture	236	147.73^{a} ± 2.34
18; Polyculture	102	137.69^{b} ± 3.57
23; Polyculture	147	137.53^{b} ± 2.97
Gender		
Female	452	128.74^{a} ± 1.69
Male	496	157.38^{b} ± 1.62

Means bearing same superscript within a class are not significantly different from each other

Table 2. Mean squares from ANOVA for harvest body weight

Source	DF	Mean sum of squares
Tagged age class	2	6127.81^{*}
Culture type	1	13378.89^{*}
Pond no. (culture type)	3	9854.75^{*}
Gender	1	195345.52^{*}
Error	941	636.55

($^{*}p<0.05$)

Since the production of required number of families was spread over three months, the age at tagging was grouped into three subclasses and used as the fixed effect in the model (Model 1). The age class had significant effect on the body weight and the animals born later (age class1) had significantly lower body weight (Table 1). The average harvest body weight of magur reared in monoculture was 146.27 ± 1.36 g and was significantly higher than the average body weight of magur reared in polyculture 137.33±2.28 g ($p<0.05$) (Fig. 1). The males were significantly heavier as compared to the female fishes (Table 1; Fig. 2). Pond effect was confounded within the culture type and they had significant effect on harvest body weight. The average body weight of two ponds within polyculture did not differ significantly among themselves, while one pond (Pond No.14) had significantly lower body weight as compared to two other ponds of monoculture.

Heritability estimates

The heritability estimates for harvest body weight in different culture environment (separately as well as combined) are provided in Table 3. Heritability estimate

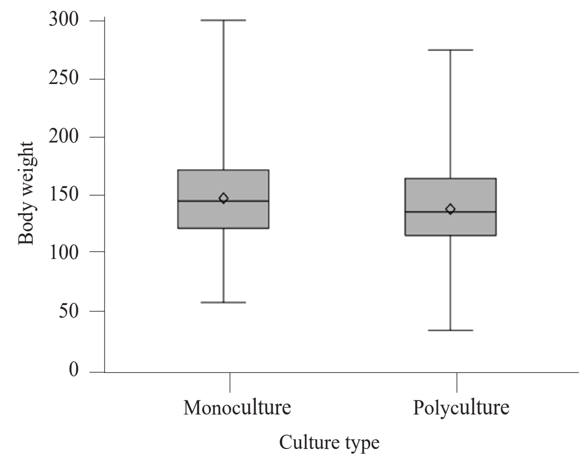
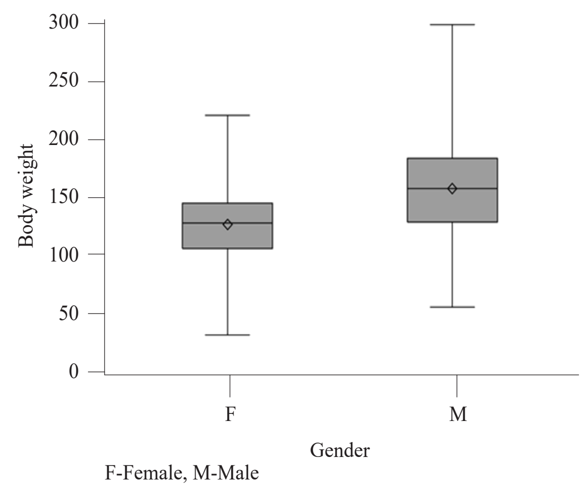


Fig. 1. Box plots of harvest body weight (g) of magur reared in mono and polyculture systems



Fig, 2. Box plots of harvest body weight (g) of female and male magur fish

Table 3. Heritability of harvest body weight of magur reared under mono and polyculture

Heritability estimates by animal model	
Combined	0.55 ± 0.76
Monoculture	0.56 ± 0.75
Polyculture	0.46 ± 0.69
Heritability estimates by single pair design	
Combined	0.63 ± 0.10
Monoculture	0.63 ± 0.09
Polyculture	0.46 ± 0.12

for body weight in monoculture was much higher (0.56) as compared to the heritability of body weight in polyculture (0.46). The standard errors of heritability estimated from animal model were higher. As expected, the heritability estimates from single pair mating design were higher as compared to the heritability estimates from animal model, however their standard errors were smaller. When full-sib family and progeny were used as random effects, the SAS Proc Mixed could not converge and hence heritability was estimated separately by single pair mating design and by animal model. In general, the heritability estimates of body weight were high.

Discussion

Non-genetic factors

In aquaculture, body weight at harvest is the most important economic trait as the fishes are traded based on their weight. Therefore the harvest body weight should be one of the main selection criterion. In India, magur is usually raised in polyculture systems mainly with rohu. Magur has a tendency to burrow into the soil, and therefore full harvest of magur requires draining of the ponds and hand picking, which is possible only after the main species is harvested, which is cultured for about one year period. Hence, in this study also magur was cultured for one year in grow-out ponds. Market size of magur ranges from 46 to 251 g (Das, 2002; Chowdhary and Srivastava, 2013; Kishore and Saha, 2013). In the present study, the mean body weight after one year grow-out period was 143.92±1.17 (Table 1) which is a good size to market.

A prerequisite of any selective breeding program is to tag the fishes for individual and pedigree identification and rear them communally in grow-out condition/s to reduce the impact of common environmental effects (Gjedrem, 2005). In the present study, full-sibs were reared in separate cement ponds with the aim of growing them to a size of about 15-20 g for PIT tagging to facilitate family identification and then rear them communally under commercial grow-out conditions for evaluation purpose. As it was not clear about the rate of survival of the young ones in the cement tanks and to avoid the loss of families, all the offspring of a full-sib family were stocked in one cement tank. This led to varying stocking density in each cement tank and in turn affected the growth rate. In this study, full-sib family production was spread over three months, prolonging the communal rearing of the fishes and it brought in wide variation in age and weight at stocking among the families. This variation in body weight might have been carried forward even up to the harvesting age which was evident from significant variation in the means of different age classes (Table 1, 2). Family-wise rearing of the fishes for a long period increases the environmental effect particular to a family. Due to the limitations of the number of cement tanks, it was not possible to grow full-sib families in replications, and therefore it was not possible to quantify the environmental effect common to a family in this study. The environmental effect common to a family has been reported for tilapia and other fish species (Ponzoni *et al*., 2011; Bentsen *et al*., 2012; Trong *et al*., 2013).

Growth performance of fishes depends on the culture practices adopted. Performance of the species in mono and polyculture is bound to vary even under optimum management practices. In the present study, the body weight of magur in monoculture system was significantly higher than that of the fishes from polyculture system. The difference could be attributed to the large variation in number of observations between the two culture systems. The number in monoculture was almost two times than that in polyculture system (Table 1). Only limited information is available on the optimum stocking density of magur in polyculture systems and for optimum economic returns, there is need to standardise the aquaculture practices of this species. To the best of our knowledge, this is the first report on performance of magur in monoculture system. Though magur has grown better in monoculture system in the present study, its suitability for monoculture system and economics needs to be further investigated.

In the present study, it was observed that the pond environment had significant effect on the body weight at harvest. The variation was mainly seen between the ponds under monoculture system. Even though efforts were taken to adopt uniform standard management practices for culture of magur, the dynamic aquatic environment was found to play a crucial role on the performance of the animals.

Sexual dimorphism has been reported in magur and males are heavier than the females. In the present study also the same trend was observed and male fishes were almost 19% more heavier than the females. It was also observed that the interaction effect between the gender and culture type, ponds and age class was non-significant (results not shown) indicating that the male and female growth rate is equal across these classes and the difference in body weight of male and female could be attributed to physiological differences.

Non-genetic factors play an important role in the production performance. Understanding their effects helps to standardise the aquaculture practices and to design better selective breeding programmes. In the present study, all non-genetic factors tested had significant effect on the harvest body weight. It is therefore essential to incorporate them in the models to estimate unbiased genetic parameters.

Heritability estimates

Heritability estimate indicates the degree of additive genetic variation to the total phenotypic variation. Knowledge about the heritability of economic traits is essential for designing selective breeding programmes for magur and this is the first report to the best of our knowledge on the heritability estimates of harvest body weight of magur. In this study, heritability of body weight was estimated by animal model and single pair mating design. Heritability estimates by both the methods were very high (Table 3). The high heritability estimates obtained with single pair mating design was expected as it contains the dominance effect, interaction between additive and dominance, dominance by dominance and the common full-sib effects (Becker, 1992). The heritability estimates from animal model were also high and accompanied with high standard error. In this study, only full-sibs were produced and thus the common full-sib effects and dominance effects got confounded with the additive gene effect inflating the heritability. As mentioned earlier family-wise rearing of the fishes was too long for a few families as breeding of fishes was spread over three months. This might have led to accumulation of common full-sib effect which may not have vanished during the 12 months of grow-out period and this also may have inflated the heritability estimate. High heritability estimates for body weight are obtained in first few generations when the natural stocks are used to produce the families. Considering these facts, the present estimates may be taken as indicative of the presence of additive genetic variance for the harvest body weight in magur, and the extent may further need to be established by producing both full and half sibs by employing better mating designs. High heritability estimates for body weight and the common full-sib effect have been reported in other fish species such as common carp, Nile tilapa and channel catfish which supports our findings (Bondari, 1983; Choe and Yamazaki, 1998; Maluwa *et al*., 2006; Vandeputte *et al*., 2008; Neilsen *et al*., 2010; Marjanovic *et al*., 2014).

According to Falconer and Mackey (1996), heritability is a property not only of the trait but also of the population, of the environment in which the individuals are raised and how the phenotype is measured. Any change in the above components affects the heritability estimates. Heritability estimates are also influenced by the method of estimation and the sample size within a method. In the present study, magur fishes were reared in mono and polyculture systems and since these management practices are treated as two different environments and the performance of the animals were significantly different, it is but appropriate to estimate the heritability separately. It was observed that the heritability estimates for harvest body weight in polyculture system was lower compared with the heritability estimate of the fishes from monoculture system (Table 3). The difference may be due to variation in the overall sample size and number of fishes per family. It was also observed that a few families were not represented in both the culture systems which may have also led to the differences in the estimates.

The present study suggests that there is significant effect of culture type on the harvest body weight of magur and males are heavier than females. Heritability estimates indicate the presence of significant additive genetic variation in harvest body weight of magur, suggesting the possibility of increasing the harvest body weight by adopting appropriate selective breeding programme.

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