Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(2): 545 – 553 (2022)
www.ejabf.journals.ekb.eg

Development of Farm Type Golden Variant of *Labeo rajasthanicus* (Pratap Sunahari) through Captive Breeding

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ARTICLE INFO

Article History:
Received: Sept. 10, 2021
Accepted: Feb. 19, 2022
Online: April 29, 2022

Keywords:
Breeding,
Golden colour,
Labeo rajasthanicus,
Ornamental fish,
Farm type fish

ABSTRACT

The artificial selection was applied for the development of a farm type golden colour variant of *Labeo rajasthanicus* from the wild type of brood stock collected from Tidi River, a tributary of Mahi-Som River System (N 24° 13.720, E 074°59.754) in Rajasthan (India).The variable numbers (1-16) of brood fish pairs in 1:1 male to female ratio of breeders were addressed in six generations. Fish of each generation were selected when they reached an average weight of 10±0.09g with a dark golden colour. The wild types and light golden colour were culled. The final/second selection for golden colour was done prior to breeding. The golden colour population in the first generation was 10.90% only, which was observed to increase to 100% in the third generation and onwards. The selection for farm type golden colour variant of *Labeo rajasthanicus* was found effective as the progeny for the fifth generation had a 100% desired colour population.

INTRODUCTION

It is desirable to have some unique external features in fishes. The external appearance, especially the colour decide the consumer's preference and price of a particular fish in the market. Similar to other animals, the colour of the body is specified by the genetic makeup (genotype) of a particular species (**Gomelsky *et al.*, 1996; David *et al*., 2004**). However, the intensity/sharpness of the colour depends markedly on a number of external features, such as type of feed, water quality, light intensity, etc. (**Wallat *et al*., 2005; Kim & Lee, 2015; Kop & Durmaz, 2008**). Thus, the skin colour in fish, and more specifically in carps, are complex traits, involving numerous genetic and environmental factors. The colour of fish can be gainfully improved using selected strategies, such as marker-assisted selection based on molecular data. The classical genetic tools have been used to improve the external traits of commercially important aquaculture species (**Pillay & Kutty, 2005**); skin colour is a qualitative trait under Mendelian control. The dominant inheritance in red Stirling strain of the tilapia

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Oreochromis niloticus (**McAndrew *et al*., 1988**) and recessive inheritance in rainbow trout for the iridescent metallic blue variant (**Kincaid, 1975**) are classical examples of Mendelian genetics. Simply, the knowledge of inheritance mode could be used to selectively breed new stocks with particular colour.

In 2010, a World Bank/GEF sponsored project was initiated (**Anon, 2014**) to explore the availability of indigenous fish species of economic importance, and *Labeo rajasthanicus* was evaluated as an important minor carp native to south Rajasthan (India). This species has potential for inclusion in composite culture. However, there was a lack of adequate knowledge about the breeding protocol and optimum rearing condition, *etc*. (**Lal *et al.* 2015**). Hence, the brood stock of this species was collected from natural habitat (Tidi River near Jaismand Lake) and captive breeding, and seed rearing protocols were developed during 2010-2013. In August 2013, only one golden coloured mature male fish was obtained from the seed produced and reared in a farm pond. Considering the importance of coloured fish in aquaculture, and especially in the ornamental fish industry, a breeding plan was implemented to develop farm type golden variant of *Labe rajasthanicus*.

MATERIALS AND METHODS

This study was conducted during 2010-2018 at the Aquaculture Research & Seed Unit of Directorate of Research, Maharana Pratap University of Agriculture and Technology Udaipur (India).

Experimental Fish: A minor carp, *Labeo rajasthanicus* native to southern Rajasthan (India) was selected for the development of farm type golden variant. It is closely related to *Labeo boggut* (Sykes), resembling it in general morphology, but differs in meristic characters: (i) number of dorsal fins rays 16 vs. 12; (ii) number of lateral transverse scales in the body 11 1/2 and 91/2 vis. 12 and 14. In captivity, both male and female, *Labeo rajasthanicus* attain first sexual maturity in their first year. It is a slow growing species that attains about 250-350g in one year under normal culture conditions.

Etymology: The farm type golden variant of *Labeo rajasthanicus* was developed at Maharana Pratap University of Agriculture & Technology (MPUAT), Udaipur (India). The local name *'PRATAP SUNAHARI'* of this species has two words: Pratap and Sunahari. The first part 'PRATAP' represents the university name, and the second 'SUNAHARI' is the Hindi meaning of golden.

Brood Stock: The mature brood fish were collected from its wild habitat (River Tidi near Lake Jaismand-N 24° 13.720, E 074°59.754) and transported to university fish farm. The collected specimens were reared at university fish farm following standard protocol of **Thomas (2003)** for the rearing of carp brood stock.

Breeding: The fully matured male and female fish were selected for breeding purposes. The potential brood stock was selected on the basis of secondary sexual characteristics. The matured fish showed sexual dimorphism; the pectoral fin of the male becomes rough, pointed and narrow genital papilla, with freely oozing milt when slight pressure was applied on the abdomen. The genital papilla was swollen and slightly pinkish in colour, with a smooth pectoral fin. The abdomen of the female was bulgy. For the post collection from the pond, the brood fish were transferred to a nylon hapa installed in a pond for spawning purpose. The brood samples were conditioned for about 6hrs in a hapa fixed in a pond before administering the inducing hormone (Gonopro-FS). No feeding was provided during the conditioning of brooders.

Intramuscular hormone injections (0.2 and 0.1ml/kg body weight to female and male, respectively) of inducing hormone (Gonopro-FS: GnRH) were given to selected brood fish using syringe No.22 and placed in hapa. The hapa was installed in a pond and a continuous monitoring of breeding behaviour was carried out after hormone administration. Specimens were removed after the completion of spawning. Eggs were collected from hapa and transferred to the incubation unit for hatching. After hatching, the spawn was harvested and stocked in well-managed nursery ponds. Once the fry attained a size of +20mm, they were harvested and counted.

For the development of golden variant, as mentioned earlier (introduction) in August 2013, one light golden coloured mature male fish was obtained from the seed reared for captive breed wild stock of *Labeo rajasthanicus*. The milt striped from this male was used to fertilize eggs of three female fish. Thus, the progeny produced was reared in earthen pond until an average growth of 10±0.09g was achieved. At this stage, golden and wild type fingerlings were counted, and only golden coloured fingerlings were further reared for developing future brood stock of this species. At maturity stage, once again healthy and golden coloured brood fishes were selected and used for captive breeding. The same simple selection procedure was adopted for a number (6 Nos.) of breeding seasons. The phenotypic data of golden body colour segregation from each mating were subjected to chi-square (χ^2) analysis at significance level of $P \leq 0.05$. The inbreeding rate for each selection generation was calculated using the procedure suggested by **Tave (1986**) for inbreed population.

RESULTS

Captive Breeding

The spawning in Gonopro-FS (GnRH) injected fishes occurred after 8.13±1.24hrs. The fertilized eggs appeared transparent enclosing a blue-green zygote forming an identifying character for the fertilized eggs of *Labeo rajasthanicus*. The unfertilized eggs of this species were white in appearance. Fertilized eggs of *Labeo rajasthanicus* were non-floating, with 3.4±0.02 mm in diameter after hardening. The fecundity of this species ranged between 1.32 and 1.43 lacs/kg body weight. The average fecundity was 1.31±0.06 lacs per kg body weight. In all the experiments, the fertilization rate was always above 90%. The fertilization rate ranged from 93.3 to 94.6, with a mean fertilization rate of 94.16±0.75% (Table 1).

The fertilized eggs were shifted from breeding hapa to D-85 type carp hatchery system for hatching, and some eggs were kept in plastic tube under laboratory condition to observe the developmental stages and hatching percentage. While observing the development of embryos, it was noted that the twitching movement started at 9.5±0.13hrs after fertilization at 27.5±1.04°C.

Table 1. Summary of *Labeo rajasthanicus* breeding indices

Parameter		Range	Mean	SD±
Weight (g) of Male	Initial	409-504	455.33	47.54
	Final	401-498	448.33	48.54
Weight (g) of Female	Initial	632-683	657.33	25.5
	Final	507-549	526.33	21.19
Spawning Time (hrs)		10.06-10.20	10.13	0.004
Nos. of eggs(lacs)		0.836-0.965	0.916	0.069
Fecundity (Lacs/kg body weight)		1.323-1.438	1.391	0.06
Fertilization (%)		93.3-94.6	94.16	0.75
Hatching (%)		95-98	96.66	1.53

Hatching stated 14.8±0.85hrs after fertilization. The hatching rate was 95-98%, while its mean was 96.66±1.53% (Table1).The hatchlings were about 4.19±0.03mm in length and light blackish in colour. On the 4th day of hatching, the size of spawn was 8.07±0.06mm when the yolk sac was completely absorbed. The survival rate of the hatchling up to the 4th day recorded 87.23±1.03%.

Development of Farm Type Golden Variant

The values pertaining to the number of brood fish used in each generation, the total fry produced and the numbers of golden colour progeny selected at both the stages i.e., fingerling and adult are presented in Table (2).A positive response to the selection for golden colour was observed in each progressive generation.

The proportion of golden colour in F1 generation was 10.90% and increased to reach 100% in the third generation. Further, the results from single pair cross showed that the segregation of wild and golden were not in the expected 1:1 ratio and was confirmed by the $\chi 2$ analysis (Table 3). The inbreeding rate for each of the selection generation is shown in Fig. (1).

Table 2. Selection response on the development of golden variant of *L. rajasthanicus*

S.No.	Generation	Breeders (No.)*	Fry (No.)	Percentage		No. of golden fish selected for next generation**	
				Wild type	Golden	I: Fingerling Stage	II: Maturity Stage
1	0	4*	16370	89.10	10.90	23	14
2	1	10	23050	15.18	84.81	325	18
3	2	16	19650	2.79	97.20	280	14
4	3	14	27500	0.00	100.00	372	18
5	4	12	24800	0.00	100.00	450	20
6	5	16	23080	0.00	100.00		

**Male: Female =1:1 (except ‘0’ generation where one golden colour male milt was used to fertilized eggs striped from three wild type females); **Fingerlings (having* 10±0.09g *weight) & Brood fish withgolden colour were only selected*

Table 3. Data on the ratios of wild type to golden individual pair for each generation

Generation	Category	Observed(Ho)	Expected (He)	χ2	CV
0	Wild	1870	1100	1078.00	3.841
	Golden	330	1100		
1	Wild	375	1250	1225.00	
	Golden	2125	1250		
2	Wild	350	1050	933.00	
	Golden	1750	1050		
3	Wild	0	1125	2250.00	
	Golden	2250	1125		
4	Wild	0	950	1900.00	
	Golden	1900	950		
5	Wild	0	725	1553.45	
	Golden	1500	725		

DISCUSSION

Compared to agriculture and livestock, aquaculture is a young science where systematic and efficient breeding programs improving traits have rarely been utilized until recently, except for salmonid species (**Gjedrem, 2005**). In agriculture and livestock, the situation is vastly different; virtually, no terrestrial farm production is based on unimproved populations. Though the basic fundamentals of fish breeding are the same as for terrestrial animals, in spite of this fact the difference exist between aquaculture and livestock production. This is attributed due to the difference in the reproductive biology of aquatic species, and special consideration needs to be taken in the design of breeding plans for these species.

Selection is applied to change the fish population for making genetic improvement, where certain individuals are allowed to reproduce and others are denied the opportunity to produce next generation. Therefore, selection is the choice of individuals to produce the next generation. In the present study, this basic principal of selection was applied for the development of golden colour *L. rajasthanicus*. In the first generation (F1), the wild type population was 89.10%, which was significantly decreased from generation to generation, with no wild type population in third generation (Table 2).The percent of wild type and light golden colour between the 1^{st} and 5^{th} generations clearly decreased to an almost zero expression. This was probably due to the selection pressure exposed on dark golden colour individuals in each generation, in which no more than 0.085% of the total progeny was selected. This agrees with the results of **Mather *et al.* (2001)**, who decreased black blotching in the Fijian hybrid tilapia in three generations.

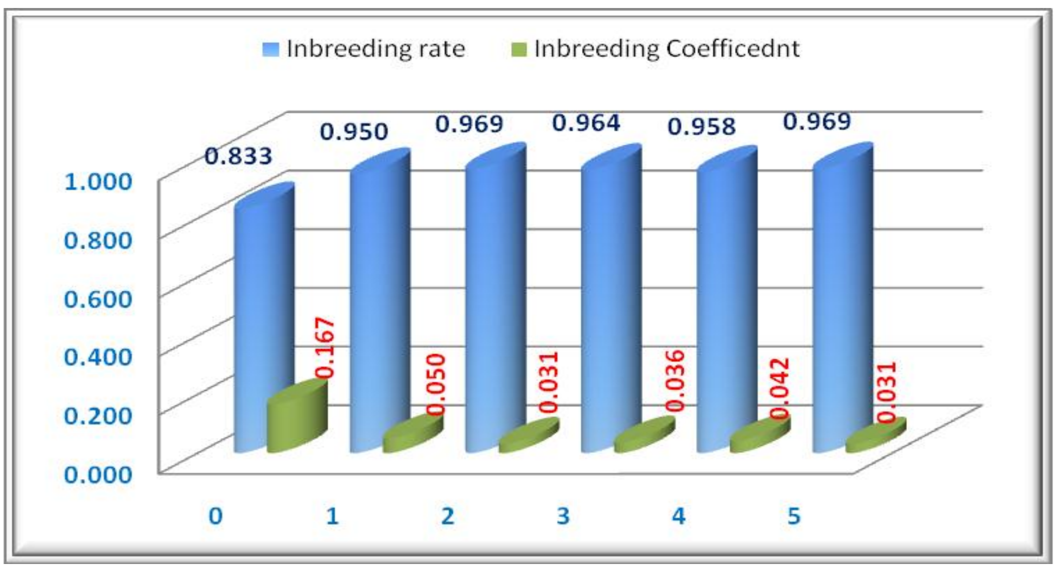

Fig. 1: Inbreeding rate in each generation of *Labeo rajasthanicus*

In the present study, the wild type phenotypes abruptly decreased beginning from the 1^{st} selection generation, with no wild types observed in the third generation. This rapid response to selection of golden colour *L.rajasthanicus* could be due to the very high frequency of alleles responsible for wild type phenotype in the initial population (Fig. 2). In addition, **Mario *et al* (2004)** suggested a sharp improvement in the red colour of the tilapia fish due to selection pressure for a particular trait. The red colour in common carp (*Cyprinus carpio*) was related to the recessive homozygous genotype (**Tave, 1985**). We achieved homozygosity in golden colour within five generations, which may be attributed to the homozygous recessive alleles or the effect of multiple genes as in the case of goldfish (**Kon *et al*., 2020**). However, it needs to be validated through progeny testing and marker based molecular studies. The use of this planned mass selection for obtaining golden colour phenotype could be recommended for mass scale production of golden colour genotype of *Labeo rajasthanicus*. It is worth mentioning that, the golden variant of *L. rajasthanicus* (Pratap Sunahari) may be promoted as ornamental fish, especially for garden pools.

In aquaculture species, most colour phenotypes of economic importance are qualitative traits under Mendelian control (**McAndrew *et al*., 1988**). Nevertheless, the results of the present study did not support the Mendelian inheritance. **Tave, (1986**) suggested that the inheritance mode of qualitative traits for skin pigmentation has a simple genetic basis, i.e., a monogenetic control, which may be recessive, completely/incompletely dominant, and co-dominant or sex-linked. The recent studies emphasize that skin pigmentation in fish can possess a more complex genetic architecture, characterized by specific genome regions that harbour genes controlling quantitative traits. In this essence, both light and dark golden colour progeny were recorded in this study which shows the possibility that colour trait in *L. rajasthanicus* may possess a complex genetic architecture, with the existence of a variable number of quantitative loci. Further analysis of these traits will clarify their particular genetic architecture.

Fig. 2: *Labeo rajasthanicus*: **(a** & **b)** adult golden & wild type; **(c)** spawn, and **(d)** fry

It is obvious that a narrow inbreeding could lead to the depression of productive traits (i.e. growth rate, survival, fecundity, etc.) due to endogamy **(Klug & Cummings,**

2000). The estimated inbreeding rate in the 5th selection generation was 0.969% (Fig.1), which is lower than the 5% proposed by **Tave (1986)** as a preventive measure to avoid negative effects from inbreeding. In the present study, the consanguinity generated through selection would definitely affect the production traits. However, due to the limited availability of brood-stock of this rare species of western Rajasthan (India), it was inevitable and unavoidable. In future, to avoid the negative impact on aquaculture traits, an emphasis is recommended on the collection of brood-stock from natural habitat and crossing the same with the golden line produced in this study.

ACKNOWLEDGEMENTS

The financial grant by World Bank/GEF for research project "Harmonizing biodiversity conservation and agricultural intensification through integration of plant, animal, and fish genetic resources for livelihood security in fragile ecosystems" is gratefully acknowledged. We also thank the university administration for logistic support in carrying out the experiment at university farm and laboratory.

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