Lessons from the Breeding Program of Rohu

Kanta Das Mahapatra[1], R. K.Jana[1], J. N. Saha[1], Bjarne Gjerde[2] and N. Sarangi[1]
[1]*Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar -751002, India*
[2]*AKVAFORSK, Institute of Aquaculture Research AS, 1430 Ås, Norway*

Abstract

For the first time in India, selective breeding work has been initiated at the Central Institute of Freshwater Aquaculture, Bhubaneswar, India in collaboration with the Institute of Aquaculture Research (AKVAFORSK), Norway. Rohu has been chosen as the model species because it enjoys the highest consumer preference among Indian major carps (IMC) although its performance was observed to be slower than other IMC. As this was the first ever selection work on any Indian major carp, many procedures and techniques for successful implementation of the programs were standardized (i.e. production of full-sib groups, establishment of model hatchery for selective breeding of carps, rearing of full-sib groups in partitioned nursery ponds, individual tagging with the Passive Integrated Transponder (PIT) tag, communal rearing, sampling, data analysis, field testing and dissemination of improved rohu). After four generations of selection, an average of 17 per cent higher growth per generation was observed in improved rohu.

Introduction

Indian major carps such as Catla (*Catla catla*), Rohu (*Labeo rohita*), and Mrigal (*Cirrhinus mrigala)* are relatively fast-growing fish in India. They are mutually compatible and their food habits are also complementary to each other so these fish gained popularity not only in India, but also in neighboring countries such as Bangladesh, Pakistan, Myanmar, Thailand, and Vietnam. After the success of induced breeding technology, a large number of hatcheries have been established in the country. Since seed is the basic input in the culture system, its production has been accorded the highest priority. While India has attained self-sufficiency in carp seed production, most hatcheries do not follow any genetic norms while producing carp seed. As a result, the Indian carp hatcheries are experiencing deterioration of the quality of carp seed because of inbreeding (Eknath and Doyle 1990).

Among the Indian major carps, rohu is one of the most preferred species in the country and commands a higher price in the market. The species is also an excellent game fish owing to its easy acceptance of anglers' bait. Andhra Pradesh, West Bengal, Assam and Orissa are the most important states for aquaculture production and rohu is most preferred species in these states. In view of its fast growth and high demand, it is possible that in the future, monoculture of the species might be undertaken by farmers instead of the present polyculture. However, its performance in terms of growth is slower when compared to other species in the multi-species culture system. Besides, rohu is highly susceptible to diseases. Thus, in India there was an urgent need for better procedures for seed production and genetic improvement in terms of growth, survival and other traits of economic importance to rohu through selective breeding.

Taking all these factors into consideration, a project on the genetic improvement of rohu, particularly for better growth performance through selective breeding, was initiated for the first time in India in 1992 at the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, in collaboration with the Institute of Aquaculture Research (AKVAFORSK), Norway. The funds required for carrying out the research were provided by the NORAD Agency, and the infrastructure (pond and laboratory) and manpower to execute the project activities were provided by the CIFA under the Indian Council of Agriculture Research (ICAR), New Delhi. The scientists of AKVAFORSK, Norway provided consultation services. The main aim of the project was to develop a national selective breeding plan for rohu and disseminate improved rohu to fish farmers of India for quality seed production.

Selective breeding of rohu

The main objectives of the selective breeding of rohu project were: (i) to obtain information about the magnitude of the genetic variation for growth and survival in rohu, (ii) to develop manpower at the CIFA with strong knowledge on quantitative genetics and selective breeding, (iii) to develop a breeding program of rohu based on the results obtained, and (iv) to disseminate improved rohu to fish farmers through a number of multiplier units.

The base population

The selective breeding project of rohu was initiated with six stocks as the base population. Rohu stocks were procured from five different river systems of India (i.e. Ganga, Yamuna, Brahmaputra, Sutlej and Gomati) (Table 1). The fry or fingerlings collected were quarantined for about two weeks during which time they were kept in cement cisterns. To these five riverine stocks, the CIFA hatchery stock was added as sixth stock. After quarantine, the fish were marked by fin clipping or M-prociane blue dye marking or a combination of both. They were stocked in communal ponds for rearing until sexual maturity.

The base population is very important in a selective breeding program. Genetic variability is essential to start any genetic improvement program. Genetic characterization of these six stocks indicated a wide variation within each **S**tock. The variations within stocks were much more significant than between-stock variations.

Table 1. Base population and year of procurement.

Stock	Base population year-classes	
	1993	1994
Local (CIFA) hatchery stock	X	
Ganga	X	X
Gomati		X
Yamuna		X
Sutlej		X
Brahmaputra		X

Figure 1. Base population from different rivers of India.

Brood fish raising and management

Fin clipped or dye marked fish were reared in monoculture ponds to raise brood fishes. Vitamin enriched feed was provided to the brood fish at 2-3 per cent of body weight. The ponds were also regularly fertilized with organic manure. Health monitoring was done frequently by checking the fish monthly. Other management practices were followed according to the general pond environment prevalent in India.

Fish breeding and mating design

The Ovaprim hormone was used for the breeding of rohu. Five hours after the hormone injection, male milt was collected separately in small vials, which were serially numbered. The milt was then refrigerated until fertilization time. After stripping of females (generally 5-6 hours after hormone injection), fertilization was carried out with the pre-determined male milt according to the breeding plan.

To date, twelve year-classes of full-sib and half-sib families have been produced with five complete generations of selection. Parent-offspring genetic ties were established between the two populations found from the 1993 and 1994 base population year-classes (Table 2). The choice of mating design, males nested within female or vice versa, was dependent on the number and body size of the female breeders available in the actual year-class (Figure 2).

Table 2. Production of different generations.

Generation	Population	
	1	2
0	1993	1994
1	1995	1996
2	1997	
3	1999	2000
4	2001	2002
5	2003	2004
6	2005	

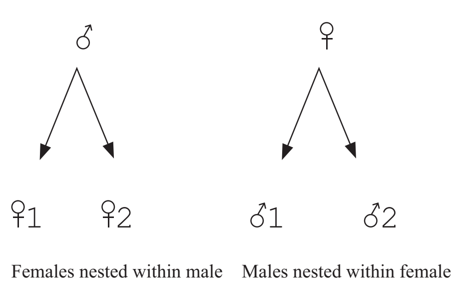

Figure 2. Nested mating design.

Production of diallele crosses

To estimate the magnitude of heterosis for harvest body weight and survival crossing, different rohu stocks produced using 3X3 diallele cross. One included Ganga, Yamuna and Local (Diallele I), and another included Local, Brahmaputra and Sutlej. This was performed for the 1995 year-class where a total of 18 crosses were produced. The local stock was common for both crosses. Thus, 17 different stock combinations were produced (Table 3).

Data analysis indicated that the total heterosis for each of the six stock crosses was low or negative and the average heterosis was also low and in most cases not significantly different from zero. In terms of survival, the heterosis was negligible and not significantly different from zero. Hence, it was concluded that genetic improvement through cross-breeding of rohu has little practical significance (Gjerde et al. 2002).

Incubation of fertilized eggs

The incubation of fertilized eggs, hatching and further rearing until fingerling size are critical stages in a selective breeding program. A combined selection method (utilization of own, full-sib and half-sib records in the selection decisions) was adopted in the rohu selective breeding program. Hence, rearing was done separately until the fish attained taggable size. During the initial phase of the project (i.e. 1993-99), the incubation of fertilized eggs was carried out in hatching hapas fixed in a pond. High rates of mortality occurred in the hatching hapas due to unavoidable environmental hazards such as a sudden change in the temperature, strong winds, and predatory fish.

Consequently, it was decided that a specialized hatchery had to be constructed for the production of full-sib family groups. The hatchery was constructed specifically to cater to the needs of the selective breeding program. The hatchery proved to be very effective with almost 100 per cent recovery of families for the last five years. This hatchery can be a model for any selective breeding of carp species (Das Mahapatra and Sahoo 2003).

Rearing of full-sib groups

On the fifth day after breeding and 72 hours after completion of the hatching process, the spawn are transferred to nursery ponds at 5000 / 100m^2. The spawn are reared separately in nursery ponds until they attain taggable size. Selection through full-sib families requires a good number of nursery ponds. Each nursery pond (200 m^2) is partitioned into two nursing areas by partition cloth. A rich plankton crop is always ensured before stocking the spawn and also during the rearing phase. Supplementary feed is also provided regularly (Saha et al. 2003)

For any selective breeding program, the number of full-sib groups is very important. A small number of full sib groups in a breeding program of rohu create a lot of problems in the later generations. The fish did not attain taggable size in small indoor tanks, so earthen ponds were utilized in the rearing program. In the estimation, a high common environmenal effect on the full-sib groups for tagging was observed.

In a recent experiment, it was also observed that rohu spawn could reach taggable size in a cement cistern of larger size (10x5x1.5 m) and large common environment effect could be avoided.

Tagging

Tagging of individual fish is essential in a selective breeding program involving family selection or a combined selection. Initially, different indigenous tags including surgical suture with plastic chips, and vinyl thread with plastic chips were tried, but they all proved to be unsuitable for rohu. Since the fish is an active swimmer, retention of the external tags was very poor and secondary infection was observed in most cases. Therefore, Passive Integrated Transponder (PIT) tags were used instead when the spawn reached a size of 10-15 g. PIT tags were implanted on their abdomen of the fingerlings with the help of a tag implanter. The individual growth status was also recorded before tagging. After implantation the fish were kept in separate tanks overnight in case of any possible mortality. After that, they were stocked in communal ponds for further growth experiments. An equal number of fingerlings were stocked from each full-sib group in the communal ponds.

Table 3. Diallele cross.

Sire strain	Dam strain				
	Ganga	Yamuna	Local	Brahmaputra	Sutlej
Ganga	X	X	X		
Yamuna	X	X	X		
Local	X	X	X	X	X
Brahmaputra			X	X	X
Sutlej			X	X	X

PIT tags were found to be very suitable for tagging of rohu. The size range of 10-15 g was also observed to be the most suitable for the tagging of this fish (Das Mahapatra, et al. 2001).

Test environments at the CIFA

At the CIFA, tagged fish were stocked in three well-prepared communal ponds of 0.1 ha each under monoculture, and two ponds of 0.4 ha each under polyculture. In polyculture, rohu was stocked along with catla and mrigal in the ratio of 1.2:1:1, respectively; this practiced continued until 1997. After 1997, monoculture of rohu was practiced in the selective breeding program.

An analysis of the data indicated that for growth and survival, the pure stock as well as crosses rank similarly (r_g = 0.74 ± 0.27) in monoculture as well as polyculture, and that development of specialized varieties for each of the two production systems is not required(Reddy et al. 2002)

Estimation of genetic parameters

The body weights recorded at tagging, sampling (6 months after tagging) and harvesting were considered. The harvest body weights of fish raised in monoculture, polyculture, and in different agroclimatic zones were considered as the same trait.

For the tagging body weight, the heritability estimate was very low while the effect common to full sibs other than additive genetics was very high. For the sampling and harvest body weights, the heritability was a medium magnitude (Table 4). Thus, the prospect for the genetic improvement of growth in rohu is promising.

Table 4. Estimation of heritability of body weight.

Body weight	H^2 ± se
Tagging	0.05 ± 0.07
Sampling	0.23 ± 0.09
Harvesting	0.23 ± 0.06

A different, less risky procedure for rearing the newly hatched larvae from different full-sib groups until tagging size needs to be developed.

Realized response to selection and genetic gain

Each year, a control group was produced using 10 male and 10 female parents with an average breeding value for the harvest body weight. The control group was reared in replicated nursery ponds. They were tagged individually and reared in the growout ponds together with the tagged fish from all the selected families. In the field tests, local hatchery stocks were also used along with the control and selected families in the rearing program.

Harvest body weight data recorded for the control animals and the selected animals were used to obtain estimates of the realized selection response per generation for the harvest body weight (i.e. as the difference in the mean performance of all the selected families and the control group).

One of the main objectives in a breeding program is to maximize the genetic gain per generation of selection. This is obtained by selecting individuals with higher genetic merit or breeding value. Different methods are available to estimate breeding values. In the rohu breeding program, the selection index procedure is used to estimate the breeding value. Information from full sibs, half sibs and individuals is considered for breeding value estimation. This procedure efficiently combines all the available information about one as well as several traits recorded on the breeding candidate and its relatives into an index of genetic merit. In the rohu project, an average genetic gain of 17 per cent per generation was observed after five generations of selection in the research center. However, a much higher response was observed in different field testing centers.

Statistical analysis

Editing of the data and basic statistical analysis were performed using the SAS statistical package.

For each year-class, breeding values for the harvest body weight (for the trait selected in each year-class) were calculated for all the breeding candidates (using own, full- and half-sib body weight records), and for an SAS program developed in the project.

Field testing of improved rohu

Field testing was initiated and carried out from 1999 to 2001 at different centers listed below (Table 5).

1. Kausalyaganga State Fish Farm
2. Rahara, West Bengal
3. Vijayawada, Andhra Pradesh
4. Ludhiana, Punjab

In all these centers, improved rohu showed significantly higher growth than the control and local hatchery stocks.

Table 5. Year-class and locality where selected families and control groups were tested.

Year class	Locality	Selected families	Selected control	Local control
1995	CIFA (Orissa)	X	X	
1996	CIFA (Orissa)	X	X	
1997	CIFA (Orissa)	X	X	
1999	CIFA (Orissa)	X	X	
	Andhra Pradesh	X	X	
	State Dept (Orissa)	X	X	X
2000	CIFA (Orissa)	X	X	
	Andhra Pradesh	X	X	X
	Punjab	X	X	
	West Bengal	X	X	X
2001	CIFA, Orissa	X	X	
	West Bengal	X	X	X
	Andhra Pradesh	X	X	X
2002	CIFA, Orissa	X	X	

Dissemination of improved rohu

Initially, the dissemination of improved rohu is planned for the following three states:

1. Orissa (through the State Fisheries Department)
2. West Bengal (CIFA, Regional Center)
3. Andhra Pradesh (Private Hatchery, Sairam hatchery)

The improved rohu, popularly called “Jayanti”, as it was named in 1997 (i.e. the 50th anniversary of Indian independence - Swarna Jayanti), has been released to several hatchery owners so that they can provide better quality seed to the fish farmers.

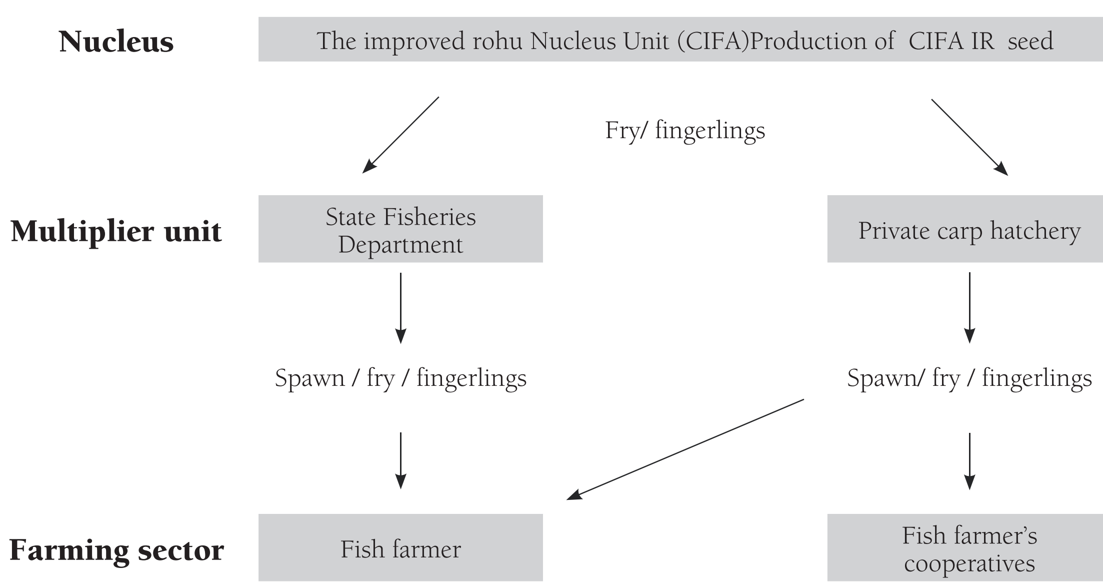

Figure 3. Dissemination plan for improved Rohu.

Dissemination plan for the Jayanti rohu

Through an effective dissemination mechanism, it is possible that research products will reach the ultimate users (i.e. fish farmers). At present, rohu dissemination has been planned through different meetings and suggestions from collaborative institutes, international institutes and peer groups. The scope of the dissemination plan outlined below has the means for modifications with change in situations of nucleus and multiplier units.

Dissemination at present will be done with a single nucleus (i.e. CIFA).

The basic elements of a multiplication and dissemination program (Figure 3) according to the production and distribution process, are as follows:

1. Distribution of ***Jayanti*** brood stock: Research Institute (i.e. CIFA) provides broodstock to selected hatcheries.

2. Multiplication: hatcheries (Government, private farmers), using brood stock from the Research Institute, produce seed (fry or fingerlings) for distribution to grow-out farmers.

3. Nursing: seed from the hatcheries are reared prior to stocking in grow-out ponds. The rearing may be conducted by the end-user farmers themselves or by multiplier units that, in turn, sell or distribute the reared seed (fry, fingerlings, yearlings) to grow-out farmers.

Addressing farmers' needs

While designing and implementing a multiplication and dissemination program, the focus of the institute is often on the improved seed and the mechanisms required to make the improved seed available to the farmers. Unfortunately, such a focus on the seed may cause the institute and its multipliers to lose sight of the farmers and their needs.

The institute will ensure that farmers are able to obtain the maximum benefit offered by genetically improved seed. This will require that the farmers be provided with adequate training, education and technical support. Although government extension programs exist, the institute and its multipliers will carefully evaluate the extension services provided to the farmers by the Government.

The dissemination plan for improved rohu will be state-specific. In Andhra Pradesh, since the private hatchery is doing a very good job, the improved rohu may be disseminated through it. Regional Research Centers (RRC) of CIFA at Andhra Pradesh are monitoring the program. Selection of an additional hatchery in Andhra Pradesh other than the SaiRam hatchery as a multiplier unit needs further study and suggestions.

In Orissa, since Government hatcheries are selected as multiplier units they will breed and supply seed to the farmers. At present, six hatcheries have been stocked with improved rohu. Additional Government hatcheries can be stocked with improved Rohu for proper distribution.

In West Bengal, the present Regional Research Center of CIFA is acting as a multiplier unit. The West Bengal State Fisheries Department and some private hatcheries may be contacted, and dissemination can be planned under the supervision of the RRC, Rahara.

Apart from these states, some other states such as Chattisgarh and Tripura, or any other suitable states or organizations may be considered for multiplier units in future.

It is also the responsibility of the multiplier units to collect feedback from the farmers. Jayanti rohu grow-out farmers are required to adhere to the conditions that are described below.

a. Improved rohu seed received will only be used for table size fish production.

b. The Jayanti rohu will not be utilized or retained in the farm for breeding and propagation purposes.

c. Fish farmers should follow the complete package including feeding and sampling procedures provided by the nucleus through multiplier units.

d. The farmers will provide feedback regarding production and sale to the multiplier unit or nucleus from time to time.

e. Any violation of the above conditions will result in legal actions by the multiplier or nucleus (CIFA).

Addition of new trait to growth in rohu

Recently another trait (i.e disease resistance) against *Aeromonas hydrophila* was initiated at the CIFA, India in collaboration with the AKVAFORSK, Norway. Standardization of the mass challenge test for rohu was completed. A wide variation in the survival percentage was observed in different full-sib groups. The project is in progress.

Conclusions

Improved Jayanti rohu- is the first genetically improved fish of India. In order to capitalize on the efforts made for the development of Jayanti rohu, its dissemination to farmers must be effective. The notion of hatcheries engaging in the production of their own broodstock may be discouraged. Experience shows that this is likely to result in inbreeding and impaired performance, and this will damage the reputation of Jayanti rohu. Therefore, vigilant effort is required for effective dissemination of improved rohu.

The experiences and lessons learned from the selective breeding of rohu are plenty, such as the production of full-sib groups in rohu carp, individual tagging methodology, selective breeding hatchery management, and data analysis. These experiences can be utilized by other carp selective breeding programs to avoid initial failures.

A lesson to be learned from other (terrestrial) species is that the processes of multiplication and dissemination occur in a more systematic and effective manner when special resources are assigned to the task.

References

Das Mahapatra, K., B. Gjerde, P. V. G. K. Reddy, M. Sahoo, R. K. Jana, J. N. Saha and M. Rye. 2001. Tagging: on the use of Passive Integrated Transponder (PIT) tag for identification of fishes. Aquaculture Research 32:47-50.

Das Mahapatra, K. and M. Sahoo. 2003. Efficacy of specialized hatchery for selective breeding of carps, p.64-69. ***In*** Final workshop on genetic improvement of rohu (***Labeo rohita***) for growth through selective breeding held at Bhubaneswar 20-21 May 2003.

Eknath, A. E. and R. W. Doyle. 1990. Effective population size and rate of inbreeding in aquaculture of Indian Major Carp culture. Aquaculture 85:293-305.

Gjerde, B., P. V. G. K. Reddy, K. Das Mahapatra, R. K. Jana, J. N. Saha, P. K. Meher, M. Sahoo, S. Lenka, P. Govindswammy and M. Rye. 2002. Growth and survival in two complete diallele crosses with five stocks of Rohu carp (***Labeo rohita***). Aquaculture 209(1-4):103-115.

Saha, J. N., M. Sahoo and J. Mohanty. 2003. Production of full-sib groups for selective breeding of Rohu (Labeo rohita), p. 59-63. ***In*** Final workshop on genetic improvement of Rohu for growth through selective breeding.

Reddy, P. V. G. K., B. Gjerde, S. D. Tripathy, R. K. Jana, K. D.Mahapatra, S. D.Gupta, J. N. Saha, M. Sahoo, S. Lenka and P. Govindswamy, M. Rye and T. Gjerdem. 2002. Growth and survival of six stocks of rohu (***Labeo rohita***) in mono and polyculture system. Aquaculture 203(3-4):239-250.