

Expanding cryobanking assisted carp broodstock management for quality seed production

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Quality seed is the most important input in the commercial freshwater aquaculture sector of the country and it is mostly supplied by hatcheries of the private and public sectors. With decades of hatchery-based carp culture and seed production intensification, a serious concern has emerged about seed quality, especially with the risks from inbreeding. Sperm cryobanking as a conservation tool, can not only provide support to captive breeding technology in the propagation-assisted rehabilitation of the target species in its natural habitat but can also assist carp broodstock management for quality seed production and sustainable fisheries and aquaculture enhancement. This report presents efforts and experiences of ICAR-NBFGR, Lucknow in customization of the sperm cryopreservation technique, primarily developed for ex-situ conservation, for upscaled fertilization through field validation, demonstrations, and capacity development of stakeholders in 11 states of the country.

Keywords: Aquaculture, Broodstock, Carp, Cryobanking, Seed

THE commercial freshwater aquaculture sector of the country is mainly dominated by carp which includes Indian major carp (IMC) and exotic carp. Seed is the most important input and it is mostly supplied by hatcheries of the private and public sectors. As per production statistics of FAO 2022, Indian major carps viz. catla (*Catla catla*), rohu (*Labeo rohita*), and mrigal (*Cirrhinus mrigala*) contribute approximately 5.44 million tonnes to the total fish production of the country. It is reported that in India the losses due to the inbreeding of fish are quite significant, however, it is difficult to quantify it in monetary terms. Inbreeding in carp hatcheries of the country is affecting seed quality as genetic management of brooders is not followed due to the lack of awareness and resources. The same stock of brooders is used repeatedly for seed production over many generations which leads to homozygosity and reduced genetic vigour. In hatcheries, a smaller number of males are maintained to

save rearing space, and the same set of males is used repeatedly in many breeding sets. It results in producing paternal half-sibs and when these half-sibs are mated, the rate of inbreeding per generation increases. It affects the quality of the seed produced and these inbred seeds do not perform to their full potential on the grow-out farm. Recently, Govt. of India has been implementing National river ranching under PMMSY for which the production of quality seed is an important input. Thus, to maintain the quality of fish seed, it is crucial to follow a good brood

husbandry practice and broodstock should be replenished at regular intervals. However, replenishment of brooders is not an easy task as it involves transportation of stock from far distance which might result in significant mortalities and will involve higher costs. Therefore, an alternate approach could be to use cryopreserved sperm of unrelated healthy stock to establish a cryobank and facilitate exchange between hatcheries for quality seed production.

Sperm cryopreservation technology: A working strategy for field validation and upscaling

Sperm cryopreservation technology has revolutionized the animal breeding program with big industry. Although fish protocols were also established almost at the same time, however, commercial application has not been adequately demonstrated. A farm with a normal breeding population may not need frozen sperm, but this technology can be an asset to



Egg collection for field demonstration at Raisen, Madhya Pradesh

alleviate milt-related problems, such as improving genetic fitness, exchange with wild germplasms, and broodstock management. This will need upscaling of the methodology, customization with local material for the convenience of use by the hatchery personnel, and identified farm to source quality sperm. To achieve this, there is a need for a cryobank, which can be operated in the upscaled mode, with quality milt collected from brooders on the farm, and stored, and a system of distribution to hatcheries is established. At the same time, capacity-building efforts are also necessary to enhance the competence of state fisheries officers/ hatchery managers.

With this background, to take the fish milt cryopreservation technology to the field and make a visible impact in fostering the aquaculture sector of the country, ICAR--National Bureau of Fish Genetic Resources (NBFGR), Lucknow made this new initiative with the support of NFDB, Hyderabad. The program was envisaged to demonstrate that sperm cryopreservation can be used as a tool for implementing genetic exchange between hatcheries. The work is aimed at establishing a working strategy for field validation of sperm cryopreservation based on the technology developed for Indian major carp. The frozen sperm is supplied to the selected state government hatcheries/ private hatcheries/entrepreneurs for enabling cross-breeding. This will help in understanding the customization required for the technique to suit large-scale fertilization. The cryopreservation and supply were done for three species of IMCs. The seed is being



Addition of thawed semen on measured volume of eggs for fertilization at Raisen, Madhya Pradesh

Table 1. *Ex-situ* conservation – Cryopreservation of fish milt and fertility trial

Scientific name	Common name	Cryopreservation status (Protocol developed, Viable hatchling and Field demonstration)
<i>Catla catla</i>	Catla	Field demonstration
<i>Labeo rohita</i>	Rohu	Field demonstration
<i>Cirrhinus mrigala</i>	Mrigal	Field demonstration
<i>L. dyocheilus</i>	Kali rohu	Protocol developed
<i>Oncorhynchus mykiss</i>	Rainbow trout	Field demonstration
<i>Salmo trutta fario</i>	Brown trout	Viable hatchling
<i>Cyprinus carpio</i>	Common carp	Field demonstration
<i>Tenualosa ilisha</i>	Hilsa	Viable hatchling
<i>Tor khudree</i>	Deccan mahseer	Viable hatchling
<i>T. putitora</i>	Golden mahseer	Viable hatchling
<i>L. dussumieri</i>	Malabar labeo	Viable hatchling
<i>L. dero</i>	<i>Bangana dero</i>	Viable hatchling
<i>Horabagrus brachysoma</i>	Sun catfish	Viable hatchling
<i>H. nigricollaris</i>	Black collared catfish	Viable hatchling
<i>Barbodes carnaticus</i>	Carnatic carp	Protocol developed
<i>Garra surendranathanii</i>	Periyar Garra	Protocol developed
<i>Clarias magur</i>	Walking catfish (Desi Magur)	Viable hatchling
<i>Heteropneustes fossilis</i>	Asian stinging catfish (Singhi)	Viable hatchling
<i>Ompok malabaricus</i>	Travancore Gulper Catfish	Protocol developed
<i>Gonoproktopterus curmuca</i>	Red-tailed barb (Kooral)	Protocol developed
<i>L. calbasu</i>	Black rohu	Viable hatchling
<i>Osteochilichthys longidorsalis</i>	Paral fish	Protocol developed
<i>Pangasius pangasius</i>	Pangas	Protocol developed
<i>Puntius sarana subnasutus</i>	Sarana	Viable hatchling
<i>Etroplus suratensis</i>	Pearlspot	Protocol developed
<i>Puntius chalakkudiensis</i>	Miss Kerala	Protocol developed
<i>Schizothorax richardsonii</i>	Snowtrout	Protocol developed
<i>Ctenopharyngodon idella</i>	Grass carp	Viable hatchling
<i>L. gonius</i>	Kuria labeo	Viable hatchling
<i>Cirrhinus reba</i>	Reba carp	Viable hatchling
<i>Pangasianodon hypophthalmus</i>	Striped catfish	Viable hatchling
<i>C. dussumieri</i>	Mozhi	Under developed
<i>L. bata</i>	Bata carp	Under developed
<i>O. bimaculatus</i>	Butter catfish	Under developed
<i>P. sarana sarana</i>	Puntius barb	Under developed

(Source: Handbook of Fisheries and Aquaculture 2011 and ICAR-NBFGR Annual Reports)

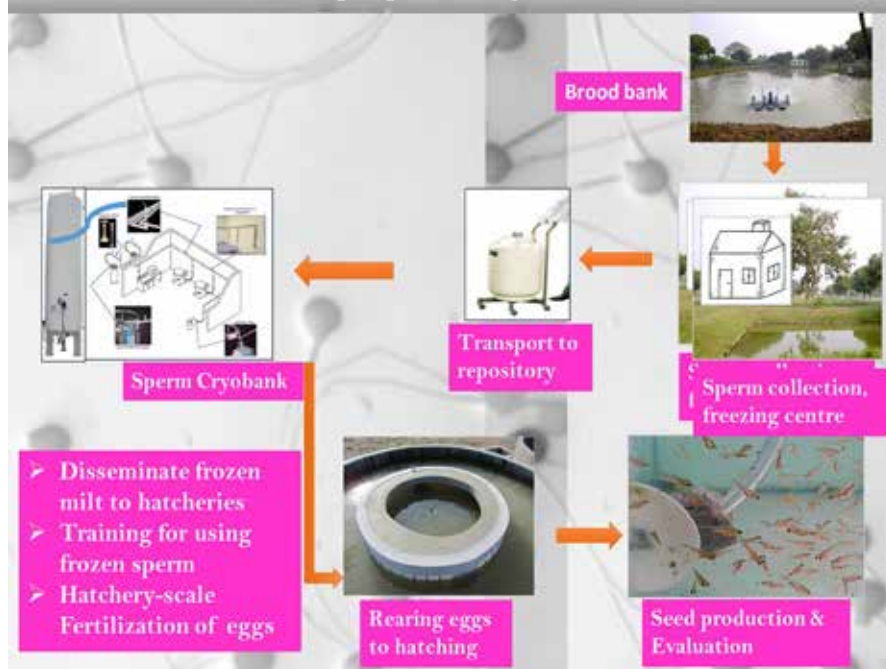
raised by the state hatcheries separately to develop broodstock. This effort developed interest and exposure among seed producers through training and demonstration on the application and prospects of milt cryopreservation and its use in artificial spawning in captivity. In the long term, cryobanks will become a mode for the distribution of improved varieties and native types with known performance. It will ensure a sustainable culture of good quality and genetically fit seed by the farmers leading to increased

fish production in the country.

Field demonstrations and capacity development

With over four decades of hatchery-based carp culture and seed production intensification, a serious concern has emerged about seed quality, especially with the risks from inbreeding. Though, the utility of the technology had been described in the literature for decades, however, there is no case study of application in commercial seed production. This program

Carp Sperm Cryobank



Various components of model carp sperm cryobank at ICAR-NBFGR, Lucknow

required customization of the technique, primarily developed for *ex-situ* conservation, for upscaled fertilization which is essential for its adaptation in commercial hatcheries. Another challenge was to complete the cross-breeding within the limited time of fertilization and carried out by semi-technical hatchery managers. Therefore, it was necessary to expose the concerned stakeholders to the application of the technology and understand their needs. Realizing these challenges, a total of 370 hatchery owners/ state fisheries officials/ young entrepreneurs/ research scholars, etc., from 20 states were exposed to the technology

through in-house training and on-farm demonstrations, during the last four years. Based on the interest, capacity, and logistics, the partners from different states were selected for applying cross-breeding through on-farm work and demonstrations. The team of scientists from ICAR-NBFGR traveled to the field with the cryopreserved sperm and necessary material. The eggs were used from the stock available at hatcheries. Initially, the hatchery owners were reluctant to this new venture and could provide a limited number of females. The sperm were cryopreserved using 2 ml cryovials (optimized in place of commonly used 0.5 cc French

medium straws). The males were sourced from the stock available at Germplasm Resource Center at ICAR-NBFGR, raised from wild collected fingerlings from the Ganga river system.

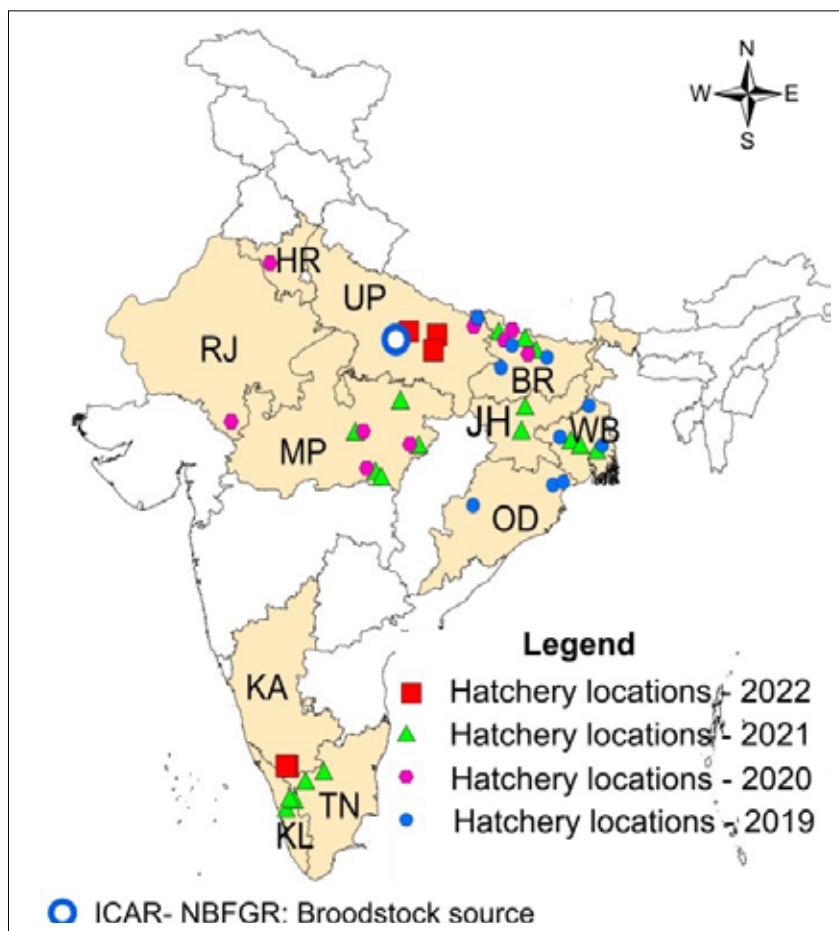
The cryopreserved milt was supplied to different partner hatcheries for seed production in commercial hatcheries. The fertilization was carried out using easy-to-use tools and on the large scale under real-time conditions of commercial seed production. In the hatchery, two populations were produced i.e., one from cryo-milt (outbred with a cross of sperm from ICAR-NBFGR) and another from fresh milt (local inbred). In total, the 126 lakhs spawn (cross-bred) were produced in 38 hatcheries located in 11 states during 2019-2022. The work demonstrated that cryobanking can be used as a technology for genetic exchange and outbreeding between hatcheries. This will need future strategies and enabling policies to establish sperm cryobanks.

Feedback from the stakeholders

The feedback from the stakeholders has been very important while planning the course of this program. With first-hand experience, the hatchery professional/seed producers have indicated an interest in exploring the new possibilities of commercial benefit such as early and late breeding and availability of seed by using cryopreserved milt, when prices are high. Under these conditions, though eggs are available, seed cannot be produced due to lack of milt. The seed producers also expected a reduction in the cost of rearing males and avoiding the risk of losses due to poaching. In the long run, seed producers were enthusiastic to expand the program to develop sperm cryobank, at individual farm levels, and cooperative or state-sponsored cryobanks established at regional levels. These benefits can be accrued through evolving a working strategy to use for broodstock improvement through enabling genetic exchange. The future might bring in the era of systematic cross-



Transfer of fertilized eggs into incubation pool of eco-hatchery at Raisen, Madhya Pradesh



State-wise distribution of hatcheries covered for field demonstration during project period (2019-2022)



Demonstration of commercial seed production using cryomilt at Ramsagar, West Bengal

seed will reach the marketable size early and thereby, have a positive impact on the overall production. The seed producers expressed the need for a similar protocol for other potential species, such as pangasius, grass carp, etc.

SUMMARY

This technological demonstration opens up a new vista for the sustainability of carp aquaculture. Quality seed is a pre-requisite for any culture system. Inbreeding is affecting seed quality, as genetic management of broodstock is not followed due to a lack of awareness and resources. An alternative approach could be to use cryopreserved sperm of unrelated healthy stock to establish a cryobank and facilitate exchange between hatcheries for quality seed production. From the point of view of genetic exchange from broodstock, it will be useful that two lines from different hatcheries produced with frozen milt of different males are exchanged, maintained, and cross-bred for seed production for sale. Using PIT tagging for identification, hatcheries can produce genetically diversified seeds for farmers by crossing. Through systematic training and demonstrations, youths can take up cryobanking as an enterprise and can take help under the PMMSY scheme of the Govt. of India. The establishment of cryobank in various parts of the country can create livelihood opportunities for semi-technical unemployed youths, with support through skill upgradation programs.

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breeding for the genetic fitness of broodstock and seed. This can be possible by establishing the Live Germplasm Resource Centers with broodstock of certified origin and evaluation and traceability data; which supply sperm or fingerlings for developing broodstock for sperm banks. This will enable certification and traceability in the carp seed value chain, and improve quality for a resilient carp seed production system in the country.

Advantages and applications of using cryomilt

The cross-breeding of two broodstock representing diverse genetic makeup is likely to improve genetic diversity, resulting in enhanced fitness and improved seed quality. When such broodstock is from a known and certified origin, it sets the base for certification and traceability. During the program, a control set was produced in the majority of the farms, however, due to constraints of separate rearing space, awareness, and lack of uniform

rearing conditions, the growth data was not comparable, though 15-30% more growth was recorded over local inbred population. Therefore, the perception of the hatchery managers was recorded through a questionnaire. Most of the respondents believed that 20-30% additional growth can be obtained by outbreeding. Partner hatcheries were convinced that the cryobanking technique can be used for early seed production wherever the male matures one or two months later in the breeding season. Some of the farmers believed that the early



Demonstration of fish milt cryopreservation at Seoni, Madhya Pradesh