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Genetic management of **Indian major carps**

GENETICS
IN AQUACULTURE
A CASE STUDY

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Authors

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1. Introduction

1.1 Indian major carp (IMC) aquaculture production, status and trends

Aquaculture plays an important role in the economic development of many Asian countries, contributing to animal protein intake, employment generation and domestic and international trade earnings. In recent decades the sector has been growing globally at a rate of over 5 percent per year. Asia has continued to dominate the aquaculture sector, producing 89 percent of the global production volumes in the last 20 years (FAO, 2020).

Forty-one species or species groups from the family Cyprinidae contributed 24.64 percent to global aquaculture production in 2020, according to data derived from FAO (2022). The five Chinese carp species (grass carp, common carp, bighead carp, silver carp and black carp) and two Indian major carps (catla and rohu) are the dominant species and contribute 82.23 percent of total carp production. A third IMC species, mrigal (*Cirrhinus mrigala*), also part of common polyculture systems in South Asia, contributes 1.91 percent of total carp production and 0.47 percent of global aquaculture production. Naylor *et al.* (2021) reported that catla and rohu contributed 4.39 percent of global aquaculture production. The three IMC are often stocked together in these polyculture systems (which may also include other species such as common carp) to utilize their differing trophic feeding niches most effectively.

In the major fish producing countries of South Asia, IMC production increased from 8.33 thousand tonnes in 1950 to 6602 thousand tonnes in 2020 (FAO, 2022a). Ninety-nine percent of this production comes from four countries, namely India (74.9 percent), Bangladesh (11.8 percent), Myanmar (10.72 percent) and Pakistan (1.55 percent). India is the second-largest global aquaculture producer and this is mainly due to growth in IMC production from 830 tonnes in 1950 to 4. 947 million tonnes in 2019. Eighty-eight percent of India's total aquaculture production is contributed by freshwater culture with 57.3 percent coming from IMC culture (FAO, 2022a).

1.2 Contribution of Indian major carps to national nutritional security of India

In Asia almost 30 percent of total animal protein intake is derived from fish (Gupta *et al.*, 2005) and low trophic fish, including small indigenous fish, play an important role in nutritional security. In South Asia, the majority of the carp production is consumed domestically in fresh form. The importance of IMC for the nutritional security of traditional fish consumers and their producers, mostly small-scale farmers, is very significant. In the Indian context, this production system needs to be stable and follow a sustainable intensification path. The IMC, especially catla, have religious and ceremonial importance in eastern India. When viewing production and consumption data the unique dietary profile of India needs to be considered. There is regional diversity in culinary habits and wide variation in annual per capita fish consumption, which ranges regionally from about 2 kg to over 50 kg (DoF, 2020a). Plans for increasing aquaculture production, including IMCs, must consider these local differences in fish consumption patterns (Lal and Pradhan, 2021).

1.3 History of the domestication of Indian major carps

Carp culture has been prevalent in the Indian subcontinent since the Middle Ages (Agarwal, 2018). Until 1960, the seed for carp farming was sourced from the wild. Wild seed collection was a major fishing activity that used specific gear developed for this purpose and occurred at identified locations along the rivers (Jhingran, 1991). Seed was collected as spawn (3-day-old larvae with yolk sac absorbed), fry and fingerlings and used to be a mixture of various species of carp and catfish. Wild-caught seed had high transportation costs from collection sites to farms. This practice had limitations, including the short period when collection was feasible and fluctuations in seed quality and quantity.

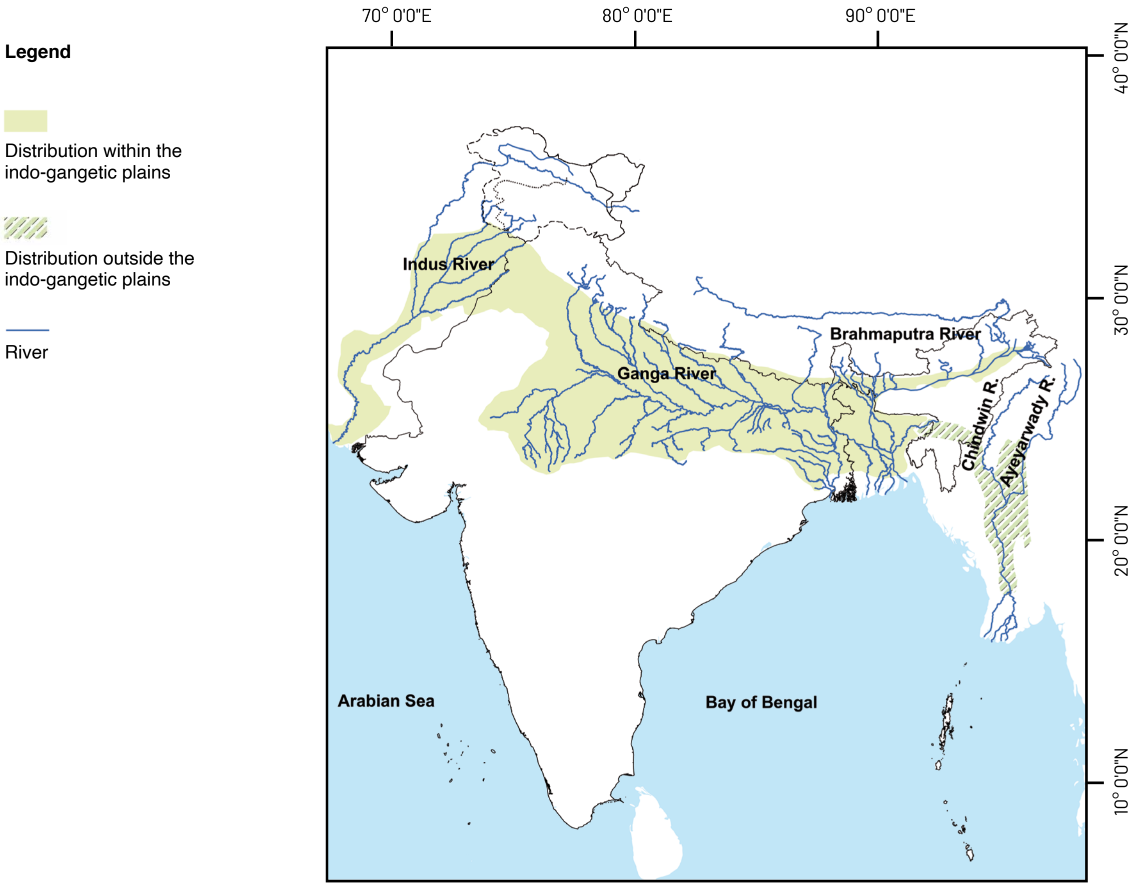
The first successful captive breeding of IMC was achieved with the hypophysation of mrigal in 1957 (Chaudhuri, 1963) and this revolutionized the whole course of seed production and carp culture. Currently, hypophysation is a common practice in IMC breeding through the use of pituitary extract or synthetic preparations containing salmon gonadotropin releasing hormone analogues (sGNRHa) (Figure 1). “Bundh breeding”, which encourages natural breeding of carps (Jhingran, 1991), with hundreds of fish pairs breeding at random, is also followed in a few places. In 1962–1963, nearly 92 percent of the requirement was met through riverine seed collection; however, this reduced to only 5 percent by 2002–2003 and 95 percent of seed requirements were met through induced breeding (Basavaraja, 2007).

Figure 1. Injecting broodstock *L. rohita* with hormone preparation sGNRHa analogue based.

1.4 Native distribution of the Indian major carps

The Indian major carps, also known as Gangetic carps, have a native distribution that includes the rivers, tributaries and floodplains of the Indus, Ganga, Brahmaputra and Chindwin Irrawaddy. These rivers flow through Pakistan, India, Nepal, Bangladesh and Myanmar (Figure 2). The IMC were also introduced into peninsular India and some other countries in Southeast Asia (Reddy 1999).

Figure 2. Map of the probable native distribution of the three Indian major carps.



Notes:

[a] The river network and basin boundary shapefile are prepared using satellite images of Google Earth platform, used with Shapefile India sourced from INDIA-WRIS and mapping work was carried out in ArcMAP 10.8.1.

[b] The dotted line represents approximately the Line of Control in Jammu and Kashmir agreed upon by India and Pakistan. The final status of Jammu and Kashmir has not yet been agreed upon by the parties.

Source (based on): Reddy, P.V.G.K. 1999. *Genetic resources of Indian major carps*. FAO Fisheries Technical Paper No. 387. Rome, FAO; Chauhan, T., Lal, K.K., Mohindra, V., Singh, R.K., Punia, P., Gopalakrishnan, A., Sharma, P.C. & Lakra, W.S. 2007. Genetic differentiation in the population of Indian major carp, *Cirrhinus mrigala* (Hamilton-Buchanan, 1882): evidence from allozyme and microsatellite markers. Aquaculture, 269 (1–4): 135–149. https://krishi.icar.gov.in/jspui/handle/123456789/5112; Aung, O., Thuy T.T. Nguyen., Poompuang, S. & Wongpathom, K. 2010. Microsatellite DNA markers revealed genetic population structure among captive stocks and wild populations of mrigal, *Cirrhinus cirrhosus* in Myanmar *Aquaculture*, 299(1): 37–43. https://doi.org/10.1016/j.aquaculture.2009.12.010; Indian Council of National Research – National Bureau of Fish Genetic Resources (ICAR-NBFGR). 2014. *Final report outreach project of fish genetic stocks*, 2008–2014. Indian Council of Agricultural Research, New Delhi. 127 pp. www.nbfgr.res.in/site/writereaddata/sitecontent/fgr-guidelines_nbfgr.pdf. Map conforms to UN Geospatial. 2011. Map of South Asia. New York, United States of America. Cited on March 2023.

2. Challenges to effective genetic management of IMC (including risks to wild relatives)

2.1 Key aspects in broodstock management

Genetic diversity is essential for the capacity of a species to adapt to a changing environment. Maintaining such diversity is not only critical for the conservation of wild populations but is also the basis for the success of any long-term breeding programme. In aquaculture it is crucial to remember that the broodstock used for seed production represents only part of the genetic pool of a species, population or farmed type. Lack of capacity in genetic management, limited knowledge of the genetic background and diversity of the broodstock and failure to target optimum effective population size (*Ne*) have unfortunate consequences. These may lead to inbreeding depression, consequently undermining the success of a long-term breeding programme. Effective population size (*Ne*) is the number of reproductively mature individuals which contribute to the next generation. Optimum *Ne* helps to maintain genetic diversity in equilibrium from one generation to the next. Effective population size is a key parameter in the management of genetic resources. The number of reproductive individuals that contribute to future generations is one of the main aspects that influence the level of genetic diversity. Genetic drift (the random variation in allele frequencies or genotypes in a population) can reduce genetic variation when the *Ne* is low. This genetic erosion in farmed species can reduce overall productivity and result in reduced production performance and loss of fitness and adaptive capacity. Negative consequences for important traits in farmed populations, such as growth, fertility, and disease resistance, have been shown to be due to loss of genetic variation. The risk of diseases can also be amplified during domestication in the absence of appropriate broodstock management (Doyle *et al.* 2019).

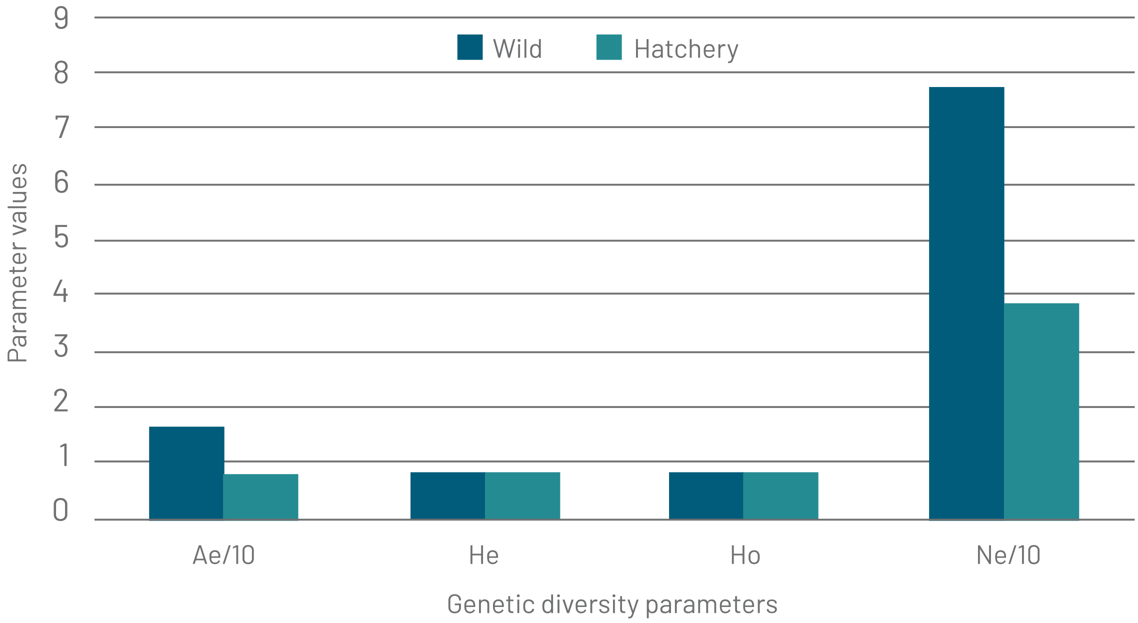
2.2 Evidence of inbreeding and its impact on seed quality

Eknath and Doyle (1990) reported a reduction in production performance among IMC due to increase in homozygosity. Based on data from 18 fish hatcheries in the Karnataka State of peninsular India these authors concluded that there was a reduction in estimated *Ne* and rapid inbreeding of stocks. Basavaraju and Mair (1996) estimated the effective population sizes of carp species in major hatcheries of Karnataka and found this to vary from 55 to as low as 11. Das (2012) reported low *Ne* in carp hatcheries in Assam, ranging from 5 to 17. FAO (2008) recommended a minimum *Ne* of 50 for routine hatchery seed production to avoid inbreeding. This recommendation should be valid if the founder population had genetic diversity. However, in the majority of hatcheries seed from other hatcheries or farms (which could be from only a few parents) is sourced for the founding population. Despite the large number of broodstock maintained in these hatcheries to produce their target quantity of seed it is unlikely that the optimum *Ne* will be attained to avoid inbreeding.

Maheshwari and Biradar (1998) reported a decline in the fertility of hatchery bred *Labeo rohita* due to inbreeding. Analysis of 10 years of data from a research farm on the breeding of related individuals and fertility revealed that there was a 71 percent decline in fertility level up to the sixth generation compared to the base population. Deepak *et al.,* (2006), examining the carp hatcheries in Karnataka and Maharashtra, found an accumulation of inbreeding in IMC.

Genetic level investigations can precisely pin-point the occurrence of inbreeding. Significant deficit of heterozygotes and deviations from Hardy-Weinberg Equilibrium (HWE) were observed in rohu collected from the Punjab province of Pakistan (Qadeer and Khalid, 2017; Sultana *et al.,* 2015). Using sixteen microsatellite markers Masih *et al.* (2014) compared rohu samples from six Indian hatcheries with those recorded in wild riverine populations. This analysis revealed a reduction of allelic diversity (*Ae*) and effective population size (*Ne*) in the farmed populations (Figure 3) compared to wild populations (unpublished data, ICAR-NBFGR).

Figure 3. Genetic diversity in hatchery and wild *L. rohita* indicating probable inbreeding.



Note

The parameters compared are the mean number of alleles per locus (*Ae; actual values are divided by 10 to fit on the scale*); expected heterzygosity (*He*); observed heterozygosity (*Ho*) and mean effective population size (*Ne; actual values are divided by 10*) for 6 hatchery and 6 riverine populations.

Source: ICAR-NBFGR. 2019. Annual Report 2019. ICAR-National Bureau of Fish Genetic Resources, Lucknow, India.

2.3 Hybridization and introgression risks

Some commercial hatcheries often induce the three IMC species to breed at the same time in a common spawning pool. This practice produces seed with a mixture of IMC species and reflects limits in space, time and effort compared to breeding, raising and transporting the seed of individual species to farmers. In these cases, the quantity of the broodstock of each species is used to suggest the proportion of the seed produced. This practice meets the demand for a mixture of seed of the three species by farmers or the middlemen distributors who accumulate and supply the seed (Jhingran, 1991). In some cases, this practice counters the excess demand for the seed of one species (usually catla) which leaves the seed of other species unsold. Some hatcheries also fertilize with the milt of alternative species when male broodstock or milt of target species is unavailable. These practices are not recommended due to the inadvertent production of hybrids within the pool, due to the overlap of time of gamete release in the three species. The unwanted production of the hybrids can be detrimental to the overall production system in the long term.

There is scientific evidence of the use of molecular markers for the detection of hybridization and species introgression in hatcheries in India (NBFGR, 1998; Jayashankar, 2020) and Bangladesh (Simonsen *et al.*, 2005). Mishra *et al.* (1998) reported 8.26 percent hybrids among the 42-day-old fingerlings, produced inadvertently during mixed spawning of IMCs. One of these studies (NBFGR, 1998) analysed genetic and morphological data from 276 samples from two IMC hatcheries and detected that up to 27.0 percent of the fingerlings were hybrids. It is interesting to note that 16.9 percent of the hybrids were identified only by molecular analysis but not morphologically. This highlights the fact that a lack of genetic characterization leads to a lack of awareness of the composition of broodstock and seed. The consequence is extensive introgression of the species which jeopardizes broodstock management and the long-term genetic integrity of the resulting farmed types.

Hybrid and introgressed farmed types can also threaten the fitness of natural populations. Farm escapes may interbreed and contaminate wild relative stocks. Such contamination of pure wild stocks can even lead to the extinction of wild genetic stocks (Allendorf *et al.*, 2001). One of the important risks of such hybridisation and introgression is the breaking of co-adapted gene complexes, consequently reducing fitness (Muhlfeld *et al.*, 2009). This increases the threat to wild relatives as climatic and habitat conditions change. Hybrids and introgressed individuals may have altered biological traits that may not suit an ecosystem or food chain. A probable negative consequence for farmed IMC is the loss of distinct feeding habits which are species characteristics and an alteration in the dynamics of polyculture farming (Mair, 1999). Sarder *et al.* (2014) reported underperformance of IMC hybrids under culture conditions and recommended the avoidance of hybridisation in IMC aquaculture.

3. Hatchery seed production: status and concerns

3.1 Structure of the hatchery sector

Carp hatcheries in both the public and private sectors have contributed to an increase in seed production of inland aquaculture in south Asia. The public sector hatcheries are functioning in almost all the states; some states even have separate corporations (e.g. Uttar Pradesh and Madhya Pradesh) that operate a hatchery network for seed production. The public departments also procure private seed and rear them for distribution to farmers. However, the private sector hatcheries outnumber and dominate the production and supply of carp seed through their distribution network in various states. The hatcheries located in West Bengal, the majority of them in the private sector, fulfill the need for outsourced seed for most of the Indian states. As well as in India the private sector is dominant in carp seed production in Bangladesh and Pakistan. With so many hatcheries having been established, seed has been made widely available. This has promoted the expansion of fish culture in ponds and the stocking of reservoirs. Backyard hatcheries use portable fibre-reinforced plastic tanks and nurse fry in small net cages or hapas. At the other end of the scale are large-scale hatcheries that have their own nurseries and stocking ponds. Small hatcheries with limited land-holdings only produce spawn and sell to nursery farmers who grow the hatchlings for sale as fry or juveniles to grow-out farms. Such practices are common in the state of West Bengal which is the largest seed producing state and supplies seed to other Indian states. Overall, the carp seed production sector is not very well-structured and does not have any accreditation system.

Jayashankar and Das (2015) reported the presence of 2000 carp hatcheries across India, producing approximately 40 billion fry as seed. Currently, it is estimated that over 3000 carp hatcheries are operating in the country. Four hundred and thirty-five new hatcheries are supported as a part of entrepreneurship development under a new Government Plan from 2016 to 2020 (DoF, 2020a). Seed production increased from 6.3 billion fry in 1985–1986 to 52.2 billion in 2019–2020 (DoF, 2022). Current seed production level is short of the estimated seed supply required in India considering the stocking requirement, including reservoirs, is about 60 billion fry (DoF, 2022). With the significant increase in seed production that has occurred the dependence on wild-caught seed has become minimal in the country.

3.2 Drivers of IMC seed production

Until now the major driver in IMC seed production in India has been the need for sufficient seed to meet increasing demand, due to the expansion of the aquaculture sector. This aim has been fairly well achieved; currently, the national IMC seed requirement is being met through hatchery breeding. Most of the value-chain is operated by owners or managers who have not received any academic or structured training and are only partially skilled. This caters to the niche requirements of low-input and semi-intensive producers for domestic trade and consumption. The farms producing IMC seed usually keep the broodstock in earthen ponds and rear the seed in earthen nurseries. The use of available technology is limited. Thus, there is scope to enhance productivity by improving quality of seed through technical innovations, which include improved broodstock management and nursery rearing DoF (2022).

4. Common practices in hatcheries

4.1 A survey of seed production

A survey of seed producers and grow-out farmers was conducted in 2021 by the National Bureau of Fish Genetic Resources (NBFGR) in order to gain a more comprehensive understanding of the IMC seed value chain and levels of quality of the seed produced in India. On-farm interviews were conducted through an open-ended questionnaire. The survey covered 107 hatcheries, 364 grow-out farms and 75 nursery farms located in the following eight states: West Bengal, Bihar, Jharkhand, Uttar Pradesh, Madhya Pradesh, Kerala, Karnataka and Tamil Nadu.

The hatchery managers addressed questions related to: broodstock management (male and female ratio, number of years broodstock are used, brood fish replacement, pedigree record of broodstock); breeding practices (spawning methods, pure or mixed-species breeding, frequency of hybridisation, segregation for exclusion of hybrids from fingerlings and broodstock); and awareness about genetic management, seed quality and certification).

Similarly, seed growers and grow-out farmers were asked questions focused on the type of culture (monoculture, polyculture); husbandry practice; seed quality and performance and the consequences for production; husbandry; and diseases. The questionnaire formats are accessible online (www.nbfgr.res.in/en/page/downloads). The geographical coordinates of the surveyed sites were recorded using a handheld Global Positioning System. The farming operations of carps were divided into different entities including those carrying out breeding and seed production, nursery rearing, and grow-out culture activities.

4.2 Survey findings – Seed producers

- Hatcheries are not always managed by the qualified personnel. Some managers are academically qualified in a fisheries discipline but the majority have been trained through a government programme. In the latter training occurs informally through work experience in hatcheries. Hatchery managers were not maintaining pedigree records of the broodstock available at the farm or their replacement history. There was no record of the evaluation of seed for growth and performance at the producer level. The results of the survey are detailed in Table 1.

Table 1. Details of the 2021 survey of hatcheries, nursery and grow-out farms in 9 states of India

State	Hatcheries			Grow-out farms		Nursery Farms	
	Number of hatcheries	Annual seed production (million)	Hatchery area (ha)	Number of grow-out farms	Grow-out farm area (ha)	Number of nursery farms	Nursery farm area (ha)
West Bengal	29	44 410	145	45	1 909	11	70
Bihar	9	2 000	55	28	55	0	0
Assam	14	138	71	75	311	0	0
Uttar Pradesh	2	500	21	3	6	0	0
Madhya Pradesh	8	1 480	655	61	816	0	0
Jharkhand	3	2 450	30	30	122	0	0
Tamil Nadu	17	1 370	81	26	99	0	0
Kerala	14	750	44.5	66	203	0	0
Karnataka	11	1 280	105	30	571	12	30
Total	107	54 378	1 207	364	4 092	23	100

- The same broodstock are used two or three times a year for seed production in the eastern states of India including Assam, West Bengal and Odisha, where the climate favours extended breeding seasons. In most of the hatcheries, the same individual broodstock were used for 3 to 4 breeding seasons (years) while in few hatcheries individual broodstock were used over 6 to 7 breeding seasons.

- The sex-ratio in the spawning pools is generally maintained at 1:1 although a few hatcheries in West Bengal also reported using less than 1 male per female. In some places of eastern India, Assam, West Bengal and Odisha, where prolonged breeding seasons are common, males are reused in the spawning activity.

- The breeding set (pure or mixed species) depends on customer demand. The farmers reported that in mixed breeding (different species induced simultaneously in the same spawning pool) generally 2 to 5 percent of the total seed is hybrid; hybrid seed is not segregated or removed. In the states of peninsular India, no hatcheries reported the practice of mixed species breeding.

- Depending upon customer demand hatcheries may either use interspecific gametes to produce hybrids or F1 hybrids are also used for producing seed in the West Bengal hatcheries. In extreme cases, to counter non-availability of milt, gametes from two species are also mixed.

- Hatchery owners are aware that mating of related broodstock is not appropriate and know the term "inbreeding". However, they are not aware about the losses or performance depression which can result from such inbreeding and do not use any operational strategy to avoid it.

- Most hatchery managers expressed the need for seed certification and guidance on procedures for enhancing seed quality. They also expressed a need for a source of certified broodstock for replacement purposes.

- In most of the hatcheries, generally, 5 to 15 percent of broodstock are replaced annually, Replacement broodstock come from their own farms but they may also be purchased from farms to which they sold seed in the past or from other grow-out farms. In most cases the broodstock intake is to compensate for mortality and increasing capacity. Only a fraction of hatcheries (in Bihar and Jharkhand) periodically bring in fish stock from other farms or from the wild specifically to avoid mating related broodstock.

- Argulus and dropsy disease were the most prevalent reported diseases.

4.3 Survey findings – Grow-out farmers

- Nursery rearing (from spawn to fry or fingerlings) is practised in certain regions of West Bengal and in one area of Karnataka. In West Bengal all of these farmers practice polyculture with stocking densities at 3.8 to 5.0 million per ha and have a major preference towards rohu and catla. Most of the farmers harvest 3 to 4 crops of juveniles per season and unsold seed is grown on to table size. In Karnataka all the seed-growers practice monoculture with a stocking density of 3.0 to 3.75 million spawn per hectare of water area.

- In grow-out farms, the average farm size is 0.5 to 32 ha with the exception of West Bengal, where the farms range from 6 to 48.5 ha. A few farms have nursery ponds where fingerlings (10–20 g) are stocked to obtain the stocking size (200–400 g) required for grow-out ponds. The culture duration for catla and rohu varied from 5 to 12 months and mrigal was harvested, after 10 to 12 months. After harvest the ponds are drained for drying and prepared for next season stocking.

- Farmers are unaware of seed quality changes that happen over the years. However, they report the differential growth that occurs among the batches of seed. The grow-out farmers are not able to assess performance systematically because they do not keep any long-term records.

- A few farmers reported that growth and survival rates have increased compared to those in the past. These achievements were attributed to the adoption of supplementary feeding practices, the use of medicines and improved husbandry practices.

- Overall, 18 percent of the farmers were not aware of inbreeding depression but knew about the reduced growth that can be caused by low seed quality. All the farmers presume that using small broodstock affects seed quality. Some farmers opined that seed produced from broodstock late in the breeding season leads to poor seed performance. A few farmers reported poor survival and growth in some batches of the seed they produced. In terms of performance few farmers complained about the reduction in growth that occurred in some batches and the same farmers emphasized the need for seed certification for enhancing monitory benefits.

- A majority of the farmers fed their fish once a day with a conventional feed prepared on-farm. This feed was comprised of de-oiled rice bran and either mustard or groundnut oil cake, with an added mineral mixture. However, some farms use commercially available formulated pelleted feed when available.

- The major diseases observed were argulus, dropsy, and the red disease (a farmers' term used to refer to Motile Aeromonas Septicaemia). A few farmers also reported fin and tail rot.

Based on the views of the seed producers and grow-out farmers views expressed during these face to face interviews, hatchery production is the primary source of seed driving carp production. There is a lack of systematic procedures (breeding plans and broodstock management). Thus, the hatcheries are likely to depress seed quality due to inbreeding and the accumulation of inbreeding over the generations. However, the practice of using equal sex-ratios during spawning in the majority of hatcheries may slow the pace of inbreeding. There is an undisputed need for appropriate record keeping at the hatchery level, including broodstock history, management and breeding. Record keeping is also required at the farmers' level and is also needed for monitoring grow-out performance, husbandry and revenue generation. To fulfil these essential requirements interventions at multiple levels that involve research, policy, development and extension agencies are needed. The foremost necessity is to develop a set of standard operating procedures to be followed by hatcheries and also standards of seed quality as a reference for recording performance by grow-out farmers. Growers may not able to pinpoint the performance of the seed, as they are not aware of the level of optimum performance and do not maintain any reference records. Farmers have attributed reduced performance, whenever it is recorded, to the possible use of small-sized broodstock. This belief may not have any proven direct correlation; it may possibly be a misconception. However, these small-sized broodstock, which may be result of accumulation of inbreeding in the closed hatcheries, can lead to reduced performance. This emphasizes the need for structured skill development, academic training or certificated courses for hatchery managers and aquafarmers. Such trained manpower would rapidly improve hatchery management and also provide the capacity to absorb upcoming technologies and procedures in the long-term.

5. A way forward for improved genetic management of IMC

Six decades of hatchery seed production that has helped to enhance carp production has contributed to nutritional security in India and other South Asian nations. Over the years, concern has emerged regarding the degradation of seed quality. This has been caused by the application uninformed practices and the lack of standard operating procedures for broodstock management. This may not only be constraining the productivity if IMC aquaculture but may affect wild IMC stock. Natural genetic variability among wild relatives in the Indo-Gangetic rivers may be eroded.

There is therefore a need to establish a system of best practices and policies for the management of hatcheries and broodstock and provided quality assurance. Such a system would improve aquaculture productivity. This will require policy initiatives that are adequately supported by technology, knowledge development and retention, and capacity building. It will also require the establishment of a network of carp germplasm resource centres for supporting the conservation and sustainable use of IMC genetic resources. Certification systems for seed with linkages to stakeholder seed producers through a centralized information technology framework would aid quality control. They would also provide growers with information on the best sources of quality seed. To achieve this system a series of recommended action points are provided below.

5.1 Policy directions relevant to seed quality

5.1.1 Existing policies

Government of India schemes, the Blue Revolution and Pradhan Mantri Matsya Sampada Yojana (PMMSY), focused on seed supply, seed quality and certification (DoF, 2020b). In 2010 the government recommended guidelines for developing fish seed certification and hatchery accreditation systems (DoF, 2010). These guidelines suggest procedures for certification and benchmarking for hatcheries and seed of various aquaculture species, including IMC. The benchmarks for carps covered infrastructure requisites; certification standards for operating procedures, water quality and disease profiles, and the identification of hybrids based on genetic markers. The guidelines also recommend broodstock replacement when broodstock genetic diversity is low or the hybridization and introgression rates are high. Hatcheries can be accredited based on certification that confirms the quality standards of the seed produced. However, certification and accreditation of hatcheries has not been adopted by the majority of states. The Indian state of Assam has made seed registration compulsory for hatchery owners and seed sellers through the Fish Seed Act of 2005. The Fish Seed Rules of 2010 extended this to all seed producing units (i.e. hatcheries, seed growers, seed importers and seed exporters). Similarly, many states have records of the hatcheries operating in their respective areas of jurisdiction, both in private and public sectors. However, there is a need for improving and harmonizing these procedures so that they produce seed of specified quality standards, as already prescribed (DoF, 2010) or as revised from time to time by the designated agencies.

5.1.2 Recommended policy measures to address genetic management and seed quality

The Government of India has recently identified priorities for the holistic development of carp aquaculture. These include improving seed quality, certification, broodbanks (source of seed to raise quality broodstock) both for farmed species and for stock enhancement through river ranching programmes. To accomplish these priorities policies with appropriate legal support are required. These must empower the development of quality standards, procedures and technical protocols. Policies must also designate authorized agencies responsible for the implementation of the whole process of seed production certification and accreditation. This would transform carp seed production from an unorganized sector to a transparent, science and technology driven system.

Some recommended actions to achieve this are suggested below:

- Register commercial seed production farms and record their geographical coordinates and farm area. Farms should also be categorized in terms of capacity, purpose and type of seed production (e.g. seed for broodstock or grow-out).
- Enhance farmer awareness through the provision of academic training/certificated courses for hatchery manager. These would accelerate the uptake of science-based management of carp seed production and improve synergies in the value chain.
- Designate certification/accreditation state level authorities to establish standards, approve technical protocols, referral laboratories and the implementation of certification processes.
- Establish standard operating protocols for hatcheries of different types. Since seed is transported across the whole country, certification and standard protocols need to be established centrally with linkage to states.
- Enable the development of practical technical protocols for the exchange of broodstock between hatcheries.
- Provide certification for husbandry practices, genetic management and disease-free status.
- Establish a network of Regional Live Germplasm Resource Centers (ICAR-NBFGR, 2016) to provide evaluated wild IMC genetic stocks and designated sources of broodstock for hatcheries/sperm cryopreservation units for use in commercial seed supply and to produce fingerlings for ranching or stocking in rivers and reservoirs.
- Enhance regional cooperation for genetic characterization and documentation of wild stocks and farmed types in IMCs across the native distribution range.

5.2 Mitigating inbreeding: available tools and procedure

Science-based broodstock management is essential to avoid the risk of inbreeding depression, maintain genetic diversity and fitness of broodstock, and limit species introgression. Consequently, this will maintain and enhance the performance of seed in grow-out. Wild stocks and farmed types, including improved strains, will need different sets of genetic management practices. Maintaining the genetic status of these types in aquaculture and the effective conservation of wild relatives are equally important.

5.2.1 Genetic management and maximizing *Ne*

The mitigation of inbreeding risks in the breeding population requires good founder stocks, optimum effective population size with a high intra-population genetic diversity and equal sex-ratios of broodstock at breeding. There have been five decades of domestication without managed breeding plans or data collection on the sourcing of broodstock and closed breeding within hatcheries. This means that the genetic variation in the existing broodstock might be lower compared to the variation in the original wild stocks from which the broodstock derived. Providing a reliable source of wild type germplasm for hatcheries to infuse genetic variation and outbreeding, may be an important factor in improving broodstock management. A well-defined strategy for the collection of wild seed material will be an essential part of the data driven strategy on genetic management of IMC genetic resources. Raising and breeding of this population in sufficient numbers and the characterisation and performance evaluation of wild and farmed types will also be essential. Record keeping and genetic analysis of broodstock history and information on the pedigree of brood fish is critically important. This information will provide critical inputs for planning the breeding programme. Passive Integrated Transponder (PIT) tagging of the broodstock is an effective way of identifying and recording individuals for breeding purposes.

Figure 4. Selection of rohu (*Labeo rohita*) broodstock using PIT tags to identify individual broodstock.

To maintain high *Ne* a large breeding population needs to be maintained. In the context of IMC, a hatchery with a production capacity of 50 million spawn seed would require over 250 female broodstock and an equal number of males. This calculation is based on the assumption that an average 2 kg broodstock is expected to spawn 200 000 eggs. However, the desired *Ne* and breeding strategy is dependent upon the overall objective. For example, the objective may be the production of seed for sale to grow food fish. Or it may be to develop future broodstock for the conservation or enhancement of wild stocks. Farms that raise their own broodstock are responsible for the long-term genetic quality of their farmed type and should aim to maintain broodstock *Ne* of 500 or more, to minimize inbreeding risk to less than 5 percent per generation (FAO, 2008). In practice batches of broodstock are induced to breed in a spawning pool. There is therefore a risk of inbreeding. Therefore, such centres should not breed less than 50 pairs (1F:1M) in a spawning batch and consciously avoid breeding between siblings.

Small hatcheries which use hapa breeding and portable tanks need not and should not develop broodstock from their own seeds. They must procure broodstock only from certified broodstock multiplication centres. In no cases should the progeny be ploughed back indiscriminately into the broodstock pool to cross with parental stock. Large hatcheries would also benefit by having regular planned exchange programmes. The *Ne* of a broodstock can be enhanced in various. These include regular planned exchanges of brood fish from other farms; introducing individuals from the same sites of the originally wild-collected brood fish; and using sperm storage and cryopreservation as tools to implement genetic exchange and crossbreeding between hatcheries.

Crossbreds, deriving from the crosses between different farmed types of the same species may be used as a means to mitigate inbreeding risks. However, such crossbreeding should be used only under planned breeding programmes for increasing heterozygosity and only for food fish aquaculture purpose and not for river stocking or conservation.

Farms for conservation or other long-term purposes, such as live germplasm resource centre, are expected to target minimum risks of inbreeding and the *Ne* should be above 1000 (FAO, 2008). However, the *Ne* can be lower if appropriately planned and sourced wild germplasm is brought regularly into the breeding farms. It would be useful for such farms to have a larger spawning pool or even to use the traditional bundh breeding system which can accommodate hundreds of broodstock simultaneously for spawning (Jhingran, 1991).

5.2.2 Molecular tools for informed broodstock management

Molecular markers are powerful tools for the direct assessment of genetic diversity. They are also meaning of detecting loss of diversity in the breeding population more rapidly than through performance evaluation. Markers are also useful to identify genetic relatedness and diversity in the founder population prior to initiating genetic improvement programmes (Hamilton *et al.*, 2019). In the case of IMC there is need to develop a set of validated microsatellite markers or single nucleotide polymorphism (SNPs) for analyzing within species diversity in aquaculture farms. For wide applicability such tools need to be cost effective and easy to use.

Molecular markers for distinguishing the different species and the hybrids are already known. One such RAPD based kit has been launched recently (Jayashankar, 2020). Most of these markers and studies have been limited to the detection of F1 hybrids but it is also important to detect introgression which is the outcome of the backcrossing of F1 hybrids with parent species or the subsequent interbreeding of hybrids. For this purpose, the use of multiple loci of co-dominant markers and establishing a hybrid index (Allendorf *et al.*, 2001) can be a useful standard reference to certify species genetic integrity in the hatcheries.

A validated genetic marker system that can be used cost-effectively as an assay tool for species conformity is required. Determining the genetic diversity status of farmed types at various points in the seed supply chain (from the wild and from germplasm centres through to small-scale hatcheries and growers) is essential for empowering small-scale farmers (Lal *et al.*, 2016). It is also necessary to assist the implementation of certification and the accreditation of hatcheries.

5.2.3 Sperm cryopreservation for germplasm exchange between hatcheries

It is relatively easy to transport frozen or chilled sperm from one hatchery to another for broodstock replacement instead of transporting fish fingerlings or brood fish. However, there is still no up-scaling plan for using preserved sperm in aquaculture, except for a very few experimental attempts in high-value species. Introducing this technology for low-value farm species, such as carps, in Asian aquaculture is a challenge.

A research programme for upscaling the sperm cryopreservation protocol and validating its utility as a tool for enabling exchange between farms, with the participation of commercial seed producers, was initiated during 2018. The first step was the conduct of a series of workshops for seed producers to enhance their awareness about broodstock management and to demonstrate the use of sperm cryopreservation procedures in carp breeding. The state departments nominated the participants in this programme through the National Fisheries Development Board (NFDB), involving 20 states. The feedback from the participants helped to identify gaps in the need to customize protocol and to identify potential benefits and available facilities. It also provided information potentially leading to the selection of partners for field demonstration.

This was followed by field demonstrations and seed production at selected hatcheries. This exercise enabled direct interaction between scientists and stakeholders under the real-time conditions of commercial carp seed production. The laboratory protocol was customized to a field-based working strategy. This aimed at fertilizing eggs on a large-scale within a small time-window, maintaining gametes fertilizable and utilizing resources that would be affordable by a normal hatchery. The milt from 10 broodstock males per batch was collected and cryopreserved in 2ml doses at the ICAR-NBFGR Live Germplasm Resource Centre. The milt was transported for use in fertilizing the eggs at recipient hatcheries. The programme was implemented over three breeding seasons between 2019 and 2021. It was successful in producing 10 million seed at 32 hatcheries located in the 10 states involved. Partners in the programme were encouraged by the simplicity of the technology. Its potential use for purposes other than genetic exchange were also shown. These included producing seed when milt is scarce and seed prices are high, reducing the raising of a large male population, etc.

The protocol used in this programme will be further refined. Field demonstrations have validated that the technology can be adapted for use as a tool for germplasm exchange. As a way forward, a strategy document for adaption will be prepared and submitted to the Government of India. It is encouraging that this technology is listed in the new PMMSY policy document as an activity for genetic improvement (DoF, 2020b).

5.2.4 Development and use of information systems in AqGR management

An FAO global assessment on the status of management of aquatic genetic resources (AqGR) highlighted the need to establish and strengthen national and global information systems in aquaculture (FAO, 2022b). This is necessary to improve the knowledge base on these resources and to support countries and aquaculture stakeholders to make more information-based decisions on AqGR conservation, sustainable use and development. A standardized nomenclature for the farmed types, not previously available, has been established for improved knowledge documentation (Mair and Lucente, 2020).

India is one among the few countries that have established a national information system. This is named the Aquatic Genetic Resource Information System of India (AqGRISI, accessible at https://mail.nbfgr.res.in/agrisi). The prototype of this information system conceptually adheres to the fact that countries in Asia-Pacific are biodiversity-rich and that comprehensive knowledge about these resources is critical for effective AqGR management. The AqGRISI is focused on wild genetic resources and records information for 3157 finfish species living in Indian waters. The information relates to species taxonomy, commercial use for aquaculture or fisheries, diseases, genetic diversity and genomic information. The AqGRISI will also serve as a platform to manage records of accessions maintained as live species in live germplasm resource centres, in addition to other repository accessions. Such information systems are the single point source for information on species of interest. They identify gaps in knowledge for researchers and assist policy makers in appropriate planning and making decisions on the management of resources. The scope information in AqGRISI differs somewhat from that in AquaGRIS, the Aquatic Genetic Resources Information System being developed by FAO (FAO, 2022b), but represents a useful baseline for creating an Indian national registry of AqGR in AquaGRIS. The latter will serve as a tool that countries can use to report information on national AqGR and monitor the status of conservation, sustainable use and development of these resources.

5.2.5 Documenting the impact of reduced performance of seed on the socioeconomy of farmers and society

A systematic study to document prevailing broodstock management practices has yet to be carried out and should include: the culture performance of seed; the impacts of genetic management practices on the social and economic standing of fish farmer; and the nutritional security. This would greatly help to guide future progress in the genetic management of IMC (Lal *et al.*, 2016). Such data would be critical to define real-time status and identify the factors responsible for poor seed quality. It would also form a framework for developing a strategic plan to make paradigm shift towards:

- the institutionalization of fish seed production;
- the production of accredited seed of optimized and predictable performance.

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Collectively carps represent the largest global aquaculture sector, contributing over 20 percent of global aquaculture production. The Indian major carps including catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirhinnus mrigala*) are cultured widely across the Indian sub-continent with the main culture system being a multi-species polyculture in ponds, often including other carp species including common carp (*Cyprinus carpio*). This production sector is supported by major seed supply systems producing over 50 billion seed per annum. This case study analyses genetic management of Indian major carps since they were first domesticated with the development of hypophysation techniques in the 1950s. A review of literature and a survey of common hatchery practices identifies significant problems prevalent in the sector brought about by a lack of application of best practices in genetic management resulting in loss of genetic diversity, inbreeding and uncontrolled hybrid introgression. These practices are likely to be impacting negatively on the productivity of the cultured farmed types and will represent a significant challenge to the sector in the long term. This case study identifies some of the root causes of poor genetic management and identifies some of the practices that could bring about an improvement in hatchery management to ensure more sustainable use of these globally important aquaculture species.

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