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K. Das Mahapatra, N. K. Barik, P. Routray

About the Book

Quality Fish Seed Production through Brood Fish Management in SAARC Countries

The Central Institute of Freshwater Aquaculture (CIFA) is a premier research Institute on freshwater aquaculture in the country under the aegis of the Indian Council of Agricultural Research (ICAR), New Delhi. The Institute is also the Lead Centre on ‘Carp Farming in India’ under Network of Aquaculture Centres in Asia Pacific (NACCA)operative under Food and Agriculture Organization of United Nation (FAO). The institute has a vision of making Indian freshwater aquaculture globally competitive through ecofriendly and economically viable fish production system for livelihood and nutritional security. The research and development activities of the institute oriented towards fulfilling four mandates i.e. to conduct basic; strategic and applied research in freshwater aquaculture; to enhance production efficiencies through incorporation of biotechnological tools; to undertake study on diversification of aquaculture practices with reference to species and systems; and to provide training and consultancy services.

Contents

SAARC Agriculture Centre is the first regional centre of the South Asian Association for Regional Cooperation (SAARC) located in Dhaaka, Bangladesh. The centre has mandate to serve eight member countries- Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka. The centre was established in 1985 with a vision to empower farmers in South Asian region and adopt more knowledge-based agriculture. The mission of the centre has been to provide timely, relevant and universal access to information and knowledge resources to all the agricultural practitioners of the SAARC Member Countries. The centre has been working to promote agricultural Research and Development (R&D) as well as technology dissemination initiatives for sustainable agricultural development and poverty reduction in the region.

Editors

K. Das Mahapatra
P. Routray
N. K. Barik
P. Jayasankar

FEATURES OF THE BOOK

- Brood stock management
- Selection of brood stock
- Breeding practices followed in India
- Socio-economic and impact assessment
- Water quality requirements of brood fish
- Breeding productivity in India
- Fish diseases with particular reference to brefish
- Antimicrobial resistance
- Principles of germplasm cryopreservation and biotechnological tools
Quality Fish Seed Production through Brood Fish Management in SAARC Countries

Editors
K. Das Mahapatra
P. Routray
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Central Institute of Freshwater Aquaculture

SAARC Agriculture Centre (SAC), Dhaka, Bangladesh

Indian Council of Agricultural Research

2012
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FOREWORD

Fish as the cheapest source of animal protein constitutes a major share in the global food basket and world fish production sector faces the challenge to boost the production to meet the protein hunger in the future. According to FAO (2009) global fish production stands at 147.5 million tonnes, of which about 40% is contributed by the aquaculture sector. Seed is the most critical factor for aquaculture development. Sustainability in aquaculture calls for the attention of the aquaculturists for improvement in every aspect involved in the system or enterprise, i.e., right from the production of quality fish seed with high survival, better growth performance, the ability to resist diseases and unfavourable environment. All these traits can possibly be built in an organism through genetic improvement or modifications.

Central Institute of Freshwater Aquaculture (CIFA) is the premier research institute in the country engaged in developing economically viable and need based technologies for enhancing aquaculture production in the country. Within a span of two decades, CIFA has contributed immensely to the field of aquaculture by developing farmer-friendly technologies for quality seed production and grow-out culture of a large variety of finfish and shellfishes, besides research on fish genetics, nutrition, disease diagnostics and post-harvest management.

Aquaculture sector in SAARC nations have lot of similarities in terms of practices, species, resource management profile and problems. Initiative of Agriculture Centre (SAC), Dhaka to bring participants from member nations like Bangladesh, Bhutan, Pakistan, Sri Lanka and India at one platform by organizing training program on “Quality fish seed production through brood fish Management in SAARC countries” at Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar during 1st-10th October 2012 provides an opportunity to understand the dynamics of the sector in SAARC countries.

On this occasion a book covering various aspects of quality seed production authored by distinguished scientists in the field is being brought out. The book covers a wide range of topics related to pertinent issues in aquaculture. I hope that the publication will help the students, teachers, planners and policy makers to advance current knowledge on quality seed production and brood stock management in freshwater aquaculture and for formulating developmental policy in freshwater aquaculture sector.

New Delhi
Dated the 20th September, 2012

(S. AYYAPPAN)
Message

Freshwater Aquaculture is dynamic and fast growing sector in the South Asia. Fish culture has helped millions of farmers in improving their food and nutritional security. The characteristics of small pond size, livelihood dependence and poverty among the fish farmers are shared by most of the SAARC Nations. Hence, aquaculture development in the region needs to be more oriented towards supporting the small scale aquaculture. Aquaculture is mostly technology driven. The development and dissemination of the technology ultimately helps millions of small farmers by making their farms more productive and efficient resulting in high income generation. Therefore, greater investment and improvement in the science of aquaculture are prerequisites for the development. Such improvement needs to be shared among the SAARC nations so as to transfer the benefits equally among the nations.

In this context, the training programme on “Quality fish seed through brood fish management in SAARC countries” organized at CIFA during 1-10 October, 2012 assumes importance on improving quality seed, which has been major concern for the sustainable growth of aquaculture. This problem is widespread across the whole of South East Asian region. The programme is expected to provide a platform to share and exchange newly developed knowledge on this critical issue.

On this occasion a book on various aspects of the brood stock management for quality seed has been prepared which contains latest development and information of this important aspect. I hope that the book will provide valuable reference materials to all concerned stakeholders in the days to come.

(B.Meenakumari)
I am extremely delighted that SAARC Agriculture Centre (SAC) in collaboration with Central Institute of Freshwater Aquaculture (CIFA), ICAR is jointly organizing regional training on “Quality Fish Seed Production through Brood-fish Management in SAARC Countries” scheduled to be held at CIFA, Bhubaneswar, India during 01–10 October, 2012.

In aquaculture sector, good brood-fish is prime source for quality seed to ensure good national and regional fish production. It is well known fact that SAARC Member states have high potentials in fisheries and aquaculture resources, but in most of the countries the production is far below their national requirement mainly due to low productivity as because of low quality seed. The quality seed production is therefore very important for the SAARC countries. Sharing of knowledge, experience and training on brood-fish management for quality fish seed production in SAARC countries would help the fish-seed producers, hatchery operators, researchers, extension workers and farmers to enhance fish production in the region.

The participants from both public and private sector of SAARC member states namely Bangladesh, Bhutan, India, Pakistan and Sri Lanka are attending this training. It would strengthen regional cooperation and make mutual benefit with each other. I extend my heartiest appreciations to the Central Institute of Freshwater Aquaculture (CIFA) to organize such type of event.

I extend my best wishes for the success of this regional training.

Dated: 27th September, 2012

Dr. Abul Kalam Azad
Director
SAARC Agriculture Centre
PREFACE

Broodstock or brood fish are the base materials on which the growth of aquaculture industry depends. Quality brood stock helps in the production of quality seed viz., fry and fingerlings. The quality and reliable supply of healthy fry and fingerlings having a sound genetic base largely depends on the successful stocking and rearing of brood fish. Therefore, brood fish collection, rearing and management are the most important part of aquaculture activities. Importance of brood fish management is to ensure quality eggs and sperm; increase fecundity; produce stronger hardy and disease free larvae and fry; spontaneous and timely supply of seeds; removal of inbreeding problem; save endangered fish from extinction; and successful aquaculture with high production potentiality.

The production of cultured fish in the SAARC region has been impressive. However the projected additional demand for the fish expected to increase by about 4 percent per year. To meet the production target it is highly essential to supply quality seed material in adequate quantity. The seed requirement may further increase if the level of production from unit area increases due to intensification in culture practices and also due to the increase of culture areas in different countries. Knowledge of recent advancements in the sector would be useful to carp breeders because it has been observed that inbreeding and mixed breeding has been practiced in many SAARC countries without a thought for the future. Production of seeds from the own stocked breeders and traditional management of hatchery systems for years together resulted in low quality seed with genetic deformities and slower growth of stocked fish in aquaculture in the region. Such problems are reported from all across Asia. Importance of broodstock management and hatchery system improvement is the major concern of aqua-culturists from most of the SAARC countries. Proper management, periodic replacement and genetic improvement of brood stock are being discussed widely in the context of these problems.

Scientists of SAARC are continuously working in this direction with different country specific problems. Here an attempt has been made to organize and bring out the best available practices in quality seed production and broodstock management of fin fish. All the contributing authors share their experiences, knowledge and know-hows, which are arranged and compiled in a book form, that will be useful to the students, researchers, planners, fish breeders and hatchery managers. Most of the pertinent and relevant chapters related to this book include broodstock management, seed quality, genetic aspects in hatchery seed production, selective breeding of carps, water chemistry, feed for the brood fish, diseases and impact assessment of technologies etc. We sincerely express our gratitude to SAARC, ICAR and CIFA for providing this platform to bring out this book. The editors also thank all the contributors and authors for their timely submission of manuscripts.

K. Das Mahapatra
P. Routray
N. K. Barik
P. Jayasankar

Bhubaneswar
October, 2012
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1. Aquaculture practices and broodstock management for quality fish seed production in SAARC countries: Status and overview

M. Golam Mustafa


1.1. Introduction

All capture fisheries are under intense pressure and social competition. On the contrary, culture fisheries are tremendously expanding regionally and globally over the last decades. The supply of quality fish seed is critical in meeting demands both in aquaculture and culture based fisheries in open waters. Production of poor quality seed due to poor brood selection, inbreeding and unregulated hybridization necessitated the preparation of fundamental rules, regulation and act for hatchery system operation and management of breeding protocol. Sharing of regulatory framework and policy documents and their consequences on brood management, breeding and hatchery operation, seed production, would be immensely useful for the hatchery manager and operator.

Broodstock or brood fish are parent fish from which fry and fingerlings are produced. The quality and reliable supply of healthy fry and fingerlings having a sound genetic base is largely depends on the successful stocking and rearing of brood fish. Therefore, brood fish collection, rearing and management are the most important parts of aquaculture activities. Importance of brood fish management is to ensure quality of eggs and sperm; increase fecundity; produce stronger and disease free larvae and fry; spontaneous and timely supply of seeds; removal of the inbreeding problem; endanger fish from extinction; and successful aquaculture with high production potentiality.

Brood fish are usually sourced internally and reared from the seeds produced in the hatchery and grow out in the fish farm. Selection of closely linked breeders caused the inbreeding problem resulting in weak, unhealthy and deformed fish seeds as well as declining growth performance. Production of seeds from own stocked breeders and traditional management of hatchery systems for years together resulted low quality seed with genetic deformities and slower growth of stocked fish in aquaculture in the region. Such problems are reported from all across Asia. Importance of brood stock management and hatchery system improvement are the major concern of aqua-culturists
from most of the SAARC countries. Proper management, periodic replacement and genetic improvement of brood stock are being discussed widely in the context of these problems.

A training programme on quality fish seed production through brood fish management in SAARC countries is being organized at Central Institute of Freshwater Aquaculture, Bhubaneswar during 1-10, October, 2012 with the broader goal of ensuring the quality fish seed production through genetic management of brood stock in the hatcheries in the SAARC region. The programme has the goal to ensure the quality fish seed production through genetic management of brood stock in the hatcheries of SAARC region. The specific objectives of the programme are to train aquaculture-seed production, quality control system in the region; exchange the aquaculture seed production system prevailing in SAARC countries; suggest ‘good regulatory’ practices for brood-stock management in hatchery industries of the region.

Present paper gives a brief overview of the brood stock management and aquaculture practices in SAARC countries.

1.2. An Overview of Aquaculture in SAARC Countries

There has been rapid increase in the demand for fish due to increase in population, income, urbanization and food habits in SAARC countries. Such high demand will have to be met primarily from aquaculture. The projected additional demand for the fish based on population growth in SAARC countries in 2020 is estimated to be 1.26 million tonnes (Dr. Michael Phillips, personal communication). The country wise estimation of such additional demand is presented the Table below.

Table 1.1. Fish demand in SAARC countries in 2020

<table>
<thead>
<tr>
<th>Country</th>
<th>Current popn. 2010 (million)</th>
<th>Predicted popn. 2020 (million)</th>
<th>Fish consumption (kg/caput/yr)</th>
<th>Additional Fish requirement 2020 (mt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afghanistan</td>
<td>31.412</td>
<td>42.141</td>
<td>0.5</td>
<td>5,365</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>148.692</td>
<td>167.256</td>
<td>19.09</td>
<td>354,387</td>
</tr>
<tr>
<td>Bhutan</td>
<td>0.726</td>
<td>0.829</td>
<td>0.2</td>
<td>21</td>
</tr>
<tr>
<td>India</td>
<td>1,224.614</td>
<td>1386.909</td>
<td>4.8</td>
<td>779,016</td>
</tr>
<tr>
<td>Maldives</td>
<td>0.316</td>
<td>0.356</td>
<td>187.3</td>
<td>7,492</td>
</tr>
<tr>
<td>Nepal</td>
<td>29.959</td>
<td>35.164</td>
<td>1.3</td>
<td>6,767</td>
</tr>
<tr>
<td>Pakistan</td>
<td>173.593</td>
<td>205.364</td>
<td>2.4</td>
<td>76,250</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>20.86</td>
<td>22.344</td>
<td>21.9</td>
<td>32,500</td>
</tr>
<tr>
<td>SAARC total</td>
<td>1,630.172</td>
<td>1,860.363</td>
<td></td>
<td>1,261,796</td>
</tr>
</tbody>
</table>
Note: Total 1.26 million metric tons additional fish will be required to meet the consumption demand. This is an underestimate increasing wealth will lead to more fish consumption. India, Bangladesh, Pakistan and Sri Lanka covered 1.24 million metric tons (>98%) of this figure. (Source: WorldFish Centre, 2012).

In Asia particularly most of the countries belonging to the South Asia are highly populated, where poverty and low family income remain amongst the most devastating problems as a common scenario. Because of land-based opportunities are limited in most of these countries, vast, varied and suitable water resources offer the best aquaculture possibilities for future food security, income generation and poverty alleviation for the hundreds and thousands of small and marginal farmers of this region.

Presented below are salient features of the aquaculture in the SAARC countries.

1.2.1. Afghanistan

Afghanistan is a land-locked country with scanty resources and fish production. People like to eat fish if available but mainly depend on livestock for animal protein. Fisheries activities in Afghanistan have been very limited, and information on the number of fishers, the species captured, yields and total catch is not available. The FAO Yearbook on Fishery Statistics has been publishing estimates of catches, rising from 800 t in 1986 to 1300 t in 1995 (FAO, 1997). The production however reduced to 1000 t in 2007. The contributions of fish to the diet, employment and economy are very low.

1.2.2. Bangladesh

Bangladesh is one of the world’s leading fish producers. The inland open water fisheries contributed 41.36 percent and that of aquaculture about 39.23 percent to the total production (DOF, 2008). The present per capita annual fish consumption in Bangladesh stands at about 14 kg/year against minimum requirement of 18 kg/year. There are about 795 native species of fish and shrimp in the fresh and marine waters of Bangladesh and 12 exotic species have been introduced. Among the fish biodiversity 10 species of pearl bearing bivalves, 12 species of edible tortoise and turtle, 15 species of crab and 3 species of lobster available in the country.

There are an estimated 1.3 million fish ponds in the country, covering an area of 0.151 million ha, of which 55.30 percent is cultured, 28.52 percent is culturable and 16.18 percent is unused. In general the size of fish ponds varies between 0.020 and 20 ha with an average of 0.30 ha. Polyculture of major and exotic carps and monoculture of Thai catfish (Pangasiodon hypophthalmus), climbing perch (Anabas testudineus), Nile tilapia (Oreochromis niloticus) and barb (Barbonymus gonionotus) and to some extent catfish (Clarias batrachus) are the most widely cultured species in Bangladesh. Three Indian major carps namely, Labeo rohita, Catla catla and Cirrhinus mrigala and one exotic carp, Hypophthalmichthys molitrix now account for more than 78 percent of total fish production from pond culture. Other common fish farming methods are cage culture, rice-fish integrated culture, and culture based fisheries in oxbow lakes and Kaptai lakes.
1.2.3. Bhutan

Bhutan has untapped inland water resources in the form of rivers, lakes and ponds. The country has over 590 natural lakes with an area of 4250 ha, the majority of them being small and located above an altitude of 2200 m. There is one man-made reservoir in Bhutan i.e. the Chukha, with an area of 150 ha. In some districts of the southern plains of Bhutan, a number of small ponds exist. The net water surface of these ponds is very small. However, steadily increasing interest of people to construct ponds for conservation of water for irrigation, as well as fish culture, is encouraging. Fish as food has wide preference for the people of Bhutan. A less productive but still significant food source is natural capture fisheries from cold-water streams, lakes (primarily trout) and warm-waters (primarily carps). The major fish producing area in Bhutan is in subtropical hills of southern Bhutan. The culture methods are primarily polyculture of common carp, Chinese carp and Indian major carps. There is no reliable production data available on fisheries and aquaculture. It is expected that there will be more reservoirs developed through dams in near future, in which coldwater fishery could be developed.

1.2.4. India

Fisheries and aquaculture forms an important sector with regard to employment, livelihood and food security. Fish products also form a significant commodity for overseas trade. During the past decades the Indian fisheries and aquaculture has witnessed improvements in aquaculture technologies, capture fisheries and overall quality improvement. Inland fisheries and aquaculture (5.0 million tonne in 2010-11) has emerged as a major fish producing system in India, with the governmental initiatives in the past three decades. Currently the average annual yield is around 2.9 t/ha. India produces over 40,000 million fry per year. Necessary capacity for feed production also exists. Carp accounts for over 80% of farmed fish. Major species cultured are rohu (Labeo rohita), catla (Catla catla), mrigal carp (Cirrhinus mrigala), grass carp (Ctenopharyngodon idellus), common carp (Cyprinus carpio), silver carp (Hypothalmichthys molitrix), catfish (Clarias batrachus), singi (Heteropneustes fossilis), rainbow trout (Oncorhynchus mykiss). Giant fresh water prawn (Macrobrachium rosenbergii) has emerged as a new species for farming with promising results.

1.2.5. Maldives

Maldives is an archipelago of nearly 1200 coral islands grouped into 19 widely dispersed atolls covering an area of nearly 90 000 km² in the centre of the Indian Ocean. The country’s Exclusive Economic Zone covers an area of nearly one million km². Marine resources are the country’s main natural endowment with economic activities concentrated on fishing and tourism. Currently fisheries account for 11% of GDP, 20% of employment and 74% of the country’s export commodities. There are no aquaculture
activities at present. However, as the geography of the country provides opportunities for aquaculture development, efforts are underway to experiment with the culture of seaweed, pearl, giant clam, spiny lobster and groupers. The potential for using mangrove areas for crab culture is also being investigated.

1.2.6. Nepal

Aquaculture in Nepal grew remarkably in 1981-82 with the commencement of Aquaculture Development Programme supported by Asian Development Bank (ADB) and United Nations Development Programme (UNDP/FAO). Since then, aquaculture is growing steadily. Although 185 species of fish have been reported in the country, fish culture is restricted to 7 carp species only. These include indigenous Indian major carps: Rohu (Labeo rohita), Mrigal (Cirrhinus mrigala), Catla (Catla catla) and exotic Chinese carps; Silver carp (Hypophthalmichthys molitrix), Big head carp (Aristichthys nobilis), Grass carp (Ctenopharyngodon idella) and Common carp (Cyprinus carpio). Besides, Nile tilapia (Oreochromis niloticus), Rainbow trout (Onchorhynchus mykiss), Catfish (Clarias gariepinus) are also becoming popular. Asala (Scizothorax sp), Katle (Neolissochilus hexagonolepis), Mahseer (Tor tor), Silver barb (Puntius gonionotus) and Giant freshwater prawn (Macrobrachium rosenbergii) are under experimentation.

Semi-intensive carp polyculture in the pond is the most common practice in the country. About 89% aquaculture production came from ponds in 2006-07. Subtropical terai is suitable for warm water aquaculture as most of the ponds (94%) are located there. Farmers grow six to seven carp species in fertilized ponds. In addition, catfish and tilapia are also becoming popular species for pond culture. Fish production from pond culture was 23,750 tonnes in 2006-2007.

Cage culture is limited only to lakes of the Pokhara valley (Phewa, Begnas and Rupa Lake) and Kulekhani reservoir in Nepal. Cage culture is very successful in Nepal except in Kaligandaki reservoir. Since fish are grown extensively in low cost cages, farmers derive high profit from cage culture. Therefore, it has become a lucrative business to farmers. Planktivore carps such as silver carp, bighead carp and rohu are common cage species. Cage culture contributed only 480 tonnes to the aquaculture production in 2006-07. Pen culture is commonly known as enclosure culture in Nepal. Enclosure culture is limited to three lakes of Pokhara (Phewa, Rupa and Begnas). These are constructed at shallow part of lakes. Enclosure culture is less productive and hence, its contribution to the aquaculture production is also low (140 tonnes) in 2006-07. Rice fish culture is practiced in terai, valleys and mid hills. Mostly common carp is stocked in the inundated rice field. Despite the high potential and promotion by government, rice fish culture did not increase. The production is also low contributing just 135 tonnes to the aquaculture production in 2006-07.
1.2.7. Pakistan

Aquaculture in Pakistan is still in its infancy, but there is immense potential for development. Aquaculture production has rapidly increased since 2000 from around 10-15 thousand tonnes to over 100 000 tonnes in 2006-07. Despite its vast fresh, brackish and marine waters, carp culture is practiced only in inland waters. Fish farming is practiced in the Punjab, Northwest Frontier Province (NWFP), and Sindh Province on a limited scale, where species such as trout, common carp, grass carp, silver carp and other carp species have been introduced, along with the native Indian carps. About 13 000 fish farms have so far been established across Pakistan, varying considerably in size. The average farm size is 5 to 10 ha. Most of the carp farms use a semi-intensive culture system.

1.2. 8. Sri Lanka

The fisheries sector plays a key role in Sri Lanka’s social and economic life. Fish products are an important source of animal protein for the population and the sector contributes about 2 percent to GDP. Brackish-water aquaculture in Sri Lanka is completely dominated by shrimp farming. The major species cultivated is *Penaeus monodon*. At present, industrial shrimp farming is mainly found in the northwestern provinces, where there are about 1 200 shrimp farms. It is estimated that about 11 000 ha are available for land-based coastal aquaculture, other than shrimp. About 10 000 ha for milkfish culture, 1 000 ha for *Artemia* culture and 50 ha for crab culture are available for brackish-water aquaculture development. Although there is potential for cultivating brackish-water species such as milkfish (*Chanos chanos*), moonies (*Monodactylus* spp.), seabass (*Lates calcarifer*), grouper (*Epinephelus* spp.), crab (*Scylla serrata*), mussel (*Perna* spp. and oyster (*Crassostrea sp*), commercial-scale culture of these species has yet to develop.

1.3. Fish seed production status in some SAARC countries

In Bangladesh hatchery produced, naturally occurred spawns and imported from other countries are the three sources of fish seed. Hatchery produced and naturally occurred sources are the two major sources, while, a few entrepreneurs are being imported seeds of high value species like catfish- *Pangasiodon hypophthalmus*, perch- *Anabas testudineus*, Tilapia (GIFT), shrimp- *Macrobrachium* sp., for aquaculture purpose. Import of fish seed compelled due to: i) irregularities of supply from the local sources, ii) comparatively lower price, ii) unavailability and banning of wild fish collection. Hence, the aquaculture is dependable for seeds on the combination of three sources in the country.
Table 1.2. Number of hatcheries and amount of spawn production from natural and hatchery sources in Bangladesh.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of hatcheries</th>
<th>No. of Nurseries</th>
<th>Spawn Production</th>
<th>Fingerlings production (Million)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Natural sources (kg)</td>
<td>Hatchery Sources (kg)</td>
</tr>
<tr>
<td>2000-2001</td>
<td>631</td>
<td>3441</td>
<td>2683</td>
<td>187343</td>
</tr>
<tr>
<td>2001-2002</td>
<td>783</td>
<td>4862</td>
<td>1975</td>
<td>276481</td>
</tr>
<tr>
<td>2002-2003</td>
<td>696</td>
<td>5960</td>
<td>1044</td>
<td>297781</td>
</tr>
<tr>
<td>2003-2004</td>
<td>756</td>
<td>7057</td>
<td>1577</td>
<td>345227</td>
</tr>
<tr>
<td>2004-2005</td>
<td>731</td>
<td>7885</td>
<td>2123</td>
<td>315892</td>
</tr>
<tr>
<td>2005-2006</td>
<td>764</td>
<td>8298</td>
<td>1723</td>
<td>407827</td>
</tr>
<tr>
<td>2006-2007</td>
<td>860</td>
<td>8712</td>
<td>2061</td>
<td>457288</td>
</tr>
<tr>
<td>2007-2008</td>
<td>873</td>
<td>8712</td>
<td>1872</td>
<td>416946</td>
</tr>
<tr>
<td>2008-2009</td>
<td>854</td>
<td>8881</td>
<td>1876</td>
<td>459804</td>
</tr>
<tr>
<td>2009-2010</td>
<td>931</td>
<td>8881</td>
<td>2203</td>
<td>459804</td>
</tr>
</tbody>
</table>

Source: FRSS, DoF.

Figure 1.1. Fry production of carps in India
(Source: Annual report 2010-11 DAHD & F, India)
In Nepal Reverie collection of indigenous major carp is not in practice. Natural and artificial breeding of common carp, Chinese carps and Indian major carps has been in practice and seed are produced from natural and artificial breeding techniques. Breeding Technology imported from China, both in public and private sector. Total production of fish seed as present are tabulated below on 2009/10,

Table 1.3. Situation of Fish Seed Production in 2009/10 (in Thousands)

<table>
<thead>
<tr>
<th>Sector</th>
<th>Hatchling</th>
<th>Fry</th>
<th>Fingerling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Public</td>
<td>93835</td>
<td>13824</td>
<td>5270</td>
</tr>
<tr>
<td>Private</td>
<td>104775</td>
<td>13699</td>
<td>6440</td>
</tr>
</tbody>
</table>

(Source: DOFD-2010)

In Pakistan before 1970, the main sources of fish seed were the natural water sources. From 1974 onwards, successful experiments on induced spawning gave an impetus to promote fish culture. As such, both warmwater and coldwater aquaculture has been developed through the establishment of fish hatcheries and nurseries in the country.

The main seed production resources in the country are fish hatcheries operated by both government and private sectors. Fish hatcheries can further be categorized on the basis of the types of cultured fish, i.e. carp hatcheries, trout hatcheries, mahseer hatcheries, etc. Presently, there are 28 carp hatcheries in the government sector whereas 117 hatcheries exist in the private sector. With regard to cold water fish culture, there are 17 trout hatcheries. One mahseer hatchery exists in the semi-cold water area of Malakand in NWFP; another full-fledged Mahseer fish hatchery is being established under a development project in District Attock in the province of Punjab. Production capacity of these hatcheries are given in the tabulated form (Table 1.4).

1.4. Fish broodstock management

Table 1.4. Fish Seed Production Capacity in four Province of Pakistan

<table>
<thead>
<tr>
<th>Province</th>
<th>Hatchery Type</th>
<th>Production capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panjab</td>
<td>Government hatcheries</td>
<td>52,000,000</td>
</tr>
<tr>
<td></td>
<td>Private hatcheries</td>
<td>22,500,000</td>
</tr>
<tr>
<td>Sindh</td>
<td>Government hatcheries</td>
<td>18,500,000</td>
</tr>
<tr>
<td></td>
<td>Private hatcheries</td>
<td>-</td>
</tr>
<tr>
<td>NWFP</td>
<td>Government hatcheries</td>
<td>11,750,000</td>
</tr>
<tr>
<td></td>
<td>Private hatcheries</td>
<td>-</td>
</tr>
<tr>
<td>Baluchistan</td>
<td>Government hatcheries</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>Private hatcheries</td>
<td>-</td>
</tr>
</tbody>
</table>

1.4.1. Major issues

Most of the South Asian countries are rich in aquatic biodiversity having a large number of fish species from fresh, brackish and marine waters. Freshwater rivers and tributaries that flow from Himalayan mountain and passing through these countries were the main source of natural seed of major and minor carps. During the middle of 60’s and early 70’s farmers were used to collect these seeds from rivers and their tributaries mostly of carps for aquaculture. Because of natural and man induced phenomena occurring in the aquatic ecosystem, the natural breeding and feeding grounds and habitats of the important river land races have been severely degraded. As a result, natural breeding activities and seed production rate have gradually declined. It is observed in Bangladesh that major carp seed collection in the natural water bodies (i.e. rivers and their tributaries) reduced from 100% in 1960-1970, to 80-60% in 1985-1989 to currently 1% due to degradation of aquatic habitat. Of course, due to improvement in the captive breeding the dependence on the natural seed sources has been reduced considerably.

The research on induced breeding or artificial breeding techniques have been conducted in in the late 50’s and subsequently refinement of the techniques has been made during the middle of 60’s. The wide scale induced breeding was practiced in the hatcheries all over India, Bangladesh and Nepal since then. The hatchery based seed production became the main source of fry/fingerling supply for aquaculture. Today the hatchery-produced seeds of various farmed species viz., carps, catfishes, tilapias, perch and shrimp/prawns are greatly contributing to the overall aquaculture production of these countries. Due to rapid expansion of fish hatcheries quantity of artificial seed availability has been increased dramatically to support aquaculture but genetic quality of those seeds have been deteriorated for poor management of hatchery population (i.e. broodstock) in most of the aquaculture species.

1.4.2. Problems identified with existing brood stock and hatchery management

In Bangladesh, as in India, most of hatcheries rear their own brood stock and usually do not recruit individuals from natural sources or exchange breeders between farms. Each hatchery, therefore, can be considered as an isolated, self-sustaining and genetically closed unit (Eknath and Doyal 1990). It is now well established fact that, in genetic closed hatchery systems, potential selective pressures exerted on finite and often small culture populations by various farm management practices such as the selection of founder stock, the number of breeders maintained, the method of replenishing brood stock, stocking density, feeding regime etc., can result in ‘indirect’ or ‘negative’ selection, inbreeding and genetic drift (Doyle, 1983). In Bangladesh, since middle of nineties, stock deterioration in hatchery population was reported because of poor brood stock management and inbreeding depression (Hussain and Mazid 1997; Hussain and Mazid
2001). On the other hand, introgressed hybrids in particularly of carps are being produced by the private hatchery operators and sold to the nursery operators and farmers. Hybrid introgression both in Chinese (Fig. 1) and endemic major carps has been proved and indicated by some recent studies in Bangladesh (Mia et al. 2005).

Subsequent to initial domestication, stock deterioration in the hatcheries occurs due to poor brood stock management. The ignorance and limited scientific background of hatchery managers/operators in fish breeding principles lead this situation. As a result, in most cases deterioration of seed quality normally occurs. So, due attention is required to be given towards the genetic management of hatchery stocks so as to harvest the benefits of the quality seed in terms of its growth performance and productivity.

1.4.3. Suggested measures

Management of brood stock following Best Management Practices (BMP) using systematic breeding plans and genetic principles is the best solution to avoid inbreeding and other related genetic stock deterioration aspects in the aquaculture hatcheries and farms. In case of important aquaculture species viz., carps, tilapias, catfishes, shrimp/prawn etc, a simple protocol for brood stock replacement should be developed and maintained to ensure that each pair of breeders will contribute only once to the next generation. The “Ne” should be maximized to minimize loss of genetic variation in a hatchery population. This can be achieved by retaining equal numbers of individuals from all spawning lots or sets for future use of brood stock and maintaining a 1:1 ratio of contributing male and female breeders. Such measures will allow the chance of family size variation to be controlled and the additive genetic gains in each generation to be equalized. The most important strategy of such type of breeding plan is that the maximum number of fish in any one generation of brood stock should contribute to the next generation, and the mating among close relatives should be avoided as far as possible.

Selective breeding is a long-term, useful and continuous strategy to improve the production performance and quality of fish. In a well-designed selective breeding program the pedigree of brood fish can be monitored to increase the accuracy of selection and to restrict inbreeding (WorldFish Center, 2004). There are different methods of selection being used worldwide for animal breeding program. For fish breeding program at least three selection methods viz. (a) Individual selection; (b) Family selection and (c) Combined selection have been suggested (Gjedrem 2005; Fjaleastad 2005; Hussain 2004; WorldFish Center 2004). The main goal of a breeding program is to produce superior individuals per generation having increased rate of heritability and genetic variability of all desirable traits viz., growth, survival, fecundity, fertility and disease resistance. And these stocks might have minimum level of inbreeding depression. Such genetic gain may of benefit to fish breeding and aquaculture (Hussain and Mazid, 2001). There are number of improved breeds of some aquaculture species have been developed
using selective breeding techniques in South Asian countries viz. BFRI rohu; BFRI silver barb and BFRI GIFT strains in Bangladesh; Jayanti rohu in India. State run central breeding nucleus is available in Vietnam (e.g. National Brood stock Centers). To protect poor brood stock and deteriorated stock seed production, Government of Bangladesh has enacted i) Fish Hatchery Act 2010 and ii) Fish Hatchery Rules 2011.

The real benefit of the breeding program, through which the genetically improved fish breeds have been developed, must reach to the target groups (i.e. the commercial hatchery/nursery operators and farmers). Genetic improvement typically takes place in a very small fraction of the hatchery population. The genetic improvement achieved in that ‘elite’ of superior animals needs to be multiplied and disseminated to the production systems (Ponzoni et al. 2009). The flow of genes is graphically illustrated below:

An effective plans and strategies for dissemination of such improved stocks need to be developed to transfer of responsibility for fish seed production from breeding nucleus unit to decentralized commercial units across the region. A system of seed supply and restoration of quality through genetic management from the Breeding Nucleus (i.e. Breeding Stations of Research Institute, Universities and Department of Fisheries) to the Multiplier Stations (i.e. Private Sector Hatcheries) and finally to the Fingerling Producers (i.e. Nursery Operators) and Grow-out Producers (i.e. Farmers) as shown in Figure 1.2.
1.5. Research priorities

For future aquaculture development in the SAARC region the following priority areas are identified for the brood stock development and management.

- Genetic improvements in the commonly farmed species especially carps.
- Advanced technologies for semi-intensive and intensive aquaculture in fresh and brackish water ecosystems.
- Domestication and breeding of new species and developing farming techniques.
- Diagnosis of diseases in aquatic organisms and their management.
- Nutrition and feed development for cultured fish and shell fishes from locally available raw materials.
- Introduction of suitable mariculture and sea-ranching technologies.
- Development of value added products, improved packaging and market chains.
- Conservation and management of inland fish stock and cataloguing of germplasm and establishment of gene bank.
- Development in harvest and post harvest technologies and quality control of fishery products.
- Innovative techniques of cage and pen culture in lakes, reservoirs and running waters.
- Development of coping strategies in fisheries and aquaculture to climate change
- Incorporation of experience of livestock and others sector in the dissemination of improved stains or breed to farmers. Adoption of the decentralized mode of the seed production of the improved stain to make the seed widely available to farmers.
- Development of improved quarantine systems to avoid risks of unintentional spread of aquatic animal diseases. The recent SAARC/ADB initiative on regional quarantine systems is a welcome initiative in this regard.
- Development and implementation of the protocol for the maintenance of quality brood and seed.

References


2. Recent advances in freshwater finfish aquaculture - Prospects and constraints

P. Jayasankar


2.1. Introduction

Fish as the cheapest source of animal protein constitutes a major share in the global food basket and world fish production sector faces the challenge to boost the production to meet the protein hunger in the future. According to FAO (2009) global fish production stands at 147.5 million tonnes, of which about 40% is contributed by the aquaculture sector. However, global capture fishery being at crossroads with over 70% of the resources exploited, aquaculture is the only option to fill up the gap of much of the future fish demand. The global conference on Aquaculture held in Thailand in 2010 has reiterated the commitment to advance aquaculture as a global, sustainable and competitive food production sector (FAO/NACA, 2012).

Indian fisheries sector has made great strides in the last five decades showing eleven fold increase, from 0.75 million tonnes in 1950-51 to 8.1 million tonnes in 2009-2010, which is a testimony to the contributions of the sector. Besides providing livelihood security to over 14 million people, the sector has been one of the major foreign exchange earners, with revenue reaching over Rs. 13,000 crores in 2011-12 accounting for about 18% of the agricultural export. Producing 5.42% of the world’s fish, India trades to the extent of 2.5% in the global fish market. Fisheries sector has registered an overall annual growth rate of 4.5%. During the previous five year plans contribution of fisheries sector is estimated around 1.10% to the national GDP and 5.4% to the agricultural GDP (Ayyappan et al., 2011), thus boosting the agricultural growth since last several years. Capture fishery in the country being almost stagnant since last three decades, freshwater sector has been shouldering the major responsibility to meet the increased demand for fish.

2.2. Growth of freshwater aquaculture in India

The share of inland fisheries sector to total fish production, which was 29% in 1950-51, has gone up to over 61% at present. India, as the second largest aquaculture producer in the world (first being China), has the major contribution from freshwater aquaculture, whose share in inland fisheries has gone up from 46% in the 1980s to over 80% in the recent years. The sector, during the past two decades, has shown an overwhelming ten-fold growth from 0.37 million tonnes in 1980 to 4.03 million tonnes in 2010. Freshwater aquaculture has been able to meet the increasing fish requirement of the...
country when the production from marine capture and other open waters has remained almost stagnant (Ayyappan et al., 2011). It is estimated that only about 40% of the available area of 2.25 million ha of ponds and tanks, 1.3 million ha of beels and derelict waters and 2.3 million ha of paddy fields has been put to use and there exists ample scope for horizontal expansion.

Foundation for blue revolution in freshwater aquaculture in the country was laid with the lunching of nationwide demonstration on ‘Composite culture of Indian and exotic fishes’ under the ‘All India Coordinated Research Project (AICRP)’ by the CIFRI with enormous success throughout the country. Freshwater aquaculture started gaining popularity as a profitable venture and attracted farmers and entrepreneurs to adopt it as a part or full time profession. The holistic development of aquaculture over the years has been realized through a series of standardization and development of methods in all fronts of aquaculture, i.e., resource survey, their characterization and effective utilization, production and rearing of seed, grow-out farming technology, nutritional improvement, disease and health management and the extension mechanism to transfer the technology to the field. Further, realized need to improve genetic quality and yield of cultured organism led to up gradation of the broodstock using the tools like selective breeding and cryopreservation techniques. The culture technology itself has undergone several modifications, ramifications and refinements over the years with incorporation of innovations made during scientific evaluations through multi-location trials to evolve to the present day’s package of grow-out farming practices to adapt to the varied kind of water bodies.

2.3. Fish seed production scenario

Seed is the most critical factor for aquaculture development in the country. Sequel of activities at Central Institute of Freshwater Aquaculture (CIFA) as well as other parts of the country, including ampouling of pituitary extract, refinement of breeding protocol, evolution of an array of hatchery technologies and hatchery models from the initial earthen pits to the latest circular eco-hatchery model and portable FRP hatchery have provided scope to produce seed in mass scale. The once highly skill-demanding induced breeding technology has been popularized as a more user friendly one which helped it spread across the length and breadth of the country. The easy availability of synthetic hormone formulations further has given fillip to the seed production. Success in multiple breeding of major carps for as many as four times in a season and off-season breeding although have shown new dimensions for increasing seed production potential, their extension for commercial production must be given due importance. At present, more than 1100 fish hatcheries are operating in the country in both public and private sectors. Total carp fry production increased from 409 million during 1973-74 to 32,254 million in 2009-10 making in the fry production of carp. However, quality assurance has not been given the due importance in majority of seed production centres. Rampant hybridization of Indian Major carps and inbreeding depression are perils of most of hatchery practices in the country now.
A National Freshwater fish Broodbank Facility (NFFB) is coming up under the funding by NFDB in the Odisha state fisheries farm at Kausalyaganga, with technical inputs from CIFA and NBFGR for supply of quality brood seed to all hatcheries, in turn leading to supply of quality seeds to the farmers. Nation-wide dissemination of improved rohu Jayanti should be ensured. Further, in order to ensure supply of quality seed of different species, the technology needs to be extended to other candidate species.

2.4. Production trends

In many parts of the country Indian Major Carps (IMCs) are often stocked together in almost equal proportions or in combination with exotic silver carp, grass carp, and/or common carp. However, in Godavari-Krishna delta of Andhra Pradesh (AP), hub of commercial farming, only rohu and catla at 80:20 are generally used as component species. Despite the fact that exotic carps have shown good growth and production, they are seldom used in the commercial system probably due to low consumer demand in many regions, except for common carp. Polyculture of IMCs alone or along with exotic carp at lower to moderate stocking density has been realising the production of 4-10 t/ha/yr. But there are wide variations across the states and production environments. Though the national average of freshwater aquaculture production of the country is about 2.9 t/ha/yr, high production levels up to 8-12 t/ha/yr are obtained by several farmers today, especially in AP and Punjab. Generally low production could be attributed to adoption of unscientific and extensive farming practice with use of minimum inputs. Low investment capacity of the farmers together with small pond size, seasonal nature, community ownership of ponds and low level of technology adoption in most cases have been the major reasons for such low production.

Production growth can also be achieved through horizontal expansion of the culture area, improvements in the productivity of the culture system by efficient input use, genetic improvement, effective husbandry, health management, etc. Multiple cropping and single multiple harvests are some of the culture systems which proved to have edge over year-long mono-cropping practice. Integrated fish farming and sewage-fed fish culture involving carps as the principal component has also demonstrated production levels of 3-5 t/ha/yr. In future, aquaculture will be more technology and input based with greater intensification per unit water bodies to produce more fish per unit area. In this context setting up of more fish feed production units at suitable locations along with mapping the potential agro-based feed ingredient availability in various zones of the country and a database on their nutritive values and nutrient digestibility would be a major step ahead.

2.5. Fish feed

Though carp culture system has undergone transformation from subsistence farming practice to almost industrial enterprise, the feeding practices has not changed much. Feed constitutes about 60-70% of the production cost in commercial aquaculture. At present, only 43.85 million tonnes of concentrate feeds are available in the country, where as the demand for concentrate feeds by the different animal husbandry sectors
is 142.68 million tonnes, with a huge deficit of 69.3%. The aquaculture sector currently uses about 20% of total available concentrate feeds in the country and by the year 2030, about 12-16 million tonnes of feed would be required to sustain a freshwater fish production target of 8.0 million tonnes per annum.

At present, about 8 million tonnes of feed is used by the aquaculture industry in the country with a feed conversion ratio (FCR) of 3.0 in carp culture. Use of dough feed results in low levels of utilization and increased wastage, thereby high resultant FCR. However, FCR as low as 1.7-1.8 was achieved during feed demonstration in the farmers’ ponds by CIFA. Fish meal and fish oil are completely replaced by plant ingredients in the diets of IMCs without affecting most wanted nutrients such as Eicosa Pentaenoic Acid (EPA) and Docosa Hexaenoic Acid (DHA) in fish flesh. Entry of commercial floating pelleted feed in recent years has been a significant development. Today carp farming is becoming less remunerative due to the increasing cost of conventional feed ingredients. Alternative cheap ingredients and farm-made feeds should receive greater attention.

### 2.6. Fish health management

Health management would play a pivotal role in the coming years for sustainability of semi-intensive or intensive systems of aquaculture. The misuse and drawbacks in antibiotics, problems of emerging pathogens, transboundary diseases, poor quarantine etc. are further adding up to this issues for moving into better health management practices. To meet the challenges of newer and emerging pathogens, there is a need to emphasize on the development of newer molecular-based, specific, sensitive and farmer-friendly disease diagnostics. Exploration of immune system of major cultured candidate species and understanding pathogenesis of important diseases would pave the way in developing suitable immunoprophylaxis using latest molecular approaches. Frontier research on immune related genes to identify host-pathogen interactions, and application of nanotechnology in disease diagnostics and vaccine development and water remediation are the priority areas.

Freshwater aquaculture has been witnessing threat of several diseases, most important being the Epizootic Ulcerative Syndrome (EUS) and argulosis; though a study conducted by CIFA has indicated the loss due to argulosis as Rs. 29,500/- per hectre meter (Sahoo, P.K. personal communication), such figure for EUS is not available. CIFAX, a chemical formulation from CIFA has been effective against EUS, but as yet suitable remedy for argulosis has not been found out. Rapid diagnostic kits for *Aeromonashydrophila* and *Edwardsiellatarda* and relevant therapeutics including herbal formulations have been developed, enabling to control diseases to some extent. There has been larger threat due to introduction of exotics and intensification of culture systems and practices.

### 2.7. System diversification

Increasing demand for fish has necessitated need based modification and improvement of the existing seasonal and perennial production systems. While the conventional method of composite carp culture is giving a yield of 4-6 t/ha/yr, intensive pond culture
with supplementary feeding and aeration has recorded yield level of 10-15 t/ha/yr. Efforts to bring small water bodies other than ponds and tanks such as derelict waters, ditches, canals, beels etc., under fish farming has led to development of several non-conventional systems such as sewage-fed fish culture (3-5 t/ha/yr), weed-based carp polyculture (3-4 t/ha/yr), biogas slurry-fed fish culture (3-5 t/ha/yr), integrated fish farming with poultry, pigs, ducks, horticulture, etc. (3-7 t/ha/yr), pen culture (3-5 t/ha/yr), rice fish farming in low land rice field, cage culture (10-15 kg/m²/yr), running-water fish culture (20-50 kg/m²/yr) and intensive pond culture with supplementary feeding and aeration (10-15 tonnes/ha/yr). Other diversified systems include periphyton based system, organic farming, use of stunted stocking materials in production system, low and high input based system, waste water system, cage culture, etc. Stunting the stock material for commercial production has become popular practice in Andhra Pradesh, the hub of freshwater aquaculture in the country. The seed are stunted for more than one year and then fattened to 200–300 g before stocking into the earthen culture ponds. Commercial culture of these fish is called “zero point culture”, the name being derived from the fact that the stocked fish have not attained 1 kg weight prior to stocking. These fish would attain a 1 kg weight in 5–8 months, depending on the density and the resources used.

Concepts like biofloc technology, cropping cycle, intercropping with compatible species, high end aquaculture with use of oxygen injection, supply of enriched diet, automatic feeder, farm automation etc. have been introduced in the aquaculture system to increase the carrying capacity and fish production within the system. Some of these systems have not only proven advantageous in terms of production increase, but also have ensured better resource utilization and ecofriendly protein production. However, in order to meet the future demand of fish in the country, all these processes need to be operated at more efficient level which warrants refinement, modification and further improvement in the production systems.

2.8. Species diversification

Looking beyond IMCs, technology for breeding and culture of medium-sized minor carps, kalbasu (Labeo calbasu), fringe-lipped carp (L. fimbriatus), Kuria labeo (L. gonius), olive barb (Puntius sarana) and exotic silver barb (P. gonionotus) have been developed, and could become components of conventional carp polyculture systems. Additional species including Labeo dussumieri, L. bata, Cirrhinus cirrhosa, C. reba and Puntius jerdoni warrant greater attention for development of technology of their mass-scale seed production and grow-out farming.

Study at CIFA on seed production and grow-out culture of some medium carp and barb species viz., Labeo fimbriatus, L. gonius, L. calbasu, Puntius sarana and P. gonionotus have shown promising results in terms of compatibility with the major carps as well as in increasing the biomass yield. These medium carps have initial higher growth rate and market acceptability at smaller size of 300-400 g which qualifies them as ideal species for intercropping in the major carp culture system particularly during the initial
six months of culture. Such practice would also help in more effective utilization of pond productivity during the initial culture months when the biomass of major carp in the pond is low. Further, these species look promising for utilization of seasonal ponds which have 5-6 months of water retention.

Other than carps, breeding and culture technologies for catfish, murrels, pabda, climbing perch, etc. are developed in CIFA. Catfish farming has remained at subsistence level in India with only magur (*Clarias batrachus*) and singhi (*Heteropneustes fossilis*) being cultured in some homestead ponds of eastern Indian states. African catfish (*Clarias gariepinus*) has entered our water systems through illegal route, and despite ban imposed by the Government of India, the species has spread into several agro-climatic regions of the country.

Another illegal entrant striped catfish (*Pangasianodon hypophthalmus*) was cleared by the Ministry of Agriculture for farming, and has grown into a major culture species, especially in Andhra Pradesh. This exotic fish was first introduced into India in the year 1995-96 in the state of West Bengal from Thailand through Bangladesh. Initially farming was carried in limited area in the states of West Bengal and Andhra Pradesh. But since 2004 its farming has increased due to the commercial importance and by 2008 it is estimated that *P. hypophthalmus* is being farmed in an area of about 40,000 ha with an expected production of 1.80 to 2.20 lakhs tons. *P. hypophthalmus* is being farmed under monoculture or polyculture with carps. In Andhra Pradesh a total of 12,000 ha are under striped catfish culture at present. With an average production of 17t/ha/ year the total annual production of this species in AP stands at 2 lakh tonnes. There is only a single hatchery for *P. hypophthalmus* in the state, and depends heavily on the seed from West Bengal, most of which might be coming from Bangladesh. Success in culture of *P. pangasianodon* may be attributed chiefly to availability of compounded floating feed. There is a growing interest among the farming community in other states as well to take up culture of this catfish in a larger extent, thus paving way for demand for its seed and for establishment of commercial scale hatcheries.

Species such as *Pangasius pangasius*, *Sperata seenghala*, *Ompok pabda*, *O. bimaculatus*, *Horabagrus brachysoma* etc. have received attention on their breeding and seed production recently. Research efforts on seed production and farming of climbing perch (*Anabas testudineus*), murrels (*Channa striatus* and *C. marulius*), feather back (*Chitalachitala*) have indicated their culture potential. However, non-availability of seeds in adequate quantities and species-specific feed formulations to suit their carnivorous habits entail concerted efforts for development of technology of mass-scale seed production and suitable feed for commercial farming of these species.

### 2.9. Ornamental fish farming

With a rich diversity of ornamental fishes with over one hundred varieties of indigenous species, in addition to similar number of exotic species that are bred in captivity, the export potential of ornamental fishes from India is of the order of US$ 30 million (Ayyappan *et al.*, 2011). However, the export of the country at present is mainly confined
Quality Fish Seed Production through Brood Fish Management in SAARC Countries

to some indigenous species from north-eastern states and a few exotics, with the share in Asia’s exports being only about 2%. Gold fish is the most common and preferred fish because of its varied colouration and morphological characteristics.

The areas adjacent to the metros, Kolkata, Chennai and Mumbai have become major breeding centres for freshwater ornamental fishes due to ready urban market and access to export business. In recent years, breeding units have been established in states like Kerala, Andhra Pradesh, Orissa and Bihar.

2.10. Genetic and biotechnological interventions

Modern developments in genetics and biotechnology have great potential to increase food production efficiency in the context of aquaculture. Development of improved rohu, CIFA IR 1 (Jayanti) by CIFA through selective breeding, demonstrating over 17% higher growth per generation after eight generations, is considered a landmark. There has been huge demand for seed of this improved variety, and to ensure supply of adequate quantity of quality seeds to the sector, a major programme, National Freshwater Fish Broodbank facility (NFFB) has been initiated, with the active involvement of CIFA, NFDB and Odisha State Fisheries Department. Further, it is also envisaged to set up multiplier units of genetically improved fish in several states for disseminating quality seeds for aquaculture.

There has been a paradigm shift in the approach to trait associated gene identification with the advent of high throughput genomics platforms and precision bioinformatics tools. Therefore, the future of marker assisted breeding schemes in fish would lie in the prediction of total genetic value. It has already been proved that selective breeding procedures can improve economic traits, such as growth, disease resistance etc. in fish. DNA marker based approaches have high potential to support the conventional selective breeding in augmenting genetic up-gradation of cultured stocks. However, there is a need to emphasize on collaborative or consortia based research with multidisciplinary approach.

In the context of Indian aquaculture, genomic resources have started coming up along with the efforts to identify and characterize candidate genes for abiotic stresses, disease resistance and many other important economic traits from fish and prawn. Disease diagnostics and control (molecular approach, control/prevention and alternate approaches for fish immunity) transgenics & value addition (ornamental fish), stem cell technology (Embryonic and spermatogonial), reproductive biotechnology (regulation, control and gene expression), nutritional biotechnology (regulation and gene expression), microbial biotechnology (probiotics and bioremediation) and nanotechnology (nano fibre based scaffold) are expected to be dished out for aquaculture applications in the coming years.

2.11. Future strategy

The strategic goals for research and development to enhance fish production from freshwater aquaculture systems include: Horizontal and vertical expansion of culture activities, improvement of seed production systems, system and species diversification
and prioritization, genetic up-gradation of prioritized cultivable species, development of feeds of prioritized cultivable species, improving water environment for aquaculture, fish and shellfish health management, water management, farm mechanization and automation, capacity building, strong extension mechanism and socio economic impact and policy research.

2.12. Epilogue

It is expected that the fish requirement by 2025 would be of the order of 16 million tonnes, of which at least 12 million tonnes would need to come from the inland sector and aquaculture is expected to provide over 10 million tonnes. Indian aquaculture has to come up with timely strategies to cope with the future challenges of increased fish demand, selective consumers’ choices, production of safe and quality fish protein, tapping the export earning, etc. These all have to be done in the face of increased land and water scarcity, competition from other agriculture sector, labour shortage, shortage of raw materials besides satisfying the code of conducts for responsible aquaculture and HACCP in farming. Therefore, the researchers and development machineries in the freshwater sector have their task cut out to maintain pace of the aquaculture development at sound level and to ensure quality fish protein to the increasing populace of the country, which they will do as is being done in last few decades. It is imperative to be pro-active and committed through adoption of the frontier research areas including development of production system for efficient use of nutrients and water, enhanced tolerance to biotic and abiotic stress imposed by climate change, achieving rapid growth rate of cultured fish following nutritional principles, development of an integrated, cost effective marker assisted breeding plan through the application of biotechnology, concerted and integrated efforts with effectiveness and efficiency to meet the ever increasing demand for fish, ensuring the code of responsible aquaculture. With the above strategies and suitable action plans in place, the aquaculture sector will be contributing increasingly towards food and nutritional security.

References


3. Carp brood stock management and quality seed production

P. Routray


3.1. Introduction

Husbandry in brood fish farming is very important for successful breeding programme. It deals with selection of prospective adult fishes and brooding them in good environment with balanced diet. Mature parent fish is the foremost and essential material basis and a key factor for artificial propagation of cultivable fishes. There are some standard brood fish rearing practices available including pond management, nutrition and health care for enhancing the breeding efficiency of the fish. In the traditional system, it is limited to produce brood stock only for monsoon breeding. But, the scientific brood husbandry provide the parent stock during pre-monsoon, monsoon and post monsoon months of the year for seed production. It is also important to maintain a large number of productive brood fish in the farm which would keep a pace to inbreeding of the stock. Such brood fish would give a good breeding response and quality seed (Eknath and Doyle, 1990). Developments have been made over the traditional method by intensified brood resources that provide mature broods during pre-monsoon, monsoon and post-monsoon months of the year.

3.2. Pond preparation and management

3.2.1. Condition of rearing pond

Ponds should be situated in drought and flood free area with sufficient water of good quality soil with water retention ability and good communication facilities. The stocking of brood fishes is generally done after 15-20 days of pond treatment. For easy catching and management, it is proper to have rectangular ponds each of 0.2- 0.5 ha size retaining a minimum of 1.2-1.5 m water during peak summer season. The bottom of the pond should be flat for easy netting.

3.2.2. Pond preparation

In lean and dry season, the ponds should be dried and ploughed, and if required excessive silt should be removed from the ponds before stocking. Otherwise at high temperature, poisonous substances such as organic acids, hydrogen sulphide, methane,
etc. are generated from the decomposition of the sediments. Ponds in which fish diseases have occurred should be sterilized in sunny days. Lime is also used for such purpose. The ponds should be free from aquatic weeds, weed fishes and predators. In case of perennial ponds suitable piscicides for eradication like mohua oil cake (200-250 ppm.) or bleaching powder (20-30 ppm. chlorine level) are being applied to eradicate the unwanted fish. The quantity of bleaching powder can be reduced to half if combined with urea at 10-ppm level. The urea should be applied a day before the application of bleaching powder (Ram et al., 1988). Hard water is not suitable for brood fish.

Various kinds of green manures are applied as base fertilizers for the regulation of water quality of the ponds after pond cleaning. Generally, 3-5 tonnes of green manure applied per hectare. It is applied into the pond water at a depth of 30-40 cm and exposed to sun light for 3-5 days. After decomposition of the manure the pond is filled with water. Otherwise raw cow dung at the rate of 5-8 tonnes, single super phosphate 100-250 kg, urea 75-100 kg and murate of potash 50 kg per hectare per year can be applied. The initial dose is one fourth of the total and the rest are in splitted doses. The lime is applied based on the pH of the medium (Table 3.1).

**Table 3.1. Dose of lime (based on the pH of water)**

<table>
<thead>
<tr>
<th>pH</th>
<th>Dose (kg/ha.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>1,000</td>
</tr>
<tr>
<td>4.5-5.5</td>
<td>700</td>
</tr>
<tr>
<td>5.5-6.5</td>
<td>500</td>
</tr>
<tr>
<td>6.5-7.5</td>
<td>200</td>
</tr>
</tbody>
</table>

After pond fertilization, the plankton density should always be above 2 ml per 50 litre of water.

### 3.2.3. Water quality for brood fish

Water is a vital resource for aquaculture operations as it is the medium in which the fish live and grow. Both the quantity and quality of water used in aquaculture are critical to the well-being of fish under culture and this becomes more important when brood fish raising is envisaged and for hatchery operation. A regular and abundant water supply is essential for any aquaculture operation. For hatchery use the water quality must be in ideal ranges as the newly hatched larvae are delicate and cannot withstand changes in the water parameters than their natural ranges. Each species has a preferred range of water quality parameters and outside of this range will suffer stress, which may lead to reduced growth, disease and even death. There is a high degree of inter-relationship between water quality parameters, which means that a variation in one water quality parameter can influence the toxicity of others. Fish can be affected by water-borne contaminants from the external environment as well as those arising from their own activities. Potential external sources of water contaminants include pollution...
from agricultural runoff, sewage or industrial sources as well as natural variations in water quality due to soil quality and the climate. Over the years water quality may be influenced by farm management, fish husbandry (including stocking density and feeding) as well as excreted wastes of the fish. Now a new challenge for aquaculture has been observed in the form of pond aging and accumulated soil salinity due to deposition of aquaculture water. Culture of fish at high densities requires close monitoring of water quality, especially DO, pH, total alkalinity and unionized ammoniato ensure that critical levels are not reached or exceeded. Periodic netting to rake the pond bottom and physical action of the fish in stirring up sediments can also have an impact on some parameters. For brood stock rearing and aquaculture, the water supply should be regular and reliable, free of pathogens, and free from pollutants of different origin viz. organic, industrial and urban. Based on the different geographical conditions and regardless of the water source, some form of pre-treatment will be required before it is used in the culture system. There are many options available for treatment of water before and after use in brood raising programmes and also during hatchery phase. These treatment options include combinations of:

- Mechanical screening (sand and gravel filters, carbon filters/charcoal, screen filters) to remove particulate matter.
- Chemical treatment (chlorination followed by dechlorination and/or aging) to eliminate pathogens.
- Ozonisation or UV sterilisation to eliminate pathogens.

3.3. Brood stock management

3.3.1. Source, number and conditions of brood fish

The brood fish may be grown in the farm or collected from rivers, reservoirs and lakes. Healthy ones should be selected to ensure production of healthy progenies. Transportation of brood fishes from distant places for induced breeding may result in injury and subsequent secondary infection that lead to mortality. Therefore transportation of brood fish during spawning season should be avoided. Parent fish should be of 2-3 years of age. The monitoring should be started nearly 5-6 months prior to the spawning season. Brood rearing is important for the recruitment of healthy prospective brood fish for carp hatchery. Number of broodstock required for breeding purposes will depend on the aims of the breeding programs. For details on determining suitable numbers of broodstock for each breeding plan a systematic genetic guideline may be followed. It is recommended to use large number of broodstock, with male : female ratio of 1:1 in order to maintain high effective population size for maintaining high levels of genetic diversity.

While importing new fish stock into the farm and to ensure that diseases are not introduced to aquaculture facilities, all new stock, regardless of the source, must be
quarantined. All aquaculture facilities, regardless of size, must have a quarantine facility that is physically isolated from the other facilities (separate location on the farm). Effluent water discharged from the quarantine facility may carry pathogens and so must not be allowed to mix with the water entering other culture systems within the facility. Farm implements and other equipment used in the quarantine facility must not be used in other areas of the aquaculture facility. New stock should be quarantined for at least 2 weeks before introduction to other areas of the farm. During that time, fish should be checked regularly for signs of disease, and should be given prophylactic treatments, such as salt (10 g per liter for up to half one hour) and formalin baths (50-100 ppm for up to 15-20 minutes). New stock should appear healthy and ideally be inspected by a qualified fish health specialist before leaving the quarantine facility.

3.3.2. Brood raising

To raise good quality brood fish, it is better to collect fast growing healthy yearlings from known source of genetic history of parent fishes and keep in quarantine for 1-2 months. Stocking density is one of the important measures for assuring good and healthy parent fish. The brood carps may be stocked together at 1500-2000 kg/ha. The stocking ratio should be 3:2:2:2:1 for catla, rohu, mrigal, grass carp, silver carp respectively. The common carp should be grown separately in the farm itself as a donor fish for pituitary glands. Spent brooders of preceding breeding season are reared as ‘prospective brood’. It is always preferred to use the initial stock for multiple breeding programmes (Gupta et al., 1990, Routray et al., 2007).

3.3.3. Rearing management

The prospective brood is to be reared in a separate pond rich in plankton. They should be checked and treated with potassium permanganate solution (5 ppm.) during netting at regular intervals to avoid secondary infections. Two or three months before the spawning period, fresh oxygenated water may be filled at 15-20 days interval. The fishes are examined periodically for their progress in maturity. They should be free from parasitic infection. If the brood condition is not satisfactory, the stock should be thinned out or the rate of supplementary feeding should be increased suitably. Periodic water exchange and re-circulation of standing water and aeration during summer months is necessary for keeping the brood fish in stress free condition. The surfacing of brood during cloudy weather is usually observed, which is due to oxygen depletion. To overcome this it is advised to aerate the water surface for oxygen diffusion. Good brood husbandry management results maturity in brood which can be used for multiple breeding (Routray et al., 2007).

3.3.4. Feeding management

In spite of the natural food, the brood fish are to be adequately fed with supplementary feed, @ 1-3% of the body weight. The grass carp brood is to be fed on non-fodder diet at
the rate of 1-3% per kg body. The main component of a non-fodder diet for the grass carp includes soybean cake 50 %, groundnut oil cake 25 %, rice bran 20 % and fishmeal 5 % (Gupta et al., 2002). Under optimum management conditions, the fishes attain proper maturity before onset of monsoon. Surplus feeding may lead to deposition of fat in the body affecting maturity adversely. The ideal formulated feed comprises of groundnut oil cake (48 %), soybean cake (40 %), rice bran (5 %), fish meal (5%), calcium dibasic phosphate (1.5 %), sodium chloride (0.3 %), multivitamin mixture (0.1%), trace elements (0.1 %) and vitamin C and E (30 mg and 200 mg/kg of feed) (Gupta et al., 1990). The other feed composition is groundnut oil cake (70 kg), rice bran (28 kg), common salt (1.5 kg), trace elements (0.1 kg), vitamin supplement (13 g/100 kg feed), vitamin C (10 g) and vitamin E (3 g). The formulated feed developed by Somashekarappa et al., 1990 for advancing catla maturity is precooked one consisting of black gram, horse gram, deoiled sunflower cake, rice bran, ground nut cake, broken rice and fish meal. Utilization of CIFABROOD a carp brood fish diet having 31 % protein has been reported to be highly effective for enhancing the maturity status of carps (Nandi et al., 2007). This feed is a scientifically developed, repeatedly tested brood diet, which helps in attainment of early maturity in carps. By using this diet 3-5 % during vitellogenic phase we can overcome the problem of non-attainment of maturity as well as increases survival and recovery of spawn significantly.

3.3.5. Environmental manipulation to advance and prolong the maturity

It is observed that maturity could be advanced in carps through brood stock management. Maturity is advanced by rearing the professional brood (spent breeders of preceeding season) at 1000 kg/ha. They are fed on a formulated protein rich (>30%) feed fortified with vitamins and minerals at 1-2% of their body weight. As water replenishment has considerable impact on gonad maturity, about 25% water replenishment should be done twice in a month preferably with water from canal/reservoir during February to May. Under these environmental conditions it is possible to enhance maturity at least 2-3 months in advance. Suitable water quality of brood ponds are described as temperature 20-35°C; colour greenish; turbidity 8-20 cm visibility; pH 7.5-8.5; D.O. 4.0-8.0 ppm; total alkalinity 80-150 ppm as CaCO₃; ammonical nitrogen NH₄- 0.2-0.5 ppm; nitrite nitrogen NO₂ < 0.014 ppm and phosphorous P₂O₅ 0.01-0.5 ppm. In the context of global warming the temperature of water in cultured ponds may rise to some extent. It has been seen in India that many times the surface temperature of water increases beyond 38 °C in summer. The condition of brood carps becomes stressful in this temperature and many brood fish die due to this (Routray per com.). Among carps, catla being the surface dweller is the most affected one where poor maturation has been observed due this. As a remedial measure catla brood fish may be reared in ponds having water depth 2-2.5m.
3.3.6. Brood fish handling and use of anesthetics

Carps are sedated or anaesthetized for a range of purposes, including sample measurement, tagging, spawning, hormone administration, milt collection and transfer between holding facilities. It is important to handle fish as gently as possible to preserve the surface mucous coating and to prevent any physical damage. Handling of fish is always stressful, and may lead to lowered immune function and subsequent increased risk of infectious disease. Thus, for certain procedures, especially with larger fish, sedation is required to minimize this stress. This is particularly important when transport from one place to another is envisaged. Stress management includes use of common drugs to sedate and anaesthetize fish that include, Aqui-S, MS222, benzocaine and 1-2 phenoxyethanol. These drugs are administered through the water and are taken up across the gills. Dose rates for these drugs depend on the species, size of fish, water temperature and level of sedation required. Care must be taken not to over sedate the fish otherwise it may be fatal. Use of these drugs should follow the instructions provided by the supplier. Exposure time should be minimized where possible to avoid death from over exposure. One of the important things to remember while using anesthetics is to aerate or oxygenate the water to maintain dissolved oxygen concentrations. Fish should be monitored closely while under anesthesia, for example, opercula movement should be regular, flaring of opercula may indicate over exposure and immediate steps should be taken to recover the animal. After required treatment or handling fish should be placed in clean, well aerated water and monitored until they recover.

3.3.7. Immune competence of brood fish

Production of larvae is sometimes limited due to low availability of healthy brood fish which is due to poor attention given to brood stock during non-breeding season. Swain

Table 3.2. Seasonal mean values and standard errors (SE) of different protein and non-specific immune parameters in blood of brood of Indian Major Carp, Labeorohita

<table>
<thead>
<tr>
<th>Non specific immune parameters</th>
<th>Units</th>
<th>Summer (March-June)</th>
<th>Rainy (July-October)</th>
<th>Winter (Nov.-Feb.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Protein level</td>
<td>g/dl of serum</td>
<td>3.10 ± 0.089</td>
<td>3.24 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.60 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/dl of serum</td>
<td>0.70 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Globulin</td>
<td>g/dl of serum</td>
<td>1.88 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.82 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.03 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lysozyme activity</td>
<td>mg/ml of serum</td>
<td>11.59 ± 1.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.93 ± 1.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.26 ± 0.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Myeloperoxidase activity</td>
<td>OD</td>
<td>0.97 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.31 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bacterial agglutination titer</td>
<td>Log&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.40 ± 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.70 ± 1.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haemagglutination titer</td>
<td>Log&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.20 ± 0.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.20 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.80 ± 0.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haemolysin titer</td>
<td>Log&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.60 ± 0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.80 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means bearing different superscript row wise are significantly different (P< 0.01)
et al. 2006 studied seasonal immune parameters of carp brood fish. The immunological and hematological parameters are non-specific to know the normal health status of *Labeo rohita* throughout the year (Table 3.2 and 3.3).

**Table 3.3. Blood protein and non-specific immune parameters of male and female *Labeorohita* during rainy season (Breeding season)**

<table>
<thead>
<tr>
<th>Non-specific immune parameters</th>
<th>Units</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>mg/ml</td>
<td>11.57 ± 0.41</td>
<td>11.24 ± 0.90</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>OD</td>
<td>0.86 ± 0.13</td>
<td>0.95 ± 0.05</td>
</tr>
<tr>
<td>Bacterial agglutination</td>
<td>Log₂</td>
<td>7.80 ± 1.53</td>
<td>7.8 ± 1.58</td>
</tr>
<tr>
<td>Haemagglutination</td>
<td>Log₂</td>
<td>6.8 ± 2.89</td>
<td>6.2 ± 0.75</td>
</tr>
<tr>
<td>Haemolysin</td>
<td>Log₂</td>
<td>4.4 ± 0.83</td>
<td>3.54 ± 0.18</td>
</tr>
<tr>
<td>Total Protein</td>
<td>g/dl</td>
<td>3.77 ± 0.15</td>
<td>3.54 ± 0.18</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/dl</td>
<td>0.87 ± 0.05</td>
<td>0.8 ± 0.02</td>
</tr>
<tr>
<td>Globulin</td>
<td>g/dl</td>
<td>2.14 ± 0.24</td>
<td>2.21 ± 0.11</td>
</tr>
</tbody>
</table>

The serum, mucus and eggs of fish contain a variety of substances that non-specifically inhibit the growth of infectious microorganisms. These substances are mostly proteins of glycoproteins, and many of them are believed to have their counterparts or precursors in the blood and haemolymph of invertebrates.

Among serum components there are many which display under various conditions, some sort of reactivity with foreign or immunologically unacceptable materials. When such antigens come into contact with blood, many serum components other than immunoglobulins may become involved in the reactions that follow. The concentration of total protein in blood plasma is used as a basic index for the health status of brood (Mulcahy 1971 and Rehulka, 1996). There are ample evidence that the serum of normal animals contains a population of molecules which are reactive against a vast array of different antigenic determinants and also exhibit heterogeneity in their physicochemical and biological properties. The natural occurring humoral immune substances include acute-phase proteins, lysozyme, interferon, properdin, lysins, agglutinins, lectin, trypsin, cytokines, metal ion binding protein and eicosanoids.

Increasing the immunity status of fishes is an effective method of preventing diseases in most farmed animals. Swain et al., 2006 have developed a product named “Immunoboost C that helps in immune status of carps. By administrating immunoboost-C to brood carps prior to spawning helped in achieving higher survival of spawn.

### 3.3.8. Indicator of gamete quality

In practice, selection of female brood is done on the basis of soft bulging abdomen and swollen genital aperture with reddish or pink colouration. In case of males milt oozes
out with gentle pressure to ventral side of abdomen. To be more appropriate catheterized eggs should be loose and uniform in size when these eggs are immersed in a solution comprised of 1 part glacial acetic acid and 3 part absolute alcohol. After five minutes the nucleus of the oocytes will be discernible. If majority of egg nucleus position is found eccentric, then it is treated as final stage of maturity.

Proficiency of male brood can be assessed by evaluating the quantity and quality of the milt. Such evaluation parameters are milt yield by volume, spermatocrit value, sperm density and motility. Milter in peak breeding season yields 1-2 ml per kg body mass when pressed gently on its abdomen. Prospective milter can yield about 10 ml milt on hormonal induction with any standard inducing agent (Gupta and Rath, 1991). The colour of the milt is cream-white to milk-white depending on the viscosity. Viscosity of the milt implies the total sperm packed cell found per unit volume and can be expressed as spermatocrit percentage. Spermatocrit value is ascertained by the help of haematocrit capillary and haematocrit centrifuge instrument. Non-induced milter produces little milt but very high spermatocrit value in the range of 90-95 percent can also be seen. On induction milt volume increases but spermatocrit range comes down to 70-90 percent. The number of spermatozoa is estimated per unit volume of milt with the help of a simple haemocytometer. The sperm count is estimated as 2.0-2.5 x 10^7, 3.0-3.5 x 10^7 and 2.0-2.5 x 10^7 per cu.m respectively in catla, rohu, and mrigal. The carp spermatozoa do not bear any acrosome. Carp spermatozoa are found inactive in testis or in seminal fluid. The same get activated when they come in contact with a hypotonic solution in their own seminal plasma. Water is a very good activator of carp spermatozoa. Thus the milt gets activated when it is released into the aquatic environment during spawning. Activated carp spermatozoa remain viable for a few seconds (Verma et al, 2009). Hence fertilization is a chance and not a choice in nature. However, during artificial insemination egg and sperm can be effectively mixed as desired to get a progeny of choice. The male and female gamete quality has been described in more detail in another chapter.

3.3.9. Health care

Overcrowding should be avoided to reduce the stress and disease outbreak in brood rearing ponds. It is detrimental to brood fish during pre-spawning and spawning periods. It is better to isolate the diseased fish from the brood pond for treatment or sacrifice. Routine check and prophylactic measures are required to avoid secondary infection during management practices. Over-age, diseased and unproductive brood are to be replaced with new stock. The water quality and feed quality are to be monitored regularly for better parental stock. Before applying any medicines it should be borne in mind that the brood fish may lose its maturity status or it may not respond properly if there is any slightest side reaction of the applied medicine is there. The details of health care for brood fish is being dealt separately in another chapter of this book.
3.3.10. Steps for better broodstock management and quality seed production

The biological potential of the brood fishes can be improved by brood husbandry practices. The following measures are to be followed.

- Yearlings are to be collected from an extensive culture system or from different natural sources and are to be kept under quarantine condition for 2-3 months.
- Select the healthy fast growing carps for brood raising programme.
- Fed formulated diet for better gonad growth.
- Avoid over-crowding during brood raising programme, which may invite physiological stress to fish.
- Avoid the yearling collection from wastewater, industrial affluent and sewage culture system for reducing the chance of disease outbreak.
- Maintain year and pond wise stock register; fishes can be reared separately; while same year class breeding should be avoided to decrease inbreeding level.
- Alongwith genetic aspects, environment and feed management should also be given proper emphasis so that the expression of the genes would be proper.
- Follow Immunization protocol.
- Avoid stress to brood stock by using appropriate sedation method.
- Increase the number of males and females, keeping spawning population closer to 50:50 sex ratio.

3.3.11. Genetic considerations for up-gradation of hatchery brood stock

Carp seed production is a complex process that requires knowledge on hormone induced breeding, population genetic principles and the art of hatchery and nursery management. Collection of brood stock in adequate number, cataloguing of their geographical origin, their genetic characterization and maintaining their pedigree record are important pre-requisites for breeding programme. These aspects are of much genetic relevance. The adverse impacts due to improper breeding practices are: Inbreeding, Genetic drift, Mixed spawning

3.3.12. Inbreeding:

The mating between the closely related individuals leads to inbreeding. It is also defined as brother-sister mating in layman language. It increases the proportion of homogeneous individuals in a population. The mating of close relatives leads to the reduction of heterozygosity to nearly 1/8th proportion in three generations. Carps are more prone to inbreeding depression in hatchery environment for their high fecundity. Indian major carps can produce about 0.25 million eggs per female per breeding season. The hatchery
environment ensures high rate of survival in the absence of competition for food and other environmental challenges. So, logically speaking brood stocks in a carp hatchery can be obtained from a single pair of parents. If the brood stocks are not exchanged or replaced periodically, the hatchery manager will breed only the closely related ones. Several reports of this has been reported from carp hatcheries in Southern India, where the brood stocks remained genetically close. The inbreeding rates in these hatcheries were found to be between 2 to 17% (Eknath and Doyle, 1990).

3.3.13. Genetic drift:

General practice of carp breeding in hatcheries includes few brood stocks that are used for breeding at a time, which leads to genetic drift. It is a phenomenon that leads to random changes in the gene frequency in a founder population, which may not carry some alleles due to sampling error. These losses of alleles reduce genetic variance in the hatchery population. Allendorf and Phelps (1980) first addressed this problem of hatchery practices leading to genetic drift in Cutthroat trout *Oncorhynchus clarki* (Wallbum). They showed the loss of alleles due to genetic drift by comparing the allelic frequencies in hatchery and their wild relatives. It has been reported that genetic drift led to the extinction of certain strain of channel catfish (Tave, 1991).

3.3.14. Mixed spawning:

To provide different variety of carp seed at one time these species are hormonally induced and released together in the ‘breeding pool’ to spawn. This practice is called as ‘mixed spawning’ (Padhi and Mandal, 1994). Sometimes mixed spawning leads to hybridization inadvertently because of their genetic kinship (Padhi and Mandal, 1994). These species produce inter-generic hybrids in nature and under captive breeding condition. The identical chromosome number, identical isozyme gene expression, and ease of producing fertile hybrids on a large scale indicate their close genetic relationship. This ‘genetic pollution’ will affect the genetic diversity and genetic integrity of these species. As a result it will be difficult to get pure stock of ‘Catla’ or ‘Rohu’, which will affect selective breeding program for genetic improvement in future.

3.3.15. Suggestive measures to improve breeding practices in carp hatchery

- Brood stocks should be partially replaced periodically in hatchery. Exchange of brood stocks between the local hatcheries should be encouraged.
- Brood stocks of different age groups should be bred together. This helps in reducing the chance of loss of some valuable alleles due to genetic drift.
- Natural stocks may be inducted periodically to increase the heterozygosity.
- The cryopreserved spermatozoa may be used, if possible, to maintain heterozygosity in the hatchery population.
• The pedigree record should be maintained to avoid the mating of close relatives.
• Crossing of different lines of fishes would increase heterozygosity.
• Separate lines of fish can be maintained by keeping the record of the families of different strains bred in the hatchery.
• Indian major carps should be spawned separately in the breeding pool to avoid inadvertent hybridization between these species.

3.3.16. Data management

One of the grey areas in brood stock management in SAARC countries is the brood stock data management and recovery. In order to effectively manage the breeding program, data on each broodfish should be maintained in a database. A database is a

Table 3.4. A sample questionnaire for initial trait list development for brood bank information.

<table>
<thead>
<tr>
<th>SI No</th>
<th>Information description</th>
<th>Example traits or characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Population sample identification system</td>
<td>Population identifier, sample identifier, ampule or straw identifier etc.</td>
</tr>
<tr>
<td>2</td>
<td>Population sampling plan</td>
<td>Population size, number of collection sites, number of individuals collected per site, total numbers etc.</td>
</tr>
<tr>
<td>3</td>
<td>Spawning information</td>
<td>Date spawned, sample and fish identification, capture method, spawning method.</td>
</tr>
<tr>
<td>4</td>
<td>Milt data/transportation system?</td>
<td>Cooling and freezing methods, shipping temperature, transit time, transportation mode, etc.</td>
</tr>
<tr>
<td>5</td>
<td>Source population characteristics</td>
<td>Origin, breeding history, life history, reproductive traits, behavioral traits, habitat preference etc.</td>
</tr>
<tr>
<td>6</td>
<td>Source population genetic characteristics</td>
<td>Sourced from, allele frequencies, heterozygosity, distinctive characteristics etc.</td>
</tr>
<tr>
<td>7</td>
<td>Source fishery characteristics</td>
<td>Location, water body type, water quality, food source, predator species etc.</td>
</tr>
<tr>
<td>8</td>
<td>Gene bank processing procedure</td>
<td>Milt quality, additives, diluents, dilution rate, freezing procedure, storage unit type, etc.</td>
</tr>
<tr>
<td>9</td>
<td>Quality control and monitoring</td>
<td>Milt testing schedule, test sample collection procedure, fertility rate, etc.</td>
</tr>
<tr>
<td>10</td>
<td>Milt replacement procedures</td>
<td>Program identification, date removed, replacement criteria</td>
</tr>
<tr>
<td>11</td>
<td>Brood improvement programme</td>
<td>Prog. Identification, date removed, milt quality, number progeny produced, brood stock improvement results</td>
</tr>
</tbody>
</table>
collection of data arranged in a systematic order for quick search and retrieval operation in subsets. Essential information includes data needed by the brood bank managers to facilitate decisions on management and research applications where brood fish or cryopreserved milt will be used. The initial data element or trait list is developed by answering in complete details a series of questionnaires as shown in Table 3.4.

3.4. Induced multiple spawning of carps

The average land holding of marginal farmers in Indian subcontinent is meagre, so it is necessary to facilitate the production potential of Indian major carps which is the mainstay of freshwater aquaculture. Carp production is highly dependent on the seed availability around the year. Carp seed production is no more confined to the monsoon months. Now a days carps (catla, rohu and mrigal) have been domesticated to spawn much ahead of monsoon and months beyond the monsoon, ensuring seed during pre-monsoon and post-monsoon months of the year. Such availability of carp seed promises success in cyclic intensive carp culture. It also can compensate the loss of seed due to natural calamities like flood and draught etc. The brood involved in pre-monsoon, monsoon and post-monsoon are not different individuals but one and the same. Multiple breeding of carps is undertaken on a regular basis at different parts of India and other countries by entrepreneurs and hatchery operators. In Sri Lanka, two times spawning of carps is a natural phenomenon.

3.4.1. Principle of multiple spawning

Multiple spawning is the timely harvesting of the mature gametes repeatedly by more than two spawning. Multiple spawning in carps takes place when the management is proper. The fishes are bred by adopting routine hypophysation technique. Major carps have been bred as many as four times between March & September. Brood recovery between first to fourth spawning within the stipulated period (March- August) is observed almost 100%. Further maturation rate declines 30-50 % which may need some more management manipulation for the purpose (Routray et al, 2007).

Pre-monsoon spawning

Success of pre-monsoon spawning depends on a precocious gonadal maturity. This can be achieved by simple brood stock management practices. Pre-monsoon breeding commences as early as March. The yield is 0.5-0.6 lakh spawn/kg body weight of fish. Second spawning of the same fish is achieved within a time interval of 40-45 days. The production rate increases to 1.0-1.5 lakhs spawn/kg body weight. Both the spawning is completed between March & May.

Monsoon spawning

A time lapse of another 40-45 days following the second spawning brings the third crop (June-July). During this period climatic conditions are more favourable than any other breeding period and yield further increases to 1.5-2.0 lakh spawn/kg body weight.
Late monsoon spawning

Quality of mature eggs cannot be maintained in situ for indefinite period. Therefore, monsoon dependent traditional brood fish are not useful for late monsoon breeding. This is possible only to spawn the same fish repeatedly with an optimum time interval between two successive spawning. The fish has already spawned two or three times in a season expected to come for late monsoon breeding i.e. between August & September. However, the yield of spawn declines when compared to monsoon breeding.

Breeding status of multiple brooder

Type of brood raised for such multiple spawning is termed as professional brood. This brood certainly spawns early and show better breeding response as compared to brood maintained for monsoon dependent traditional breeding. The brood 4-5+ years of age show consistent yield of spawn. Therefore, it is essential that the overage fish should be excluded from the multiple brood rearing programs. Further, to buildup a stock of professional brood, a continuous process for successive multiple spawning program may be initiated. These professional brood carps may be selected for developing a layer type carp for future that will enable year round availability of fish seed. However, it is important to mention a cautionary note for the breeders that the spawn produced from multiple breeder (3rd time) has comparatively low growth than the 1st and 2nd spawning. So for quality seed production it is essential to supply seed from multiple spawning programs for two times from a single fish.

Care of multiple brooders

Spent brood monitoring programs is an important aspect for better survival and quick recovery of the spent brood. Top most care is suggested at every stage for handling brood as follows.

(i) The brood should be transported in the canvas bags (hammock) along with water.
(ii) Hormone administration should preferably be intraperitoneal which reduces the injection stress.
(iii) Stress should be minimized in the spawning pool by providing required flow and duration of water supply in the pool for spawning.
(iv) Spent brooders should be removed from the spawning pool as soon as breeding operation is over.
(v) Spent brooders should be treated at regular intervals with potassium permanganate solution (5ppm). This keeps & checks secondary infection and also quickens recovery from spawning stress.
(vi) Feeding and other management practices should be followed meticulously for subsequent maturity.
3.5. Transport of brood fish and seed

Transport of brood fish and carp seed are integral parts of aquaculture. During transport the most important parameter is the oxygen availability in the water. Oxygen requirement of fish ranges from 100-1100 mg/kg/hour. Oxygen requirement depends on many factors during transport and also during storage. It depends largely on fish species, size of fish, physiological condition of fish and on several environmental parameters. In general, oxygen consumption increases with the increase in water temperature, after food consumption, under severe stressful conditions. Oxygen consumption decreases with weight of fish and also depends on the physiological condition of fish.

**Conditioning for transport**

Before packing for transport for short and long distance by the open or closed system, the young fish need to be conditioned to enable them to survive in the restricted space to which they are unavoidably confined during transport. The principle behind conditioning is that the fish should, before packing, rid themselves of all food existing at different stages of digestion in their alimentary canal, the rectal content most of all, before packing and should become accustomed to the conditions of overcrowding prevailing during transport.

Containers for conditioning may be boxes made of rust free wire netting of appropriate mesh size, bamboo or cane wicker work, wooden barrels or boats with perforated sides and bottoms, enclosures of nylon netting and cloth hapas. A cloth hapa is the most common type of container used for conditioning because of its efficiency, portability, ease of use in carp hatchery work. Split bamboo or cane work containers are also very useful but are of more permanent nature.

The conditioning site may be a shaded area in a pond or indoor hatchery tanks with aeration to prevent sudden fluctuation in water temperature. The optimum temperature for conditioning carps is between 26-29 °C. The period of conditioning should depend on the size of the larvae, fry and fingerlings. Generally conditioning period of six to twelve hours is employed for carp fry and fingerlings. For carp spawn mostly just mouth opened spawn is transported so no conditioning is required but when older spawn is transported one to two hours conditioning is required. For brood fish, 48 hours starvation is preferable before transportation.

**Transport of brood fish**

The transportation of rood fish is stressful to the fish as their movement and restricted space make them vulnerable to injuries. They must be tranquilized before transportation; otherwise they dash against the wall of the transport containers and injure themselves. The least expensive method of tranquilizing them is the use of cold water (5-10 °C) as a transportation medium. But this method is not applicable to warm
water tropical fish like carps. Most fishes cannot tolerate full strength of tranquillizers for more than an hour. So tranquillizers are used in a diluted solution and maintained for a long time.

For tranquillizing brood fish are put in a full strength tranquillizer (1:20000) solution of MS 222 (i.e., 5 g MS 222 in 100 water), which very soon anaesthetize the fish (sleep). After 15-20 minutes when the fish are fully tranquilized, the solution is diluted by adding water, the quantity of which depends on the hardiness of the fish. The dilution is two times (1:40000) for hardy fish such as com carp and big head, 2.5 times (1:50000) for mildly sensitive fish such as grass carp, and five times (1:100000) for very sensitive fish such as silver carps. For Indian major carps such as catla, rohu and mrigal, dilution range is generally between 1: 40000 to 1: 60000. Tranquilizers such as 2-Phenoxy ethanol at a rate upto 2% is also used during transport of brood fish. During long distance transportation, the water in the container must be oxygenated and temperature in the container should be in the range of 20 – 29 °C in tropical conditions. Simple devices such as hammocks and bags made of half sack can be conveniently used for transporting brood fish over very short distances (Gupta et al., 2008).

**Transport of seed**

The transportation of fish seeds usually takes place from the hatchery to a farm which has rearing facilities. Indian major carp larvae (spawn) and fry can be transported in plastic bags with oxygen under pressure. In one plastic bag 25000 to 50000 spawn or 3000 – 5000 larvae or fry is packed along with 5-7 l of water and 15-20 l of oxygen under
pressure. It is important to prevent splashing of water in the bags during transport of carp larvae (spawn). About 100000 just feeding carp fry after 72 h of hatching can be transported in 100 l of water. This would mean that 2 million fry can be transported in a 2 m³ container. The oxygen consumption pattern of fish is shown in Table 3.5. Similarly, the utilizable oxygen in polyethelene bags is given in Table 3.6. To prevent the water temperature from rising, it is advisable to use an insulated container or use a wet cloth or grass mat. Recently vehicle mounted tanks are used in different parts of SAARC to transport (Fig. 3.1).

Table 3.5. Oxygen consumption of carp fry, fingerling and broodfish at different water temperatures.

<table>
<thead>
<tr>
<th>Weight of fish (g)</th>
<th>Oxygen consumption (mg/kg/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature range (22–25°C)</td>
</tr>
<tr>
<td>0.3</td>
<td>1230</td>
</tr>
<tr>
<td>0.4</td>
<td>1180</td>
</tr>
<tr>
<td>0.5</td>
<td>1150</td>
</tr>
<tr>
<td>1.0</td>
<td>1000</td>
</tr>
<tr>
<td>2.0</td>
<td>900</td>
</tr>
<tr>
<td>3.0</td>
<td>830</td>
</tr>
<tr>
<td>5.0</td>
<td>740</td>
</tr>
<tr>
<td>10.0</td>
<td>660</td>
</tr>
<tr>
<td>20.0</td>
<td>550</td>
</tr>
<tr>
<td>30.0</td>
<td>530</td>
</tr>
<tr>
<td>40.0</td>
<td>520</td>
</tr>
<tr>
<td>50.0</td>
<td>500</td>
</tr>
</tbody>
</table>

Oxygen consumption of broodfish

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>Oxygen consumption (g/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22-25°C</td>
</tr>
<tr>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>6</td>
<td>1.3</td>
</tr>
<tr>
<td>8</td>
<td>1.6</td>
</tr>
<tr>
<td>10</td>
<td>1.9</td>
</tr>
</tbody>
</table>
Quality Fish Seed Production through Brood Fish Management in SAARC Countries

The brood stock in many carp farms is not reared following any brood management methods. It is presently being reared like table fish production where somatic growth is given more importance. So brood stock management and their nutrition and health should also be given priority to get quality fish seed apart from the maintenance of elite genetic stock having proven qualities. Maintenance of record / data of the hatcheries is very poor or almost not there. As, some of them are not educated properly, many of them needs training on this aspect also. Replenishment of brood stock is not done from the beginning. However, some hatcheries are doing replenishments from designated brood banks (only in Bangladesh and India) since last four years. Presently there are 21 seed hatcheries declared as brood banks in Bangladesh under the control of DoF (20) and BRAC (1). Broodstock management and quality seed production are the two integral parts of aquaculture development. Brood quality deterioration can eventually be seen in the performance of seed and overall productivity. A recent approach for good brood stock management is the establishment of brood banks of carps and other fishes where the genetic purity is maintained and the quality of seed is indirectly assured by supply of brood seed to the consumers.

References


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<table>
<thead>
<tr>
<th>Width of plastic hose cm</th>
<th>40 cm</th>
<th>50 cm</th>
<th>60 cm</th>
<th>70 cm</th>
<th>80 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume liter</td>
<td>Oxygen g</td>
<td>Utilizable oxygen g</td>
<td>Oxygen g</td>
<td>Utilizable oxygen g</td>
<td>Oxygen g</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>10</td>
<td>6</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>40</td>
<td>21</td>
<td>20</td>
<td>12</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>32</td>
<td>30</td>
<td>12</td>
<td>40</td>
<td>35</td>
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<tr>
<td>60</td>
<td>45</td>
<td>40</td>
<td>34</td>
<td>57</td>
<td>50</td>
</tr>
<tr>
<td>70</td>
<td>60</td>
<td>50</td>
<td>30</td>
<td>76</td>
<td>70</td>
</tr>
</tbody>
</table>

The brood stock in many carp farms is not reared following any brood management methods. It is presently being reared like table fish production where somatic growth is given more importance. So brood stock management and their nutrition and health should also be given priority to get quality fish seed apart from the maintenance of elite genetic stock having proven qualities. Maintenance of record / data of the hatcheries is very poor or almost not there. As, some of them are not educated properly, many of them needs training on this aspect also. Replenishment of brood stock is not done from the beginning. However, some hatcheries are doing replenishments from designated brood banks (only in Bangladesh and India) since last four years. Presently there are 21 seed hatcheries declared as brood banks in Bangladesh under the control of DoF (20) and BRAC (1). Broodstock management and quality seed production are the two integral parts of aquaculture development. Brood quality deterioration can eventually be seen in the performance of seed and overall productivity. A recent approach for good brood stock management is the establishment of brood banks of carps and other fishes where the genetic purity is maintained and the quality of seed is indirectly assured by supply of brood seed to the consumers.

References


4. Induced breeding of carps: Use of hormones and hormonal analogues

Khuntia Murmu


4.1. Introduction

Availability of quality seed in required quantity and quality at any point of time is the basic requisite for success of any aquaculture operation. Before the development of induce breeding technology during late fifties, carp seed were collected from the river stretches and was the only source of seed for aquaculture. The three species of Indian major carps such as catla, rohu and mrigal do not breed in confined water while in natural condition, changes in the environmental factor is the major factor that induces the breeding. The environmental factors that play important role in the reproductive cycle include photoperiod, water temperature and other water quality parameters, weather cycles (rainfall), etc. Though the matured carps experience surge in the reproductive hormone level in their circulation during the breeding season, it does not reach to the threshold level to induce spawning in confined water and therefore needs an extraneous supply of hormone. Lin and Peter (1996) have reported that inadequate secretion of GtH-II from the pituitary gland of carp is the main reason preventing them from spawning in confined water.

Spawning induction is initiated either by putting the fish in an appropriate environment or by changing its internal physiology by hormones administration (Rottmann, et al., 1991). First induce breeding of Indian major carp by hypophysation was reported in 1957 by injection of crude pituitary extract (Chaudhuri and Alikunhi, 1957) and gradually this technique was improvised to increase spawning efficiency. Later, use of partially purified gonadotropin (Sinha, 1971; Chaudhuri et al, 1977), LHRH analogue (Kaul and Rishi, 1986; Sahu and Biswas, 1988), prostaglandin and other hormones (Chander, 1985; Bhowmiket al., 1986 and Singh et al., 1991) were found to be better alternative of the pituitary extract for induced breeding. In due course of time it was understood that gonadotropin-releasing hormone (GnRH) and dopamine inhibiting factors in combination regulate the GtH-II secretion in fish (Sahu and Biswas, 1988; Lin et al., 1991; Lin and Peter, 1996). Based on this several inducing agents have been developed like ovaprim, ovatide, wova-FH, etc.
4.2. Hatchery technology

Seed production technology for the Indian major carps has evolved from the stage of hapa breeding to the modern day eco-hatchery model. Hapabreeding was the commonly used method until 1980 which was having its inherent problems like dependence on fluctuations in water level, temperature, damage of hapa by crabs, etc. Subsequently, hapa were replaced by glass jar hatcheries during 1980s. Use of Chinese-circular carp hatcheries for carp breeding came into practice during early 1990s in which all the natural condition required for spawning could be simulated. Based on the principle of eco-hatchery, Central Institute of Freshwater Aquaculture developed the portable FRP hatchery with 1.0 million spawn production capacity. The FRP hatchery with its advantage of portability has become popular among small and marginal seed producers and reached to farmers of most parts of the country within five years. Till-date there are 1740 carp hatcheries including 125 FRP portable hatcheries existing in India producing enough spawn to produce 32.25 billion fry making the country self-sufficient in fry production.

4.3. Mechanism of reproduction

The internal mechanism of fish that regulates the reproduction is the brain-hypothalamus-pituitary-gonad axis (Rottmann et al., 1991). Stimuli of reproductive importance are received from the environment and translated by the brain and routed to the hypothalamus. The hypothalamus produces gonadotropin releasing hormone (GnRH) and also gonadotropin release inhibiting factors. Dopamine inhibits the release
of gonadotropin. Gonadotropin releasing hormone (GnRH) stimulates the pituitary to produce and release gonadotropic hormones (GtH). Gonadotropic hormones (GtH) act on the ovaries and testes. Steroids and prostaglandins are the local ovarian mediators of GtH action which leads to release of the eggs. Higher levels of GtH in blood trigger two important ovarian processes: 1) final maturation of the egg, which is stimulated by steroids (e.g., progesterone) that are produced by the follicle, and 2) rupture of the follicle (ovulation), which is stimulated by prostaglandins. Steroids also induce spermatiation in the male.

**Hypophyseal approach**

The pituitary gland produces and stores gonadotropic hormones (GtH), which play a very important role in ovulation and spermatiation. Injected pituitary bypasses the brain-pituitary link, causing in a surge in blood GtH levels to act directly on the ovaries and testes which induces spawning (Fig. 4.1). The pituitary is collected from the fish by making a hole in the foramen magnum area of the skull with a knife. Usually homoplastic pituitary gland extract induces better than the gland collected from a different species. As the hormone content is high in sexually mature fish just before spawning, brood fishes are sacrificed to obtain the pituitaries which is a main problem when adult fish are less in numbers. Fresh pituitary glands can be used immediately or can be preserved by either freezing or acetone-drying. Such glands are used for preparing the hormone extract which is injected to the female brooders twice within a span of 6-8 hours while the male is injected during the second injection to female.

Pituitary extract of common carp or salmon are available commercially and widely used for induced spawning. When the donor species is closely related to the recipient fish, the chance of successful induced spawning is greater. That is why, carp, goldfish, Chinese carps, catfish, etc., are more likely to spawn successfully when injected with carp pituitary extracts (Rottmannet al., 1991). The main drawback in these hypophysation is the variable amount of LH that may be present in the pituitaries used for the extract, which leads to inconsistent results (Yaron, 2002). An improved preparation is the lyophilised carp pituitary extract, in which the LH content is calibrated by radio immune assay (RIA) and spawning experiments (Kulikovsky, et al., 1996). The main disadvantage of the hypophysation is the risk of pathogen transmission from donor to recipient brood stock.

**4.4. Purified gonadotropin**

Use of human chorionic gonadotropin (HCG) with mostly LH activity is another form of gonadotropic approach for inducing spawning. In fish, the injected gonadotropin acts just like the natural GtH produced by the fish pituitary. The supplied HCG also bypasses the brain-pituitary link, acting directly on the ovaries and testes (Fig. 4.1). However, this preparation has the drawback in the possibility of generating an immune
response in the recipient fishes, when the same treatment is applied in subsequent years (Zohar and Mylonas, 2001).

As HCG shows species specific potency in the broodfish, sometimes it is used in combination with pituitary extract of common carp. The combination has shown to have improved potency in comparison to either of these preparation used alone. These two hormones can separately be prepared and injected or can be mixed.

4.5. Hypothalamic approach:

a. **Luteinizing hormone-releasing hormones (LHRH):** LHRH of mammal have been injected experimentally to act like the fish GnRH. In this case a larger dose and frequent injections were required. Synthetic LHRH analogs, referred to as LHRHa or GnRHa, have been manufactured. These hormones last longer in the fish system and have potent stimulation effects on ovulation and spermiation in fishes. Therefore, only one or two small doses are needed to induce spawning. LHRHa stimulates the fish pituitary to produce and release the GtH necessary for spawning (Fig. 4.1).

b. **LHRHa + dopamine blockers:** LHRHais not species specific but some fish do not respond to injections of LHRHa alone (e.g., goldfish, redtailed black shark, rainbow shark). Dopamine inhibits the release of hormones from the pituitary, effectively blocking the pituitary positive response to injected LHRHa. There are some drugs that act as dopamine blockers, either by preventing the release or by inhibiting the binding of dopamine. Experimental results indicate that the use of dopamine blockers prevents this negative feedback, enhancing effectiveness of LHRHa for these species (Fig. 4.1).

The above inducing agents used in the hypothalamic approach have many advantages over the hypophyseal approach. GnRH analogue have high potency and therefore small amount is sufficient for inducing LH release and synthesis. Due to low dose of small molecular size, GnRH does not generate any immune response in the recipient fish. It is a standardised preparation that will have constant results dependent only on condition of recipient fishes. Further as a synthetic peptide, there is no risk of transmitting disease. Its advantage over HCG is that GnRH elicits a stimulatory effect on other pituitary hormones, especially gonadotrophic hormones, thus facilitating the effect of LH. (Marchant et al., 1989). However, the main limitation is its dependence on the amount of LH stored in the pituitary. Fishes that are not exposed to gonadal steroids early in the breeding season may fail to properly respond to the administered GnRHa. Therefore, to facilitate the LH releasing activity of GnRHa in carp, it is combined with a dopamine receptor antagonist such as domperidone, pimozide, or metoclopramide (Drori et al., 1983; Peter et al., 1988; Yaron, 1995) which is popularly known as the Lin-Pe method.
4.6. Conclusion

Fish requirement in India by 2025 would be in the tune of 16 mmt. With the stagnation of marine capture production at little over 3.0 mmt over last few years, the increased fish requirement of the country needs to be produced from the inland sector. The country has to produce at least 12 mmt from the inland sector of which aquaculture is expected to provide over 10 mmt. This warrants development of aquaculture in all possible front in order to support both its horizontal expansion and vertical increase in the productivity. The increased production has to be realized against the future challenges of increased land and water scarcity, competition from other agriculture sector, labour shortage, shortage of raw materials besides satisfying the code of conducts for responsible aquaculture and HACCP in farming. This needs systematic planning and its effective implementation through execution of viable programmes.

While development in all the above areas are the prerequisites for realizing the increased aquaculture production, availability of the quality fish seed with the desired characters as mentioned above and in adequate quantity are the basic requirement for the development process. Today, the technologies are available for captive breeding and mass-scale seed production of most of the commercially important freshwater fishes and shellfishes, be it the seed of major carps, minor carps, catfishes, freshwater prawn, freshwater mussels, brackishwater shrimps and finfishes like *Lates calcarifer* and *Etroplus suratensis*. Possibility of genetic improvement in terms of increased growth realisation and disease resistance has become a reality as in Jayantirohu (genetically improved) developed through selective breeding programme. Cryo-preservation of carp milt has been proved not only to overcome inbreeding, but also for ensured genetic improvement through male gamete exchange and reduction in the cost and maintenance of brood fish. All these technologies, supported with strong research and developmental back up, could cater the seed requirement for the horizontal as well as vertical expansion of the aquaculture sector.

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5. Genetic tools for stock improvement in fish and shell fish

Kanta Das Mahapatra


5.1. Introduction

India possesses rich fish germplasm resources, accounting for about one-tenth of the 20,000 and odd species of fish known in the world. The Ganga river system in North, the Brahmaputra in the East, the Sutlej, the Narmada and the Tapti in the West and the Mahanadi, the Godavari, the Krishna and the Cauvery in the South are very rich sources, harbouring bulk of the important fish fauna (Jhingran, 1982).

Systematic and scientific research in aquaculture was initiated in India during the late 1940s. One of the major constraints for aquaculture was the availability of seed in both quantity and quality, particularly of the commercially important fish species such as the Indian major carps, viz., catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*). However, success in induced breeding of these carps during mid to late 1950s through the administration of pituitary hormones under controlled conditions and the gradual development of this technique has paved the way and there after, adequate quantity of pure seed of these carps was available for large scale culture purposes. Second step in the progress of Indian aquaculture is the development of different packages of suitable and effective culture technologies. These multispecies intensive culture technologies have demonstrated production levels in the culture sector ranging from 10-15 t/ha/yr.

The research to exploit the genetic potential has also been initiated with the success of induced breeding and composite fish culture technologies of Indian carps. Several interspecific and intergeneric hybrids have been produced and evaluated for their culturable traits. Simultaneously, cytological and biochemical genetic studies were also carried out, not only in Indian major carps but also in other commercially important species.

It was in early 1980s research on genome manipulation (chromosomal engineering) has been taken up, and standard protocols have been developed for Asiatic carps to induce gynogenes and produce inbred lines of gynogens and polyploids (triploids/tetraploids).
Selection work in carps (rohu) has been initiated for the first time at CIFA, India from early 1990s in collaboration with the Institute of Aquaculture Research (AKVAFORSK), Norway and is being continued till date. Genetic engineering, gene transfer technique, marker assisted selection in fishes has also initiated in India.

Stock improvement through genetic means is a novel approach and highly essential in the present context. Exploitation of genetic potentials of the carps has been initiated seriously from early 1980s particularly through genome manipulations and later through selective breeding from 1990s, to add to the already developed culture technologies, for further enhancement of fish production in the country through quality improvement of stock.

The primary goal of aquaculture genetics is to produce “quality fish seed”. In genetic term, ‘quality seed’ may be defined as “those having better food conversion efficiency, high growth potential, better ability for adapting to changing environmental conditions and to resist diseases’ (Padhi and Mandal, 1996). It is anticipated that culture of ‘quality seed’ will improve aquaculture productivity further.

5.2. Genetic research for aquaculture development

Genetics have to play a greater role in aquaculture in the coming years. Sustainability in aquaculture calls for the attention of the aquaculturists for improvement in every aspect involved in the system or enterprise, i.e., right from the production of quality fish seed with high survival, better growth performance, the ability to resist diseases and unfavourable environment. All these traits can possibly be built in an organism through genetic improvement or modifications.

There are several genetic improvement methods, starting from simple hybridization to sustainable prolonged selective breeding and from modern genome manipulation techniques, to gene transfer technique, which are useful tools for improving the quality of aquaculture products as in agriculture and veterinary animals.

5.3. Hybridization

Hybridization is a simple means to improve the genetic status. By combining the haploid genome of two different parent species belonging to either same genus (inter-specific) or two different genera (inter-generic) and some times even between two strains of the same species (Intra-specific) hybrids can be produced. The hereditary transmission of the growth character from species less growth potential to new hybrids and its expression in different stages of life is a matter of primary importance in any hybridization cross.

Hybridization work among Indian major carps, between Indian major carps and Chinese carps and among Chinese carps has been initiated with a view to study the positive or useful traits like smaller head, wider body, more flesh content, pond breeding habit like Cy. carpio, advantageous feeding habits etc. Initially six inter-specific and 13 inter-generic hybrids have been produced for evaluation.
In India, some work on hybridization has been done and some useful interspecific and intergeneric hybrids have been produced among the different major carp species within and between different genera (Table 5.1 and 5.2). Though almost all the hybrids exhibited intermediate traits, some interspecific hybrids like *L. rohita* × *L. calbasu* & the reciprocal hybrids and intergeneric hybrids like *L. rohita* × *C. catla*, *C. mrigala* × *C. catla* and *L. fimbriatus* × *C. catla* have shown certain useful characteristics in terms of higher quantity of meat, broader feeding spectrum etc. Some of these hybrids were recommended for culture in reservoirs based on their feeding habits (Chaudhuri, 1971; Ibrahim, 1977; Natarajan et al., 1976; Bhowmick et al., 1981; Basavaraju et al., 1994).

### Table 5.1. Hybridization among Indian major carps

<table>
<thead>
<tr>
<th>Intra-specific</th>
<th>Inter-specific</th>
<th>Inter-generic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among the rohu of:</td>
<td>L. rohita × L. bata</td>
<td><em>L. rohita</em> × <em>C. reba</em></td>
</tr>
<tr>
<td></td>
<td>L. rohita × L. calbasu</td>
<td><em>L. calbasu</em> × <em>C. mrigala</em></td>
</tr>
<tr>
<td></td>
<td>L. rohita × L. fimbriatus</td>
<td><em>L. calbasu</em> × <em>C. reba</em></td>
</tr>
<tr>
<td></td>
<td>L. calbasu × L. bata</td>
<td><em>C. catla</em> × <em>L. fimbriatus</em></td>
</tr>
<tr>
<td></td>
<td>L. calbasu × L. rohita</td>
<td><em>C. catla</em> × <em>L. rohita</em></td>
</tr>
<tr>
<td></td>
<td>L. gonius × L. calbasu</td>
<td><em>C. catla</em> × <em>L. calbasu</em></td>
</tr>
</tbody>
</table>

### Table 5.2. Inter-generic hybridization

<table>
<thead>
<tr>
<th>Among Chinese carps</th>
<th>Between Indian &amp; Chinese carps</th>
<th>Between Indian carps &amp; Common carp</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. molitrix</em> × <em>C. idella</em></td>
<td><em>L. rohita</em> × <em>H. molitrix</em></td>
<td><em>C. carpio</em> × <em>C. catla</em></td>
</tr>
<tr>
<td><em>A. nobilis</em> × <em>H. molitrix</em></td>
<td><em>C. catla</em> × <em>H. molitrix</em></td>
<td><em>C. carpio</em> × <em>L. rohita</em></td>
</tr>
<tr>
<td><em>H. molitrix</em> × <em>C. catla</em></td>
<td><em>L. rohita</em> × <em>C. idella</em></td>
<td><em>C. carpio</em> × <em>C. mrigala</em></td>
</tr>
<tr>
<td><em>C. catla</em> × <em>C. catla</em></td>
<td><em>C. idella</em> × <em>L. rohita</em></td>
<td><em>L. rohita</em> × <em>C. carpio</em></td>
</tr>
<tr>
<td><em>L. mrigala</em> × <em>C. idella</em></td>
<td><em>L. mrigala</em> × <em>C. idella</em></td>
<td><em>C. mrigala</em> × <em>C. carpio</em></td>
</tr>
<tr>
<td><em>C. idella</em> × <em>C. catla</em></td>
<td><em>A. nobilis</em> × <em>L. rohita</em></td>
<td></td>
</tr>
<tr>
<td><em>A. nobilis</em> × <em>C. catla</em></td>
<td><em>A. nobilis</em> × <em>C. catla</em></td>
<td></td>
</tr>
</tbody>
</table>

### 5.4. Hybridization between Indian major carps and common carp

Common carp (*C. carpio*) and IMC hybrids show some positive traits for culture practices. All these hybrids are reported to be sterile, as IMC chromosome number is 2n=50 while
C. carpio var. communis, chromosome number is 100 and in the hybrids, because of aneuploidy condition, sterility occurs (Khan et al. 1989). Sterile common carp has its own advantages in culture ponds as unwanted increase of population due to pond breeding habit can be checked.

5.5. Intergeneric hybrids among Indian and Chinese carps

Hybridization was also attempted among C. catla, L. rohita and C. mrigala with C. idella, H. molitrix and A. nobilis. Many of the hybrid progeny does not survive beyond one week except Catla X Silver carp hybrid. However, high rate of mortality occurred and many hatchlings are observed to be deformed particularly on the caudal region (Ibrahim, et al., 1980). In the hybrid progeny resulting from Mrigal X Grass carp cross, the embryonic development was normal. However, the larvae gradually became abnormal with bent body and died within eight days after hatchling (Ibrahim et al., 1980).

5.6. Hybridization among cat fishes

Ramaswamy, 1958 produced intergeneric hybrids by crossing Male Heteropneustes fossilis and Clarias batrachus and the reciprocal crosses. He observed certain distinct characters in them. Later on viable reciprocal hybrids of the catfish Heteropneustes fossilis and Clarias batrachus were produced. The fertilization rates varied between 60-75%. But high rate of mortality occurred at hatchling stage. All the progeny of these reciprocal crosses closely resemble to C. batrachus i.e. dominance of dorsal fin and the accessory air breathing organ. However, chromosome complement of the hybrid was observed to be 53 (Padhi et al., 1995).

5.7. Hybrid index

To evaluate the tendency of hybrid trait towards the parents, the hybrid index calculation appears to be effective. Formula of Hybrid index is

\[ Hi = \frac{(H_v - M_1)}{M_2 - M_1} \times 100 \]

Where Hi= Hybrid index

Hv = hybrid value

M1= Value of maternal parents

M2= Value of paternal parent

5.8. Impact of hybridization

Adaptability of any hybrid to the environment is determined by genetic introgression i.e. flow of genes from one species gene pool to another species. If introgression is < 0.1% it may help in increasing capability of adaptation against natural selection. A large amount of gene flow may disrupt the adoptive gene complexes, which have evolved overnight to permit a species to effectively use its particular environmental
niche, so while releasing any hybrid to the nature, care should be taken to evaluate it properly. Reduction of genetic diversity can also occur because of indiscriminate hybridization. Because of mixed spawning practice (spawning of all required carps in a single pool / bundh) inadvertent hybridization took place. Due to identical chromosome numbers, hybrids are often produced among these closely related species in hormonally induced breeding programme. The F1 hybrid if fertile could backcross with the parental species leading to genetic introgression. The hybrids produced inadvertently in the commercial hatcheries are disseminated to the different parts of the country and are also often released to open waters to improve fish production. There is, therefore a significant risk of genetic introgression by backcrossing with native fishes (Padhi and Mandal, 1996). The long term impacts of genetic pollution will be reduction of genetic diversity in these species. Although, the actual data on production of hybrid carps and their status are not available, the situation is very alarming as almost all the seed producers are engaged in mixed spawning.

5.9. Cytogenetics

In India, Cytogenetic studies were carried out, mostly dealing with karyomorphological studies, covering over 200 species. Beside these, some investigations on comparative karyomorphological studies were also carried out with regard to hybrids and parent species, particularly Indian and Chinese major carps in relation to hybrid viability and fertility/sterility (Manna, 1984) Reddy et al., 1990; Reddy, 1991; Zhang and Reddy, 1991). Karyotype of Catla catla and Labeo rohita have been studied by different workers (Khuda-Bukhsh and Manna, 1974, Zhang and Reddy, 1991 and Jana 1993). All the worker have reported that the diploid chromosome number is 50. According to Zhang and Reddy, 1991 and Jana, 1993, Catla karyotype consists of 12 metacentric, 16 sub-metacentric and 22 sub-telocentric chromosomes. In Rohu, they have reported that the karyotype consists of .10 metacentric, 18 sub-metacentric and 22 sub-telocentric chromosomes. Both Mrigal and Kalbasu chromosome numbers are also was reported to be 50.

Karyotype of hybrids of Indian Major Craps was also studied for Kalbasu- Rohu (Krishnaja and Rege, 1975), Kalbasu-Catla and Rohu-Catla ((Khuda-Bukhsh and Manna, 1976), Catla-Rohu (Jana, 1993). In these hybrids the chromosomes number does not differ from parents but the types of chromosomes under each type differs. John and Reddy, 1986 studied the chromosome karyotype of Rohu-common carp hybrid. The Chromosome number observed to be 76 and in hybrids of commoncarp-Rohu, Commoncarp-Catla and Common carp- Mrigal, the chromosome number varies from 74-76 (Reddy et al., 1990), The common carp-Rohu / Catla/ Mrigal was observed to be sterile (Khan et al., 1989). The sterile nature may be due to aneuploid nature of their genome which, might have resulted from incomplete karyogamy. Aneuploidy of a karyotype is due to the loss or gain of one or two chromosomes (Reddy, 1999)
Biochemical genetic studies were carried out mainly to understand the genetic pattern and closeness among Indian carps and the inheritance pattern in hybrids of these carps.

5.10. Genome manipulations

Beside these classical approaches, genetic improvement methods like genome manipulations through chromosomal engineering appears to be promising tools in the development of modern aquaculture. Chromosomal engineering usually gives rise to gynogenesis or androgenesis and polyploidy (triploids/tetraploids). Gynogenesis & androgenesis are effective shorter routes to produce highly inbred lines for intraspecific hybridization through topcrossing to get heterosis effect and to produce monosex individuals. Polyploidy helps in producing sterile individuals. Monosex or sterile populations have several applied values in aquaculture.

Genome refers to the set of chromosome complement of a given individual. Through genome / chromosome manipulation a new genetic status can be attributed to an individual by altering set (s) of chromosome. Chromosome manipulation involve addition of extra set (s) of chromosome to the existing set of chromosome, resulting in triploid or tetraploids individual or replacement with a duplicate set of chromosome from the same individual, the resultant product being either gynogens with maternal inheritance or androgens with paternal inheritance.

5.10.1. Gynogenesis

Gynogenesis is the process of embryonic development with solely maternal genome and without paternal genetic input. It is a specialized form of parthenogenesis wherein embryos develop after activation of egg by the genetically inactivated sperm but there is no genetic contribution from the paternal genome. However, the resulting zygotes are haploid. Restoration of diploidy may occur spontaneously in natural gynogenesis or artificial gynogenesis can be induced through shock treatment (thermal / pressure).

5.10.2. Natural gynogenesis

In some fish species of family Poecilidae i.e. *Poecilia formosa* and Cyprinidae i.e. *Carassius auratus gibelio* gynogenesis has been the method of reproduction (Gold. 1979). Natural gynogenesis has also been reported among members of Pleuronectidae family. Crosses between grass carp and silver carp also resulted in gynogenetic offsprings ( Mantelman, 1973 and Reddy, 1995)

5.10.2. Induced gynogenesis

Gynogenesis can be artificially induced by eliminating / denaturing the genetic material (DNA) of the sperm through irradiation either by UV or Gamma rays and activating the eggs with irradiated milt. Giving thermal (cold/ heat) or hydrostatic pressure shock can restore diploidy. Artificial gynogenesis has been successfully induced in many species of dish including carps ( Purdom, 1969, Stanley *et al.*, 1975, Chourrout D, 1980, John *et al.*, 1984 and 1988 and Reddy *et al.*,1993)
5.10.3. Types of gynogenesis

Restoration of diploidy in a haploid gynogen egg can be achieved in two ways. In the first case it can be achieved by suppressing metaphase-II in the second meiotic division in other words by preventing the extrusion of the second polar body. This type of gynogen induction is known as meiotic gynogenesis. In the second case gynogenesis can be achieved by blocking the first cleavage and they are termed as mitotic gynogenesis.

Both meiotic and mitotic gynogenesis was successfully induced in *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* and *L. calbasu*. Meiotic gynogenesis was successfully induced in Indian Major Carps for the first time in *L. rohita* and *C. catla* (John *et al*., 1984) and in *Cirrhinus mrigala* (John *et al*., 1988). The first report on successful induction of mitotic gynogenesis was reported by Reddy *et al*., 1993 for inducing *C. catla* and *L. rohita* with the help of milt from common carp and mrigal respectively. The yield of mitotic gynogens as reported was 15-30% up to fry stage and 0.66-10% up to fingerling stage with heat shock and 3.0-4.3% up to fry and fingerling stage respectively through cold shock treatment. The average survival rate ranged from 1.13% with heat shock to 4.3% with cold shock. Success of induction of gynogenesis is species specific to type of shock treatment. In case of Rohu, yield of gynogens is better in cold shock than heat shock. However, yield of meiotic gynogens in Indian Major Carps is higher than mitotic gynogens (John *et al*., 1984 and Reddy *et al*., 1993)

Qualitatively, meiotic gynogens requires about 4-5 generations to achieve complete homozygosity (Nagy *et al*., 1984). Where as same result can be achieved with one generation with mitotic gynogens, as blocking of mitotic division before first cleavage results in retention of two identical replicated mitotic product. Different kinds of induced gynogens are shown in Table 5.3.

5.10.4. Top crossing of gynogenetic line

Once the induction of gynogenesis is over, the gynogen population may be reared separately. Because of their high homozygocity, proper feed and aeration is required for growth and maturity. The resulting gynogenetic offspring may all develop to female where homogamety exists.

One group of gynogens may be treated with Methyltestosterone (MST) for reversal of sex from female to male. MST have to be provided along with feed to the gynogenetic larvae from the day of yolk sac absorption for varying period (2-3 weeks or more) depending on the species. This will result in production of genetic female with physiological male. This can be crossed with gynogenetic female to produce next generation offspring. The highly homozygous gynogens can be top crossed with heterozygous stock to realise heterosis effect.
Quality Fish Seed Production through Brood Fish Management in SAARC Countries

Table 5.3. Details of induced gynogenesis in Indian Major carps.

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of gynogenesis</th>
<th>Shock treatment</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nature</td>
<td>Intensity</td>
</tr>
<tr>
<td><em>C. catla</em></td>
<td>Meiotic Mitotic</td>
<td>C.S.</td>
<td>12°C</td>
</tr>
<tr>
<td><em>C. catla</em></td>
<td>Meiotic Mitotic</td>
<td>H.S.</td>
<td>39°C</td>
</tr>
<tr>
<td><em>L. rohita</em></td>
<td>Meiotic Mitotic</td>
<td>C.S.</td>
<td>12°C</td>
</tr>
<tr>
<td><em>L. rohita</em></td>
<td>Meiotic Mitotic</td>
<td>H.S.</td>
<td>39°C</td>
</tr>
<tr>
<td><em>C. mrigala</em></td>
<td>Meiotic Mitotic</td>
<td>C.S.</td>
<td>12°C</td>
</tr>
<tr>
<td><em>C. mrigala</em></td>
<td>Meiotic Mitotic</td>
<td>H.S.</td>
<td>39°C</td>
</tr>
<tr>
<td><em>L. calbasu</em></td>
<td>Meiotic Mitotic</td>
<td>C.S.</td>
<td>12°C</td>
</tr>
<tr>
<td><em>L. calbasu</em></td>
<td>Meiotic Mitotic</td>
<td>H.S.</td>
<td>40°C</td>
</tr>
</tbody>
</table>

C.S.: Cold shock  
H.S.: Heat shock

5.10.5. Androgenesis

Androgenesis is a process which, results in all paternal inheritance. Like gynogenesis, androgenesis also occurs in nature and can be induced. Induction of androgenesis is difficult than gynogenesis.

5.10.5.1. Natural androgenesis

Natural or spontaneous Androgenesis has been observed in certain hybrid-related individuals or those with remotely related individuals or those with not so compatible genome as in the cross between *C. carpio* female and grass carp male. Indian carp being highly compatible among themselves, no such instances were ever reported in any of the hybrid crosses.

5.10.5.2. Induced androgenesis

Eliminating maternal genome in a similar way as is done for gynogenesis can induce androgenesis. The process of inducing androgenesis involves the genetic inactivation of egg and its activation with normal sperm of the corresponding species. Shock treatment has to be administered to restore diploidy. But the mechanism of restoring diplody in androgenesis is quite different from that gynogenesis. The process probably involves dispermy or other mechanism.

Attempts of inducing androgenesis in Indian Major Carps have been made earlier but were unsuccessful mainly due to the difficulties in the effective irradiation of the egg. The egg consists of huge mass of yolk that prevent the proper exposure of the DNA to UV rays in the nucleus, which specially in carps is oriented towards one side of the cytoplasm and usually facing downwards covered by cytoplasm and yolk. UV irradiation of egg may need a longer duration of exposure that may be lethal.
5.10.5.3. Utility of gynogens and androgens

Gynogenesis and androgenesis provide shorter routes and are effective methods to build homozygous inbred line in a faster way. By top crossing these inbred lines with heterozygous stocks, progeny with higher growth rates 30-40% over normal ones can be expected. Production of monosex population is also possible through gynogenesis and androgenesis.

5.11. Polyploidy

Polyploidy is a process in which there will be addition of one or more set (s) of chromosomes to the original diploid complement. Like gynogenesis and androgenesis, polyploidy also occurs in nature and can be induced. In nature, polyploidy occurs when very distantly related fish species are crossbred. For example cross between grass carp and big head carp. The hybrids of this cross are reported to be triploid. However, none of the inter-specific or inter-generic hybrid crosses among Indian carps have been reported to produce such triploids. Polyploid is induced in the same way as diploid gynogenesis. However, unlike the latter the former is induced by subjecting the fertilized egg (by normal sperm) to the usual shock treatments. Triploidy can be induced by preventing the extrusion of second polar body, while tetraploidy is induced by blocking the first cleavage in the zygote.

5.11.1. Artificial induction of polyploidy

Reddy et al., 1987 made the first attempt of inducing polyploidy in rohu, Labeo rohita by using colchicine. They could induce only tetraploids and mosaics. However, Reddy et al, 1990 successfully induced triploidy in Rohu, tetraploidy in Rohu and Catla by using thermal shocks. Triploidy was induced in Rohu by administering heat shocks to the zygotes, seven minutes after fertilization at 42± 0.5 °C exposed for a duration of 1-2 minutes. However, the incidence of triploidy was only 12 %. Rohu zygotes exposed to heat shock, prior to first cleavage at 39±0.5 °C for two minutes yield 70% tetraploids and those exposed to cold shocks of 10-15 °C for 10 minutes yield only 30-55% tetraploids. In catla, heat shock to the zygote at 40 °C for two minutes prior to first cleavage yielded 30-65% tetraploids.

Zhang, 1990 induced tetraploidy in C. mrigala for the first time. It was reported that heat shock at 39-40°C for two minutes to the embryo after 22-25 minutes of fertilization could induce tetraploidy in mrigal as well as in Rohu. However, the percentage of tetraploidy ranged from only 10-40% and 60% triploidy could induced by applying heat shock at 40 °C for two minutes after 4 minutes of fertilization.

5.11.2. Utility in aquaculture practices

Sterility is one of the great advantages in modern fish culture, as energy spent for maturation of gonadal development may possibly be diverted or utilized for increased somatic growth, especially in species like common carp, which have shorter maturity
cycle. Triploid common carp have shown significantly higher growth rate than its normal diploid counter part, Reddy et al., 1998. Because of its sterility, overpopulation is also controlled in the culture ponds.

Sterile triploid grass carps can be safely released in open water systems to check the aquatic weeds without fear of its establishment through reproduction or its influence on the indigenous fauna.

5.11.3. Allopolyploidy

Allopolyploidy is a process in which there will be addition of set (s) of chromosomes to the diploid hybrid genome. Here male and female of different species are used. Allotriploidy can be achieved by preventing the extrusion of second polar body so here female genome contribution is 2n and male contribution is n. Allotetraploidy can be achieved by blocking the first cleavage in the developing hybrid zygote. Allopolyploids may be possible to attribute some additional traits to the hybrids such as faster growth rate and disease resistance. Genome manipulations at molecular level, leading to gene transfer technology appears to hold a great promise for the development of aquaculture.

5.12. Manipulation of sex to increase production level

Sex determination methods are known for very few fishes. Sexuality in fishes has been shown by hermaphroditism, unisexuality, bisexuality etc. However, sexuality is under a low grade of differentiation in majority of fishes. In some species sex chromosomes morphologically distinct and can be identified but in majority of cases it is not morphologically distinct. Although sex determination is primarily under genetic control, environmental factor such as temperature, salinity, photoperiod and crowding can also determine the sex in some fishes.

Manipulation of sex to culture single sex can increase production level. Monosex population in aquaculture practice is gaining importance because of following reasons:

- **Growth:** In certain species of fish male and female shows variable growth. In tilapia, male grows faster than female while in carp females grows faster then males. So higher production can be achieved through monosex population.

- **Population control:** Population control of common carp can be achieved through monosex population culture.

- **Sexual maturity:** In common carp due to early maturity of fishes, growth efficiency and flesh quality declines, which can be checked through monosex population culture.

So sex reversal can be one of the methodology to improve production level.

Fishes are different from other animals because it can fertilized externally and
embryological development takes place without the protection of an internal womb or a hard shelled egg. This combination allows aquaculturists to manipulate the sex phenotypically. During early embryology, an embryo is phenotypically neither male nor female. It does not possess ovaries, testes or other characters associated with reproductive systems. Instead, an embryo possesses embryological precursors of ovaries and testes (primodial germ cells) and at this stage the embryo is ‘totipotent’ because it could develop to either male or female. At a specific time during embryological development, a chemical signal originated from a gene or sets of genes and this signal informs the totipotent tissue to develop in which way it should develop. Once this is over, the tissue completes its development the fish becomes either phenotypic male or female. After this, it is not possible to alter phenotypic sex.

There is a specific time when phenotypic sex can be altered and the timing is species specific. If a fish ingests or absorbs anabolic steroids during this period the steroid can direct the development of the totipotent cells.

The direct alteration of phenotypic sex by administration of hormone is a common approach to alter the sex.

![Diagram of hormone administration to spawn](image)

(General method of production of monosex population)

The hormone administration to spawn started from first feeding day to 30-60 days. For sex reversal to male, 17 α methyltestosteron (MST) was applied while in case of sex reversal to female 17 α estradiol is applied for 40-60 days. The ration of hormone is species specific. The hormone can be supplied to the fishes through dietary supplement, immersion of spawn in the hormone or through injection. The amount of hormone consumed by each fish is negligible and most is eliminated rapidly. Rainbow trout 67% of ingested 17 α methyltestosteron eliminated within 24 hours (Cravedi et al., 1989) So use of these hormones may not create any health hazard to the fishes. But some times it is risky from a market aspect in a health conscious society.
In XY sex determination system, monosex population can be produced by creating supermales (male having ‘YY’ chromosomes instead of ‘XY’). Mair et al., 1997 developed a strategy for production of all male population in *O.niloticus*.

### 5.13. Selective breeding

Role of selective breeding in increasing production level is well established in Agriculture and animal husbandry. Today the high yielding crops and land animals are totally depending on genetically improved domesticated breeds. This has not been true for aquaculture. Proper exploitation and utilization of genetic potential is lacking in aquaculture. Less than 5% of the total output of the aquaculture production is coming from improved breeding programme. Aquaculture species are thus genetically much closer to their wild counter part than the land animals and plant species. During the last few years it has been well documented that high selection response can be obtained in fish as well as in shell fish for economic important traits like growth, disease resistance, flesh quality etc.

Before success of induced breeding through hypophysation in late fifties rivers are the main source for seed collection of Indian major carps. With the introduction of induced breeding technique, hatcheries have been able to produce enough quantity of carp seed. The hatcheries in India hardly follow any genetic norms to produce carp seed. A limited number of brood fishes are used repeatedly for successive generations. As a result the quality of carp seed is showing negative effect of inbreeding i.e. slow growth rate, disease proneness etc. The potentials of genetics have already exhibited promising trends in aquaculture too. The correct breeding procedures followed through selective breeding in the case of Atlantic salmon and rainbow trout in Norway, channel catfish in USA and Nile tilapia in Philippines are the standing examples in this regard. The selective breeding programme of rohu (*Labeo rohita*) in India too is another example of the kind (Reddy *et al*., 2001).

### 5.14. Natural selection

Fitness of an individual can be determined by its contribution to the next generation. This is also known as adaptive value or selective value. Natural selection selects the fitness as the character. This fitness also changes with change of environment. The animal with highest fitness in the changed environment, will produce at a higher level and have a higher survival rate than less fit animal. The effect of natural selection over generation is to establish a population adapted to the new environmental condition. Thus adoptability is a response of a population rather than of individual. If the change in the environment condition represent large changes and take place rapidly then a population may be lost or destroyed due to non adaptiveness of the population to the changed environment and new condition.
5.15. Artificial selection

Artificial selection was created by man to improve genetic status of a population. Objectives of a selective breeding programme is to change the average performance of the targeted trait i.e. growth rate, disease resistance, better flesh quality, feed conversion efficiency etc. of the population in a favorable direction. Artificial selection can be performed in several ways.

1. **Stabilizing selection** where selected phenotype are around the mean and both the extremes are discarded. Example: fat content of a fish where high and low fat content are less desirable.

![Stabilizing Selection Diagram](image)

2. In the **diversifying selection** both the extremes are selected through which subpopulation could emerge. It is rarely used in animal breeding.

![Diversifying Selection Diagram](image)
3. **Directional selection** is the most common type of selection form used in aquaculture sector. Selected individuals are taken from positive direction with high ranking animals to produce better offspring every generation (Gjedrem, 2005).

Assuming no change in the environmental condition the average phenotypic value of progenies of selected parents is increased.

5.16. **Inbreeding**

Inbreeding occurs due to mating of closely related individuals. Genetically inbreeding leads to homozygosity. Almost all individuals carry deleterious recessive genes, which are hidden and not expressed in heterozygous state. Related individuals are likely to share common genes and probability of pairing of deleterious recessive genes gets enhanced with the increase of closeness between the parents. Due to which inbreeding depression occurs to the population with decrease growth efficiency, disease resistance and survival.

5. 17. **Effective population size**

Effective population size is one of the most important concepts in the management of a population in that it gives an indication about the genetic stability of the population. It depends upon several factors such as total number of breeding individuals, sex ratio, mating system and variance of family size. The effective population size can be calculated by following formula,

\[
N_e = \frac{4N_f N_m}{N_f + N_m}
\]

Where \(N_e\) = Effective population size

\(N_f\) = Number of female brood fishes used for seed production
\[ N_m = \text{Number of male brood fishes used for seed production} \]

Effective population size is inversely related to inbreeding

\[ \Delta F = \frac{1}{2N_e} \]

So \[ \Delta F = \frac{1}{8N_f} + \frac{1}{8N_m} \]

Where \( \Delta F \) = Rate of inbreeding per generation

\( N_e = \text{Effective population size} \)

\( N_f = \text{Number of female brood fishes used for seed production} \)

\( N_m = \text{Number of male brood fishes used for seed production} \)

So to improve the genetic status of any population, hatchery managers should decrease inbreeding rate and increase effective population size. Selective breeding plays vital role to improve genetic status of fish in a positive direction which has already been demonstrated in case of salmon at Norway, tilapia at Philippines and rohu at CIFA, India.

5.18. Objectives of selective breeding

Objectives of a selective breeding programme is to change the average performance of the targeted trait i.e. growth rate, disease resistance, better flesh quality, feed conversion efficiency etc. of the population in a favorable direction.

5.19. Selection process

Selection is an age-old process in nature. Fittest organism survives and other eliminated. Selection also can be achieved artificially. In this process best individuals are selected as parents so that parents pass on their superior genes to their progeny and better progeny can be obtained. Selective breeding based on principle of quantitative genetics. Which indicated that that phenotype of an individual, which can be measured or scored, could be partitioned in to two components. One attributable to the influence of genotype i.e. the particular assemblage of genes possessed by the individual and other one attributes to the influence of environment i.e. all non-genetic components.

\[ P = G + E \]

Where \( P \) = Phenotype of an individual

\( G = \text{Genotype} \)

\( E = \text{Environmental (Non-genetic) component} \)

Quantitative phenotype exhibit continuous variation, the only way to study them is to analyze the variance that exists in a population. The phenotypic variance \( (V_p) \) that is
observed for a quantitative trait is the sum of the genetic variance \((V_G)\) and \((V_P)\) and the interaction that exists between the genetic and environmental variance \((V_{G+E})\).

So \(V_P = V_G + V_E + V_{G+E}\)

Genetic variance is the component of interest in selective breeding program. \(V_G\) is further subdivided into three component i.e. additive genetic variance \((V_A)\), dominance genetic variance \((V_D)\) and the epistatic genetic variance \((V_I)\).

\[ V_G = V_A + V_D + V_I \]

\(V_A, V_D\) and \(V_I\) differ from each other according to their mode of inheritance. Dominance genetic variance is the variance that is due to the interaction of the alleles at each locus. Because of this, \(V_D\) cannot be inherited; it is created anew in each generation. Since it is the interaction between alleles at each locus so \(V_D\) is a function of the diploid state as alleles occur in pairs. During meiosis homologous chromosomes and allelic pairs are separated during reduction division and the chromosome complement reduced to half. So \(V_D\) is not transmitted to next generation.

Epistatic genetic variance \((V_I)\) is due to interaction of alleles between two or more loci. Epistatic interaction occurs across loci so it is also not transmitted from parents to offspring. This also created a new in each generation.

\(V_A\) or additive genetic variance is the additive effect of genes. It is the sum of the effects of each allele that is responsible for phenotype. It does not depend on specific interaction or combination of alleles so it is not disrupted due to meiosis so additive genetic variance transmitted from parents to offspring. It is transmitted in a reliable and predictable manner. \(V_A\) is also called the variance of breeding value.

5.20. Heritability

Heritability describes genetic component that is not disrupted by meiosis. The proportion amount of phenotypic variance \((V_P)\) that is controlled by \(V_A\) is called heritability \((h^2)\).

\[ h^2 = \frac{V_A}{V_P} \]

Once you know the heritability response of selection can be predicted as

\[ R = S \times h^2 \]

Where \(R=\) response to selection,

\(S = \) selection differential (difference between offspring and parent generation)

\(h^2 = \) heritability of the trait
5.21. Selection methods

Several selection methods are available for obtaining additive genetic improvement. The methods differ with respect to which type of relatives that provide information used for the selection decisions. The objective of all the methods is to maximize the probability of correct ranking of animals with respect to their breeding value, an estimate of each individual ability for producing high / low performing offspring. The breeding value of an individual cannot be estimated on basis of the phenotypic value of the trait(s). In fish such records are usually obtained from the individual itself (Individual selection / mass selection), full and half sibs (family selection) or from all three sources of information (combined selection)

5.22. Selective breeding of freshwater prawn *M. rosenbergii*

Selective breeding of *M. rosenbergii* was initiated at CIFA in collaboration with WorldFish center, Malaysia in the year 2008. Under the program base population was collected from three sources i.e. Kerala, Odisha and Gujarat. Base population was established after performing 3X3 diallel cross. Three generation of selection completed at CIFA and average 4% per generation genetic gain was observed. Field trial in farmers pond also showed positive result. Multilocal field trials are under progress.

5.23. Constraints to breeding programs

5.23.1. Inbreeding accumulation

Due to high fecundity in a selective breeding program, rapid accumulation of inbreeding is very common for fishes. When individual selection method is followed without identification of animals, it may be difficult to avoid inbreeding. By using tagged individuals with known pedigree, it is possible to avoid relative mating thus secure a slow build up of inbreeding. However, it is possible to reduce inbreeding by dividing the breeding population in to sub populations. After certain generation crossing of subpopulation can be made to avoid inbreeding. By introduction of unrelated stocks is another efficient way of reducing inbreeding.

5.23.2. Negative correlated effects

When several traits are included in the breeding goal, genetic correlation between traits is very important. Some important traits like disease resistance and growth may be negatively correlated. Then care should be taken while selecting the fishes. When negative effects are detected the breeding program must be changed and action should be taken to repair the damage already done.
5.23.3. Natural calamities

Some times in breeding nucleus due to natural calamities a great damage may occur due to loss of animals. In that case second nucleus may be considered so that protection of selected animals can be made. Safeguard of selected stock should be given priority in a breeding program.

5.23.4. Genotype-environment interaction

Genotype-environment interaction plays a significant role, as it needs to develop more than one strain. However, there is possibility that strains over time may become more sensitive to environmental variations. So periodically investigation on genotype-environment interaction should be studied by testing breeding value under common farming conditions and on several private farms.

5.3. Guidelines/ tips to the hatchery managers

For quality seed production, proper care is needed by the hatchery managers. Main aim of any hatchery manager/seed producer should be to increase population size and reduce inbreeding depression & to maintain purity of seed. Guidelines were formulated to address the issues (Das Mahapatra, 1998)

- Mixed spawning should be prevented to protect the genetic purity of carps’ gene pools. Indian major carps should be spawned separately in the breeding pool to avoid inadvertent hybridization between these species.

- Crossing of different lines of fishes would increase heterozygosity. Separate lines of fish can be maintained by keeping the record of the families of different strains bred in the hatchery.

- Importance should be given to own brooder raising programs. Seed from different days of breeding may be taken for own stocking rather than from same day spawn for own brooder raising.

- 50:50 sex ratio may be maintained and same ratio may be used in the breeding purpose.

- Female breeder of 3-5 years of age and male of 2-5 years may be used in the breeding program. Broodstocks of different age groups should be bred together. This helps in reducing the chance of loss of some valuable alleles due to genetic drift

- Replenishment of brood stock from other sources and crossing may be done for offspring production. Natural stocks may be inducted periodically to increase the heterozygosity level of the hatchery stock. Net working of hatcheries can be made
and exchange of fish among hatcheries may be done and cross breeding of different hatchery stocks of same species will improve the quality.

- Same yearclass, parent offspring mating, repeated use of same breeder may be avoided.
- Cohort breeding procedure may be followed to improve the quality of carp seed.
- Cryopreserved milt may be used to reduce space for male brooder raising
- Record of the hatchery (pond wise) should be maintained to avoid the mating of close relatives.
- Water, feed and disease management is also essential for quality seed production.

5.24. Conclusion

An assured way to sustainability in aquaculture is the systematic exploitation of genetic resources of the economically important species of fish & shellfish. Exploitation of genetic resources should include identification of different populations within the species, if any, conservation and exploitation of the potentials of each species in question right from the production of quality seed by following correct breeding procedures, with high survival and better growth performance, the ability to resist disease attacks and unfavourable environmental conditions etc.

Genetics, thus from the classical selection methods to the modern genetic engineering techniques, have a much greater role to play in stock improvement and development of aquaculture in the coming years to obtain sustainable yields.

References


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6. Principles and practices of selective breeding in rohu (*Labeo rohita*)

Kanta Das Mahapatra


6.1. Introduction

Selective breeding has been the basis of nearly all of the genetic improvement programs that have taken place in terrestrial plants and animals and has contributed significantly to the improvement in productivity and food security. This is the classical approach to achieve genetic improvement in domesticated species. The approach utilizes intra-specific variation available in the founder population and applies selection for high performance with respect to desirable traits in the subsequent generations. Although aquaculture organisms differ from mammals and birds in several important ways (e.g. higher fecundity and smaller post-embryonic size), the principles of selective breeding can also be applied to their genetic improvement. During last few years, it has been well documented that high selection response can be obtained in fish as well as shellfish for economically important traits like growth, disease resistance, flesh quality etc.

For the first time in India, selective breeding was carried out to genetically improve one of the most preferred carp species (rohu). Rohu is one of the most preferred species by the consumers in the country. However, its performance in terms of growth is slower when compared to other species in the multispecies culture system. Besides, rohu also is easily susceptible to diseases. Taking all these into consideration, a project on genetic improvement of rohu, particularly for better growth performance through selective breeding has been initiated at the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar in 1992, in collaboration with the Institute of Aquaculture Research (AKVAFORSK), Norway with funding support from NORAD. The main objective of the project was to develop a national selective breeding plan for rohu and dissemination of improved rohu to the fish farmers of India for quality fish production.

Under the program a range of selective breeding techniques (production of fullsib groups, individual identification by PIT tags, communal pond rearing, estimation of breeding value and ranking of individuals in different year classes) were standardized for rohu. The project continued till 2003 under different phases. The main objectives of the project were to obtain information about the magnitude of the genetic variation for
growth and survival of rohu; To develop manpower with strong knowledge on quantitative genetics and selective breeding; To develop a breeding program of rohu; To disseminate improved rohu to fish farmers through multiplier units.

After 2003, the project continued as through internal funding as ICAR Institute based project and selection continued for further generations.

The present paper deals with the principles and practices followed in the genetic improvement programme of rohu in India.

6.2. Base population establishment

Base population of *Labeo rohita* have been collected from five different rivers of North India, viz., Ganga, Gomati, Yamuna, Sutlej and Brahmaputra, which are the native habitat of rohu. Rohu stock from the fish farm of CIFA has also been added to the gene pool. Thus the base population for the selection project consisted of rohu from six stocks (Table 6.1).

All these stocks were collected at fry to fingerling stages and reared separately in earthen ponds for a period of one to two months before they were physically marked (dye marking) and stocked in communal ponds for raising to brood fish. Genetic characterization of these six stocks indicated a wide variation within each stock.

<table>
<thead>
<tr>
<th>Stocks</th>
<th>Base population year-classes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1993</td>
</tr>
<tr>
<td>CIFA stock</td>
<td>X</td>
</tr>
<tr>
<td>Ganga</td>
<td>X</td>
</tr>
<tr>
<td>Gomti</td>
<td>X</td>
</tr>
<tr>
<td>Yamuna</td>
<td>X</td>
</tr>
<tr>
<td>Sutlej</td>
<td>X</td>
</tr>
<tr>
<td>Brahmaputra</td>
<td>X</td>
</tr>
</tbody>
</table>

Marking was done using dye, fin clipping and a combination of both. Ganga rohu were dye injected with M-procian blue at the base of the right pectoral fin and Yamuna rohu at the base of the left pectoral fin. In the case of Gomati and farm rohu the right and left pelvic fins were clipped respectively, while the Sutlej rohu were marked with the blue dye, inject at the base of the left pectoral fin by clipping the right pelvic fin. The reverse has been done to mark the Brahmaputra rohu, i.e. by injecting blue at the base of the right pectoral and clipping the left pelvic fin (Gupta, 1994). During 1993, the first groups of full sibs were produced from the local and Ganga stocks as these two stocks were matured. Two or three males were mated with one female (paternal half sibs). However, during the year 1994, full-sib groups (families) were produced from all the six stocks of rohu: two or three females were mated to a male, producing maternal half-sibs. (Reddy
et al., 1998). Later on in other year classes paternal half sibs were produced. Approximately 60 full sib families were produced in each year class by diallele cross (Table 6.2).

After a period of one year, data pertaining to growth (length and weight) was collected and analysed to evaluate the individual breeding value and to rank the fish. The data collected indicated that growth and survival of the wild rohu stocks were equal to or better than the farmed stock. This indicated that the present procedures followed by hatcheries for seed production are improper. A substantial additive genetic variation has been observed for growth rate and survival of rohu, both under mono and polyculture systems, thus indicating that growth rate and survival of rohu can be improved through selective breeding. Competition for food may prohibit rohu from exhibiting its growth potential in polyculture. Proper feeding and pond management is essential while testing fish in a breeding programme. This preliminary observation has strengthened the ongoing selective breeding program given proper direction to it.

The study also indicated that stocks and full-sib groups within stocks ranked consistently high for growth performance in mono and polyculture, which suggests that there is no need for the development of special rohu lines for different systems. The technique of breeding followed for ovulation of females and sperm collection (milting) from males using synthetic hormone (Ovaprim) to obtain the gametes.

In order to estimate the magnitude of heterosis for harvest body weight two 3 X 3 diallel crosses were made. The local stock was common to both diallel crosses (Gjerde et al, 2002).

**Table 6.2. Rohu stocks included for two diallele crosses**

<table>
<thead>
<tr>
<th>Male parent</th>
<th>Female parent</th>
<th>Ganga</th>
<th>Yamuna</th>
<th>Local</th>
<th>Brahmaputra</th>
<th>Sutlej</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganga</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yamuna</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Brahmaputra</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Sutlej</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

However, negative to very low heterosis effect could be achieved indicating pure breeding to be the selection method for genetic improvement in rohu.

**6.3. Development of specialized hatchery for selective breeding**

One specialized hatchery was constructed at Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar, India to produce spawn of fullsib families of rohu carp (*Labeo rohita*) and mass production of genetically improved rohu spawn. While following combined selection method, fullsib families have to be produced and
reared separately from fertilized egg to fingerlings (10-15g) i.e. till taggable size for individual tagging. The specialized hatchery was developed for individual fullsib family spawn production i.e. fertilized egg to spawn. Mass scale spawn productions have also been under taken for dissemination of improved rohu. Water requirement study was conducted for full sib family production as well as for mass scale spawn production and it was worked out to be 10.54 and 81.07 m³ for one fullsib family and a million of spawn, respectively. The specialized hatchery improved the production of fullsib families and spawn recovery percentage to a greater extent. Recovery of fullsib families increased from 70-82% to 94-100% in different year classes. Efficacy of the hatchery for fullsib family and mass scale spawn production in selective breeding of rohu program for last 12 years was studied. The same model of hatchery can also be utilized for any other carp selective breeding programme.

6.4. Tagging

While working on selective breeding of rohu *Labeo rohita* at the Central Institute of Freshwater aquaculture (CIFA), different indigenous tags were tried, but all proved to be unsuitable for rohu. Rohu being one of the most actively swimming fish, retention of external tags was not practicable and very often results in secondary infection. Considering all these factors, an internal tagging system with passive integrated transponder (PIT) tags were tried and found to be quite suitable and effective.

The size of PIT tag is 11 mm. The PIT tag consists of an antenna coil that has 1200 warps of specially coated 0.0254 mm diameter copper wire encapsulated by glass ampoule. The PIT tags are passive in nature having no power of its own and rely upon an external source of energy to operate. The PIT tag excitation energy comes from a tuned loop, which is part of the tag interrogation system. This system transmits an alternating current via the loop at 400 KHz, which establishes a magnetic field, when a PIT tag passes into the magnetic field for as little as 25 milli second and induction current is established in the transponder antenna coil. The current energizes the transponder integrated circuit, which modulates the transponder coil, current at frequency of 40-50 KHz in accordance to a program code. The signal from the PIT tag is received by the loop of the interrogation system or mini portable reader. Every PIT tag has its own unique identification code, which can be easily read with the portable reader.

Before the implantation of tag, fingerlings were collected and kept overnight in well aerated water without giving any feed so that the digestive tract will be clear and thus creating more space in the body cavity of the fish to implant the tag without any injury to the visceral mass. During the tagging operation, each fish/fingerling was anaesthetized individually with a 0.3% MS 222 solution. Doses may slightly vary depending on the size of the fish

*Ten to 15g fingerlings were found to be quite suitable for tagging (Das Mahapatra et al., 2001). However, smaller sized fishes (4-5g) are also tagged subsequently with minor modification for tagging procedure.*
6.5. Test environment for rearing
Tagged fish were stocked in three communal ponds of 0.1ha each under mono-culture and two 0.4 ha ponds under polyculture practice. In polyculture, rohu was stocked along with catla and mrigal in the ratio of 1.2 : 1 : 1, respectively. This practice continued till 1997 and only monoculture was done after wards, as a high correlation between the performance of rohu in mono and polyculture was observed.

6.6. Statistical analysis
The data was analysed using SAS (SAS Institute Inc., 1990). For each year class full sib, half sib and individual data was considered for breeding value estimation. Individuals with average breeding value was chosen as control and in every generation they were compared with the offspring of selected (higher breeding value) individuals.

6.7. Estimation of genetic parameters
Total heterosis for each of the six crosses was low or negative, whereas average heterosis was low and mostly not significantly different from zero. It was concluded that genetic improvement of rohu by crossbreeding would be futile (Gjerde et al., 2002).

At tagging, the heritability ($h^2$) for body weight was very low whereas the environmental effect common to full sib families other than additive genetics very high For sampling and final sampling the heritability was moderate (0.23 ± 0.08)

The phenotypic and genetic correlations between sampling and final sampling weights were 0.92 (± 0.01) and 0.98 (±0.01), respectively, indicating that they were virtually the same trait.

6.8. Field testing
Field trials were arranged in several parts of India viz., Rahara, West Bengal; Ludhiana, Punjab; Vijayawada, Andhra Pradesh; and State Fisheries Department, Orissa. In 2005 and 2006 demonstrations were also arranged at farmers’ ponds in different states of India. A comparison of improved rohu was made with local rohu and with the control group from the research center. The following results were recorded from the field trails at different locations (Table 6.3 and 6.4).

6.8.1 Odisha
On-farm field trial in all four places of Odisha showed significantly higher growth of Jayanti rohu over local rohu in Keonjhar a very significant growth of Jayanti rohu was obtained over local rohu. In some places Jayanti rohu also showed higher growth efficiency than Catla, which generally grows much faster than normal rohu.
Table 6.3. Results of field trials of Jayanti rohu in Odisha

<table>
<thead>
<tr>
<th>Place</th>
<th>Year</th>
<th>No. of ponds</th>
<th>Stocking combination</th>
<th>% of higher gain of JR over NR</th>
<th>% of higher gain of JR over Catla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alisiasan</td>
<td>2005</td>
<td>2</td>
<td>JR+LR+Catla</td>
<td>P1-98% p2-120%</td>
<td>18</td>
</tr>
<tr>
<td>Bhadrak</td>
<td>2005</td>
<td>2</td>
<td>P1 - LR+ Catla ; P2 - JR+Catla</td>
<td>117%</td>
<td>20</td>
</tr>
<tr>
<td>Kendrapada</td>
<td>2005</td>
<td>2</td>
<td>P1 - LR+ Catla</td>
<td>100 %</td>
<td>-</td>
</tr>
<tr>
<td>Keonjhar</td>
<td>2006</td>
<td>84</td>
<td>JR + Catla + Mrigal; LR + Catla + Mrigal</td>
<td>128%</td>
<td>56</td>
</tr>
</tbody>
</table>

(JR-Jayanti rohu, LR- Local rohu, P1- Pond number 1, P2- Pond number 2)

6.8.2. West Bengal

In West Bengal third generation of Jayanti rohu seed was obtained from the multiplier unit, Rahara (Regional Centre of CIFA). On-farm field trial was conducted in 4 ponds (0.08-033 ha). Participatory members are 6 fish farmers and 24 unemployed rural youth. Stocking density was maintained at 5000 fingerlings / ha. Species composition was catla- 20%, Jayanti rohu- 35%, local rohu –35% and mrigal 10%. Here fin clipping was done to identify local rohu. Total culture period was nearly 8 months.

Table 6.4. Absolute growth increment/day (g) of fishes considering total culture period

<table>
<thead>
<tr>
<th></th>
<th>Catla</th>
<th>JR</th>
<th>LR</th>
<th>Mrigal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond1</td>
<td>3.18</td>
<td>2.01</td>
<td>1.66</td>
<td>1.74</td>
</tr>
<tr>
<td>Pond2</td>
<td>1.64</td>
<td>1.30</td>
<td>0.99</td>
<td>1.01</td>
</tr>
<tr>
<td>Pond3</td>
<td>1.59</td>
<td>0.79</td>
<td>0.51</td>
<td>0.64</td>
</tr>
<tr>
<td>Pond4</td>
<td>1.07</td>
<td>1.82</td>
<td>1.71</td>
<td>1.74</td>
</tr>
</tbody>
</table>

6.9. Dissemination of improved rohu

The improved rohu, popularly known as “Jayanti”, was named in 1997 (i.e. 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. Brand name registration to improved rohu as “Jayanti rohu” was registered under Kolkatta Court by CIFA, Bhubaneswar during 2008.

Initially dissemination of improved rohu was carried out in three states (Andhra Pradesh, Odisha and West Bengal). The nuleus is located in CIFA and the multiplier units were given broodstock fingerlings from CIFA to be used for the production of Jayanti seed. Multiplier units are raising the brood fishes, producing seed and distributing the seed to the fish farmers. Besides, the CIFA provides limited number of
seed across all parts of the country at present about 12 states have already received seeds but in limited numbers.

CIFA is planning to have other multiplier units in different parts of India for distribution of improved rohu seed.

![Diagram of Dissemination plan for improved Rohu](image)

6.10. Selective breeding of rohu for disease resistance (Aeromoniasis)

Apart from growth, disease resistance against *A. hydrophila* was also included in the rohu breeding program from the year 2004 as second trait. A project was undertaken in collaboration with AKVAFORSK, Norway in this regard from 2004-2007.

A reliable challenge test has been developed to aeromoniasis after collecting the bacterial isolates from different regions of India and testing their pathogenicity through a series of challenge tests. Ten different pathogenic isolates of *Aeromonas hydrophila* were collected from different regions of India. One of these isolates (Ah #4) was found most suitable based on pathogenicity, and thus selected for all the further challenge studies.

In order to develop a challenge model for rohu, an experiment investigating the portal of entry of *A. hydrophila* was conducted. Fish were challenged by various routes viz., immersion, scale removal (5 scales) + immersion, abrasion (1 cm) + immersion, high stocking density (10 g fish/L with aeration)+ immersion, immunosuppression (after 3 days of cyclophosphamide 0.1mg/0.1ml ip/100 g fish)+ immersion, scale removal + high stocking + immersion, and abrasion + high stocking + immersion.

Most of the challenge methods produced nil or little mortality except the abrasion + high stocking + immersion method where necrosis, sloughing of tail (erosion),
development of deep ulcers beyond the abrasion region exposing vertebral column, ventral congestion, congested operculum, secondary contamination like saprolegniasis were noted. Since none of the above methods was found to be much useful for challenge test in rohu, intraperitoneal route was then investigated. The LD$_{50}$ dose was calculated to be 5´ $10^6$ CFU/g fish. Thus intraperitoneal challenge method was selected for mass challenge study.

Fish from as many as 40, 47 and 51 families from 2003, 2004 and 2005 year classes, respectively, were challenged with *Aeromonas hydrophila* at 5 x $10^6$ CFU/g fish along with the control families from respective year classes. All challenge tests were performed in duplicate. A wide variation in percent survival among the families was observed in all the three year classes. The ranges of percent survival were 17.39 – 75, 33.33 – 90 and 20 – 70.37 for 2003, 2004 and 2005 year classes, respectively. Coding of dead and survival animals were done as 0 and 1, respectively. Apart from that, time intervals for mortality was also noted during the experiments. Hourly mortality recorded during the challenge tests showed that most of the fish died within 24 hours of challenge (Fig. 6.1). The fish were watched for 30 days for mortality. The cause of mortality was confirmed by reisolating bacteria from the kidney of 10 % dead fish.

![Fig. 6.1. Survival curve of rohu in the challenge test](image)
Based on the results of a binary threshold model analysis, the heritability (± SE) for survival in the controlled challenge test was estimated to 0.11 ± 0.04, indicating low but significant additive genetic variability. These results, further substantiated by the considerable difference in mean survival between offspring produced from HR and LR breeders in the divergent selection experiment, clearly suggest that significant improvements can be obtained for innate resistance to aeromoniasis in rohu carp through systematic selection.

Variation in innate immune response in different families was also studied. A wide range of variation was observed in different immune parameters under different year classes (Sahoo, et.al, 2008)

A wide range of variation i.e. 18-77% in 2003 year class, 33-90% in 2004 year class and 20-90% in 2005 year class was observed with respect to survival among various families of rohu. The genetic variation was analysed for survival as binary trait based on whether the animal dead or alive at the end of test. The heritability of the order 0.114 ± 0.038 was estimated through sire dam model (Das Mahapatra et. al, 2008). However, the effect common to fullsib other than additive could not be estimated as it was 0 or very close to 0.

Correlation coefficient of predicted breeding value for survival observed to be very high (0.84-0.95) in different year classes. Resistant line was produced from top 15 families having higher breeding values and susceptible line was produced from the families with lower breeding value from the bottom. Both the groups were challenged with the same dose of *A.hydrophila* in triplicate. Realised response of the order 31.1% could be achieved in the first generation. It is observed that through selective breeding it is possible to improve the resistance of rohu against aeromoniasis.

Further positive correlation (0.43) was observed between growth and disease resistant trait i.e against aeromoniasis in the rohu breeding program.

6.11. Conclusions

The opportunities for selective breeding program in aquaculture species are very encouraging. Genetically improved seed can be a vehicle for production improvement in aquaculture sector. Dissemination of improved rohu “Jayanti” through multiplier units in different states can directly improve production level. Awareness and knowledge on selective breeding is highly essential in the present scenario to improve unit area productivity.

References


7. Marking and tagging of carps

J. N. Saha


7.1. Introduction

Marking or tagging has a long tradition in fisheries management. Tagging and marking fish are essential techniques for any fisheries biologist (PIT tag marking procedures manual, 1999). Marking a fish allows biologists to gather a wide variety of information. Certain marking techniques allow fish to be tracked giving biologists a better understanding of movement and migration patterns (PIT tag marking procedures manual, 1999). Other mark and recapture methods provide population estimates, fish growth, and estimates of fish and natural mortality.

In recent times the trend has been towards integrating tagging information directly into stock assessment models and away from the estimating factors like growth, movement, exploitation and abundance as “stand-alone” parameters (Seeb et al., 1986). As the methodologies have improved, information obtained from tagging has increased in quantity and breadth. In this session we are looking for novel approaches used for marking and tagging of carps for effective fisheries management and stock assessment.

Identification of an individual animal or a group of animals within a population is important in fishery research especially in selective breeding program (Jenkins et al., 1990). Unlike land animals, in highly complex underwater fishery resources, the arrival of wrong inference is not infrequent. Fish marking or tagging plays a vital role for identification of particular individual especially when they are pooled in a common place. Efficient marking system in genetic research point of view is relevant for proper identification of individual such as strain, brood stock and family etc. The very objective of marking/tagging fish is to enable their number to be estimated indirectly or to follow the fate of labeled individuals to determine its age, growth and migration etc.

7.2. Types / methods of tagging

There are different methods of marking fishes such as fin clipping, dye marking, cold branding, anchor tag (Floy tags), Visible Implant Elastomer (VIE) tag (Close et al., 2002; Astorga et al., 2005), Petersen discs tag (Peterson et al., 1994), Passive Integrated Transponder (PIT) tagging etc. depending on the type of fish and study (Roberts et al., 1973; Refstie et al., 1975; Prentice et al., 1990a; 1990b; 1990c). In case of fishes without scales, it is convenient to mark them with dye or cold branding, without involving
much complication or sophistication. However, these methods of fin clipping offer only limited combinations and hence, can be used to mark only limited individuals. When the study involves marking of hundreds or thousands of individuals of different groups or stocks it requires individuals tagging having a separate number. This involves attachment, insertion, or injection of a foreign object to or into the body of the fish. For mass tagging especially in selection study two types of tags are generally used i.e floy tag and PIT (Passive Integrated Transponder) tag.

7.2.1. External tag

These tags have the advantages of being seen without dissection of the fish. Also many external tags allow for individual recognition. But, these tags can cause higher mortality by attracting predators, interfere with locomotion by protruding, and make the organism more susceptible to disease and infection.

7.2.2. Anchor tag (Floy tags)

Such tags are applicable for long-term studies on migration on adult migratory species. These tags are exactly like tags used to attach prices to clothing. The tags are inserted with a gun. It is important that anchor tags penetrate deep enough into the fish so that the T-bar interlocks with the skeleton.

Floy tag has proven successful in tagging of tilapia fingerlings in selective breeding of tilapia in GIFT project (Genetic Improvement of Farmed Tilapia) (GIFT Technology Manual., 2004). The Floy fingerling tag, which is a small, flat, oval-shaped piece of plastic, is tied to a vinyl thread that holds it. The tag is especially designed for fingerlings. Individual identification is based on numbers and (or) letters printed on either side of the plastic laminated chips. The tag is placed externally on the body of the fish. The needle should be carefully inserted underneath the scale between the sixth and seventh spines of the dorsal fin, above the lateral line. Prior to insertion of floy fingerling tags, the fishes are anesthetized. Floy tags are of several colors like red, pink, yellow, white etc. However, it is not recommended to use red or any other dark color since it might be difficult to read the numbers at harvest.

7.2.3. Visible implant elastomer (VIE)

The Visible Implant Elastomer system was developed by Northwest Marine Technology, Inc (NMT) biologists in the early 1990’s while they were seeking better fish marking methods than traditional external tags and fin clips, which may have adverse impacts on the growth, survival and behavior of fish (Visible Implant Elastomer Tag., 2008).

The VIE tag system provides internal colored tags that are visible externally. The system uses a bio-compatible, two-part, elastomer material. After mixing, the elastomer is a viscous liquid that is injected into tissue with a hypodermic syringe; most species of fish, and many other animals, have suitable areas of transparent or translucent tissue. Within hours or days this material converts into a pliable solid. The elastomer holds the pigment in a well-defined mark, without damaging surrounding tissue. By the use
of different marking sites, and perhaps two or more marks on each individual, development of numerous group or individual codes is possible. and the use of appropriate lighting can significantly enhance detection of tags.

The VIE tag lies beneath the skin or deeper within the tissues, without a permanent wound or lesion. It has been demonstrated to have minimal impact upon subsequent growth and behavior of fish and other animals. The system was initially used with salmonids, exploiting an area of transparent tissue behind the eye. Since then it has been used on hundreds of species of fish, amphibians, crustaceans and other animals, with many body locations being used.

To inject a tag, the syringe needle is inserted into the marking location, and is slowly withdrawn as the material is injected, so that a long narrow mark is created. It is important that the tag created is fully contained within the target tissue; extrusion of the material from the needle must cease before the needle withdrawn so that material does not project through the needle wound, as this is likely to cause rapid loss of the tag.

In transparent tissue such as the adipose, eyelid of salmonids the VIE tag can be injected fairly deeply. However, if the material is being injected into fully or partly pigmented tissue it is important to place it just beneath the skin. Tagging shrimps in the last abdominal segment has been very successful. The bases of fins and beneath the jaw are also good sites in many species. Overall, VIE appears to be a very successful marking system for crustaceans. The material was injected into the musculature of the sixth abdominal and showed a very high retention rates through multiple molts (Bailey et al., 1998; Astorga et al., 2005; Bushon et al., 2007; Reeves et al., 2009).

It has been demonstrated by Leblanc & Noakes (2012) that VIE tags did not negatively affect the growth and survival of rainbow trout (25–40 mm) and visibility and retention rate were excellent. A caudal fin ray was as an implant location for 25–40-mm fish. Tagging could be done on the upper and lower fin rays too.

Steingrimsson and Grant (2003) also reported two possible locations for VIE tags at the base of the dorsal fin of young salmonids: one anterior and one posterior on either side, which would increase the number of individual tag codes. Prior to tagging, the fish is anesthetized using tricaine methanesulfonate (MS-222; 50 mg/L MS-222 buffered to pH 7.0 with 125 mg/L NaHCO3). VIE material is biocompatible and carries no known human health hazards.

Advantages of VIE tags

- It can be applied to very small fish, amphibians, crustaceans and other animals
- Its high retention rates
- It has no effect on survival and growth
- It is less expensive
Quality Fish Seed Production through Brood Fish Management in SAARC Countries

- It can be applied quickly
- Tags can be detected visually but tags may become difficult to detect in ambient light if growth is considerable and pigmented tissue is laid down over the tag.

Disadvantages of VIE tags
- It has limited coding capacity but using different colours coding capacity can be increased
- Detection of tags is not always easy due to pigmentation of tissue

There are ten VIE colors are available. Six (red, pink, orange, yellow, green, blue) are fluorescent; the other four (black, white, purple and brown) are not (Dewey et al., 1996; Visible Implant Elastomer Tag., 2008).

7.2.4. Petersen discs

These tags were used during the first 60 years of tagging. Although tag construction has changed towards less expensive material but the design of the tag have remained unchanged. The disc is applied under the dorsal fin of the fish with a pin and pliersis the various length tag used for the fish of different thickness. The Peterson disc has also been used on mollusks by gluing to the shells with epoxy cement. Peterson disc remains on the animal for life. Disadvantage of it is long application time per tag (Peterson et al., 1994).

7.2.5. Marking

There are different marking techniques, which can be easily adapted for marking different stocks of fishes. Of these, the fin clipping and dye marking techniques are the least complicated and less expansive methods. In case of rohu stocks, both fin clipping and dye marking and a combination of two have been used and was found to be very effective.

Fin clipping can be done at both pectoral (left & right) and pelvic (left & right) sides. The fin should be cut with fine sharped scissor at the base of the fin. The cut portion of the fin is treated with potassium permanganate solution to avoid secondary infection. The clipped fin regenerates with a slight curve at the clipped site and it can be easily identified. When the fin is fully or partially regenerated, a second time clipping may be done. Clipping can be done for any number of times.

7.2.6 Dye marking

A super saturated suspension of M-Procaine blue (Biological stain) in distilled water is injected at the base of the pectoral / pelvic fins (Khan et al., 1988). The dye suspension should be just below the skin not into the muscle. The dye marks usually get faded at regular intervals and may be remarked with fresh dye if necessary. After dye marking the fishes are dipped in 5-ppm potassium permanganate solution as a precaution to prevent any infection caused due to injuries during the process of dye marking.
During breeding season a temporary mark can be done on the brooders’ scalp. Fishes are numbered on the scalp with the help of a suitable soft marker like copying pencil, a narrow or smooth tipped wood or bamboo “pen” temporally to identify the brooders during spawning or stripping time.

7.2.7. Cold branding

There is considerable interest in the use of freeze branding for identification of farm animals. For aquatic animals freeze branding is very rarely used but Norwegians are using these freeze branding for identification of salmonids and trouts as these fishes are not having bigger scales so it is easy to get freeze brand on their body, but presently they are also using the PIT tags.

7.3. Passive integrated transponder tags (PIT)

Many researchers use PIT tags and readers to study migration habits (Armstrong et al., 1996) of fish. A PIT tag is a radio frequency device that transmits a unique individual code to a reader where it is displayed in a numeric or alphanumeric form. The tag has no internal battery, hence the term “passive”. The reader powers or excites the tag circuitry by radio frequency induction and receives the code back from the tag. Radio frequency identification does not require line of sight, tags can be read as long as they are within the range of a reader. PIT tags were designed for positive identification; because they are passive they are not capable of long-distance tracking. The implant site is dependent upon the species, size of the animal and the size of the tag.

The PIT tag consists of an antenna coil that has 1200 warps of specially coated 0.0254 mm diameter copper wire encapsulated by glass ampoule. Read range depends on antenna dimensions (PIT tag and interrogator), energisation power, modulation type, duplex mode of operation and semiconductor fabrication technology utilised on chip (IC efficiency). It is the combination of these factors that will determine the overall read range of the system. Read time is governed by, interrogator antenna type (tube, panel), energisation power and by number of data bits, modulation type and duplex mode of the PIT tag. The 125kHz to 135kHz band has been widely adopted for PIT tags for animal and fish use. This is generally referred to as the ‘low frequency’ (LF) band.

PIT tag size may be different from 7 mm onwards and it is to be used as per specimen size. PIT tag size for rohu is 9-11 mm. Mini portable reader is compact and battery operated or can be operated directly from any AC current. The PIT tag implanter consists of hard aluminums material with 12-gauge needle attached to a spring. The PIT tag reader contains two coils, an excitor coil through which current is driven to create an electromagnetic field that energized the tag within read range. The transponder sends the ID code back to the receiving coil via signal modulation. The reader then amplifies the ID code and converts it to the digital form, decode it and display it on the display mirror either as numeric or alphanumeric number. The tag can be easily read through soft and hard tissue, liquid, glass, plastic and with difficulties through metals. Marking
with PIT tag is a permanent marking. It will be retained inside the fish body cavity as long as desired. It can be reused but before recovering the tags, the specimen has to be sacrificed.

In selective breeding of rohu *Labeo rohita* at Central Institute of Freshwater Aquaculture (CIFA), different indigenous tags were tried, but all proved to be not suitable for rohu. Rohu being one of the most actively swimming fish, retention of external tags was not practicable and very often, secondary infection appeared. Considering all these factors, an internal tagging system with Passive Integrated Transponder (PIT) tags is being used and found to be most suitable and effective (Mahapatra et al., 1998; Mahapatra et al., 2001; J.N.Saha, 2011)

The US Dept. of Agriculture (USDA) and US Food & Drug Administration (FDA) have only approved the use of PIT tags in fish with a condition that the portion of the animal containing the implanted device would not be used for human food. So, they recommend using the body cavity location for implantation.

### 7.3.1. Anesthesia and its concentration

Anesthesia fish is one of the vital point for tagging / marking of fishes. For tagging of rohu fingerlings, Tricaine Methanesulfonate (MS-222) is used for anesthetizing rohu. Tricaine Methanesulfonate (MS-222), is the most effective chemical for anesthetizing fish. The dose depends upon the size of the fish. During the tagging operation, each fish/fingerling has been anaesthetized individually with a 0.3% MS 222 solution. Doses may slightly vary depending on the size of the fish. Once the fingerling is unconscious it is taken out from the solution for tagging. Time of anesthesia should be minimum so that the recovery could be at faster rate after implanting of tag in the body cavity. For mass tagging anesthesia time should be minimized (Summerfelt et al., 1990).

The FDA (US Food & Drug Administration) has approved Tricaine Methane sulfonate (MS-222) as an anesthetic chemical for fishes and other cold-blooded animals. The recommended concentration of MS to anesthetize salmonids is about 40 mg/l (Schoettger, R.A. and A. M. Julin. 1967). The required concentration will vary depending on water temperature, species of fish, and stress level of the fish. As water temperature increases, so does fish metabolism, which means the drug is absorbed at a faster rate. So, at warmer water temperatures fish require less MS. MS can slow movement of the opercula enough to affect the flow of water over the gills, thereby affecting the oxygen exchange rate between water and blood, causing a state of asphyxia. But it can cause permanent damage or death due to strong concentration / to leave the fish long time in the anesthetic bath (Wedemeyer, G. 1970). So, it is always recommended to use a light dose of anesthetic chemical at the beginning and test a few fish at first to determine the correct concentration.

Baras et al., (1999) used 2- phenoxy-ethanol as an anesthetic chemical at a concentration of 0.4 mL/L for tagging juvenile Nile tilapia *Oreochromis niloticus* (1.9–13.7 g) with passive integrated transponder (PIT) tags.
7.3.2. Effect of PIT tags on growth and physiology

A series of experiments has been carried out at the Central Institute of Freshwater Aquaculture (CIFA) under the Indo-Norwegian project on ‘Selective breeding of rohu’ to determine the efficacy of passive integrated transponder (PIT) tags for marking of rohu *Labeo rohita* (Ham.). Six groups of rohu fingerlings with weight ranging from 2 to 20 g were tagged with PIT tags to determine a suitable size range for tagging. The experiments showed that 8 to 15 g rohu fingerlings were found to be quite suitable for tagging with PIT tag. Later experiment shows that 6 to 10 g is also suitable for PIT tag. Rejection of PIT by rohu was observed very less 0.05% (Harvey et al., 1989; Mahapatra et al., 2001).

Baras, E and others (2000; Greenstreet et al., 1989; Moore et al., 1990; Brannas et al., 1994; Peterson et al., 1994) conducted an experiment on the effect of PIT tags on growth and physiology of age-0 cultured Eurasian perch *Perca fluviatilis* of variable size (Lucas, M.C. 1989; Martinelli et al., 1998). It has been showed that the minimum size ranging from 1.67 to 10.62 g (55–96 mm) was suitable for PIT tagging in juvenile perch. The survival, gonadal development, and capacity of tagged perch to store abdominal fat was not affected by the tagging or tag presence, or tag to body weight ratio. After four months of tagging, no internal damage had been observed due to tagging or had been expelled, despite about 95% of them becoming encapsulated by host tissues. Negative effects from tagging were restricted to slower healing rates, and depressed growth of fish with high tag to body weight ratios during the first post-tagging days and the growth was compensated within 2 weeks. Small fishes took more time to heal their incision as well as covering of incision than larger. Rejection of PIT was not observed during the 126 days experiment with perch. X-ray photographs showed that most of the tags were located near the abdominal musculature not higher than mid gut. Tags showed no vertical migration in the body cavity but moved slightly anterior as fish grew. Some tags were found close to the anterior or posterior edge of the body cavity. But position of tags on average in the body cavity of rohu was observed in more posterior side than anterior.

The survival of Nile tilapia tagged with injectors was low (10–50% at 10 d) and surgically implanted fish had much higher survival rates (78–100% at 10 d). It is because of difficulty of controlling the penetration of the syringe into the intraperitoneal cavity following the piercing of the body wall. Suturing reduced the risks of tag expulsion and protrusion of the viscera. These results demonstrate that surgery is suitable for PIT tagging small juvenile Nile tilapia, 1.9–13.7 g (Baras et al., 1999).

7.3.3. Protocol for PIT tagging

Before the implantation of tag, fingerlings were collected and kept overnight in well aerated water without giving any feed so that the digestive tract will be clear and thus creating more space in the body cavity of the fish to implant the tag without any injury to the visceral mass.
During the tagging operation, each fish/fingerling to be anaesthetized individually with a 0.3% MS 222 solution. Doses may slightly vary depending on the size of the fish.

Once the fingerling is unconscious it is taken out from the solution for tagging.

Pre-tagging data pertaining to length and weight are recorded before tagging.

The tag number is noted by using the tag reader.

The tag is implanted in the abdominal cavity of the fingerling intra-peritoneal through a 12-gauge needle attached to a spring-loaded syringe.

Only the tip of the needle should be inserted into the peritoneal cavity.

After tagging, the fingerlings are kept in well-aerated water. Additional aeration also can be given through an aerator.

After complete recovery, the fishes are dipped in 2ppm potassium permanganate solution for ½ to 1 minute and release in a tank with well-aerated water.

The tagged fishes are kept overnight under observation for any possible mortality.

After 24 hours observation, fingerlings are stocked in the respective grow out ponds.

Now very small 4-5 g fishes can be tagged. One slit may be cut carefully on the abdomen through sharp blade and tag can be pushed through the hole by finger.

Experimental results have shown that carp fingerlings weighing 10-15g are quite suitable for tagging with PIT tags.

7.7. Conclusion

The PIT tag is an effective, safe and secure device for marking carps though it is costly but it is most suitable tag available at present for mass tagging in fish. The PIT tag can be reused any number of times if it is not broken or lost. A higher survival rate of the tagged fish can be achieved under field conditions through effective management practices, so that the recovery of PIT tag will be higher. Thus, for selective breeding studies, the PIT tag appears to be the most effective and flawless.

References


8. Impact of improved carp strain in India

Nagesh Kumar Barik and Kanta Das Mahapatra


8.1. Introduction

Southeast Asia has been a region of high growth for freshwater aquaculture in the recent times. Over a period of time, aquaculture as a tool for agricultural growth, nutritional security and rural development is finding its place. The fish farming has become the fastest growing food producing sub-sector in the world and today around 90% of aquaculture production takes place in developing countries (FAO 2004). Positive impact from aquaculture production can arise at different levels viz., i) higher productivity, income and better livelihoods at the producer level, ii) increase supply of fish and a reduction in prices at the consumer level, and iii) increase in trade and export of fish as well as employment generation that benefit overall development (Ahmed and Lorica 2002, Dey et al. 2006). India being the largest country in Southeast Asia, a large part of the development of aquaculture in the region depend on it. The country has seen a long term growth rate of 5.6 % since 1951 and since late seventies the growth has been faster and steadier. From the onset of the green revolution, the agriculture sector grew at about 2.36 % (Bhalla & Singh, 2009), meat production increased by about 3.5% but the aquaculture growth was remarkable at about 5.6 % per annum. During this period Aquaculture production in India increased from 0.2 MT in 1973 to 5.07 MT in 2010-11 (DAHDF 2012). Such high growth rate was primarily driven by the technology development as aquaculture has been transformed from traditional ‘trap and hold’ system to commercial intensive feed based aquaculture.

Amidst, these euphoria, there are hidden dangers and issues that has emerged as impediments to the continual growth of the aquaculture sector in India. As the sector matures into an organized one with heavy dependence on external inputs, the vulnerability of the sector to the changing context of production i.e. environment, market, trade, price etc has increased. The issue of reduction of profitability has been increasingly being heard from the commercial farmers. Similarly the need for improvement of quality of seed, new species, improved fishes, quality feed are entering into the policy discourse of the country. Farmers are looking more towards the options for increasing efficiency
and reducing cost of the fish production. The aquaculture sector needs to look beyond growth and shift focus towards quality of inputs and outputs for higher profit of farmers and better quality of fish to consumers.

In this context, the role and impact of improved strain of carp has been discussed in this paper. The paper is primarily based on the research and development of improved rohu in India. Indicators and information were derived from the research work carried during 2005-2008 under the collaborative project on the WorldFish Centre and ICAR.

8.2. Structure of aquaculture production India

Presently, India produces 8.3 million tones (MT) of fish in 2010-11 out of which 5.0 MT is from inland fisheries. Freshwater aquaculture contributes about 80 percent of it. The fisheries sector contributed Rs. 67.91 billion to the GDP (at current prices) during 2009-2010, which is 0.96 percent of the total GDP at factor cost and 5.41 percent of the GDP at factor cost from agriculture, forestry and fishing. The share of fisheries sector in the total GDP at factor cost in current prices increased from 0.40 percent in 1950-51 to 0.96 percent in 2009-10, recording an increase of 140 percent. The demonstrated high growth encouraged planners to invest more in the sector. At present a financial resources available to the fisheries sector is quite large. The net allocation in the 11th plan (2007-2012) was Rs 277 billion out of which about Rs 155 billion was given to NFDB and Rs 35 billion on the development of the inland fisheries and aquaculture which was significantly higher than that of 10th plan (2002-2007). The sector is expected to continue in the high growth path to support overall growth of agriculture sector.

The aquaculture development in India is primarily driven by the technological progress. In a short span of time the aquaculture system has been transformed from traditional system to commercial aquaculture. The research institutes under Indian council of agriculture research, state and central agriculture universities are involved in the research on aquaculture. The Central Institute of Freshwater Aquaculture (CIFA) has been centre for the research and development of the freshwater aquaculture in India. Many national wide projects were launched to take the research to the farmers ponds. Initiation and remarkable success of the All India Coordinated Research Project on Composite Fish Culture and Fish Seed Production in 1971 had been the turning point in the annals of freshwater fish culture in India (Jhingran 1975). The successful results of which not only instilled the confidence in the State and Central governments but in the farmers also (Sinha 1975). A number of development programs followed; the most notable are the creation of the Fish Farmers Development Agencies (FFDA), and the World Bank Fish Seed Hatchery Project. The FFDA at the district level was initiated in 1973 to provide administrative and infrastructural support, training to beneficiary, mobilization of inputs and extension support to fish farmers and also for arranging institutional finance though bank credits. At present 429 FFDA are operating in India. Since inception of the scheme till 2010-11, about 8.71 lakh hectare has been brought
under fish culture and the scheme has benefitted about 14.17 beneficiaries (DAHDF, 2012) and raised the productivity to about 2.9 t/ha among adopted farmers. In last plan period National Fisheries Development Board (NFDB) was established to develop the fisheries sector of the country which is a major source of finance and support for aquaculture development in India.

The development of aquaculture has not been uniform across the country. Most of the development has occurred in the eastern part of the country. Out of 30 states of the country 6 states contributes 73 % of inland fish production and two states i.e West Bengal and Andhra Pradesh produce almost half of the fish in the country. There is a large difference among the states in terms of productivity (Table 8.1). The states like Andhra Pradesh and Punjab achieved high productivity of more than 7 t/ha where as it is not unusual to find a productivity of less than 1 t/ha among large number of farmers around the country. The average productivity of 2.9 t/ha reported by the adopted farmers of FFDA does not reflect the actual productivity as non supported farmers are achieved much lower productivity.

The Indian aquaculture is primarily based on the Indian Major Carps (IMC) but in the recent times the new introduced species like Pangus has taken a major place in the aquaculture productions. In addition a large number of other carps and catfishes are cultivated but in small quantities across the country.

Table 8.1. Contributions of selected states to inland fisheries in India

<table>
<thead>
<tr>
<th>Selected states</th>
<th>percentage of total inland fish production</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Bengal</td>
<td>30.1</td>
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<tr>
<td>Andhra Pradesh</td>
<td>17.9</td>
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<tr>
<td>Uttar Pradesh</td>
<td>7.7</td>
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<tr>
<td>Bihar</td>
<td>7.6</td>
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<tr>
<td>Odisha</td>
<td>5.2</td>
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<tr>
<td>Assam</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>73.0</strong></td>
</tr>
</tbody>
</table>

8.3. Structure of seed production system in India

The aquaculture starts with the availability of the seed for culture. As per fish seed committee, India produced 20.45 crores seeds in 1964-65 which increased to 75.2 crore in 1980-81. (IIM, 1985) to 40,000 million fry at present (2010-11). Many programmes like National fish seed programme, World Bank programme, Fish farmers Development Agencies (FFDA) programmes are being initiated to produce sufficient seed for the aquaculture. As more and more of the water bodies are brought under aquaculture, the demand for the fish seed was growing in an exponential rate. In every state the efforts are being made to establish the seed production system to produce seed. In the
initial stage, the seed production has been under the public sector. The centrally sponsored schemes like National Fish Seed Programme (25 seed farms with 250 ha), Worldbank Programme (27 farms with 435 ha), FFDA programme (45 seed farms with 225 ha) were being launched to develop fish seed capacity in the country. Hence by 1980 about 900 ha of seed farms are being developed in the country with a total rearing capacity of 941 million seeds. Since then large capacities in the seed production has been created in the country to meet the increasing demand for seed. The curve below shows the rate of expansion of the fish seed sector in the country. It has an average growth rate of 5.8 % in last 35 years (Fig. 8.1).

![long term seed production in Mfry](image)

Fig. 8.1. Seed production trend (in million fry)

The seed production is primarily concentrated in one state as about 56% of the seeds are being produced by the state of West Bengal. About 82% of fish seed is being produced by five states i.e West Bengal, Andhra Pradesh, Assam, Odisha, Bihar and Uttar Pradesh. These states are concentrated in the eastern part of the country (Table 8.2). Therefore, only few states and few hatcheries within the states are contributing significantly for the fish seed. Such practices has an inherent vulnerability of spread of poor quality seed across the country. Therefore, the seed production needs to be much broad based and decentralized so that a genetically diverse seeds are available across the country.

<table>
<thead>
<tr>
<th>States</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Bengal</td>
<td>56.2</td>
</tr>
<tr>
<td>AP</td>
<td>3.6</td>
</tr>
<tr>
<td>Assam</td>
<td>13.3</td>
</tr>
<tr>
<td>Odisha</td>
<td>2.7</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>4.9</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>2.1</td>
</tr>
</tbody>
</table>
The comparatively fish and fish seed follows similar trend as the long term growth rates are 5.9 and 5.6 %, respectively (Fig. 8.2). These growth rates indicate ability of the seed sector to respond to the growing need of the fish production. Moreover, there are hidden capacities within the seed sector to increase the seed production in the short run whereas in the long run more of the seeds farms are being created across the country.

![Fig. 8.2. Comparative growth of inland fish and seed in India](image)

8.4. Demand-supply scenario for fish in India

India is having a population of 1.2 billion at present with the per capita income of Rs 53.3 thousand (Approx. 1000 US$) in 2010-11. There is an ever increasing demand for fish in the country and the growth in the demand is estimated to be 4% per annum (NCAP 2008). It has also been predicted that there will be reduction in the capture fisheries production of the country and therefore the whole of the additional demands needs to be met through the aquaculture development of the country. To match to the growing demand of the fish, the fish production has to be doubled within a span of 7 to 8 years. The projected annual growth of the freshwater fish production for the period of 2005-2020 is 3.99 percent which is among the highest in the Asian countries (Dey et al 2004). The projected per capita increase in the consumption is 0.97 for the same period indicating that there will be a secular increase of about 1% in the real consumption of the fish among the Indian population. The higher demand stimulates faster technical progress as it creates opportunity through market and price. In India this is a realistic scenario for aquaculture. Globally, aquaculture is recognized as the primary source of growth in the fisheries sector (Delgado et al, 2003). The study found that the income elasticities of fish demand were positive and high and it is higher for the low income group. Therefore, a high fish demand is expected with higher economic growth and shifts in the dietary pattern. But, the lower income group demand more fish and demand will be higher for the low value fish like carps (Kumar 2005). Therefore, an increase in
demand is anticipated for the fish and most of these demands are to be met through the freshwater aquaculture. Aquaculture needs to explore the strategies of horizontal, vertical as well as qualitative expansion in coming times.

8.5. Importance of improved strain in India

The poor quality seed is an emerging issue for Indian aquaculture. The deterioration of the genetic quality is most important reason for it. The inbreeding depression is estimated to be as high as 2 to 17 % among the hatchery operators in India (Eknath and Doyle, 1990). The quality seed has been an issue discussed in many forums and issue has been raised more prominently by the advanced and experienced farmers. They have observed that the fish seeds available from the market are poor leading to high mortality, lack of growth, uninform growth etc. Unfortunately, there are no systematic studies on the level of quality deterioration over a period time but, the quality seed has been emerged as an issue affecting aquaculture in India. The genetic improvement of fish is a basic strategy to improve the genetic quality of fish which has been proven successful elsewhere across the world (Gjedrem, 2012).

8.6. Performance of genetic improvement programmes across world

Across the globe few successful cases of genetic improvement programmes are leading examples of the feasibility of the producing improved strain of fishes. A significant productivity increases have been achieved for a few commercial species such as salmon, trout, and tilapia. Developed countries have particularly benefited from selective breeding. At the industry level, breeding has increased the productivity of the Norwegian salmon industry by more than 60 percent and reduced the average cost of production of Atlantic salmon by 65 percent during 1985–95 (Aerni, 2001). Genetic improvement of tropical finfish is a recent phenomenon, which began with the application of selective breeding technology to Nile tilapia (O. niloticus) at WorldFish in the 1990s (Dey et al., 2000). Similar technology is currently being applied to a range of Asian carps under collaborative agreements between WorldFish and countries in the region. These efforts have resulted in a total of 85 percent growth increases over six generations for O. niloticus, and an average value of around 10 percent growth per generation for three carp species in Asia (ICLARM, 2001). The selection response per generation was observed to be 10-13% for rainbow trout, 14% for Atlantic salmon, 15% in Tilapia and 12-18% in channel catfish (Gjedrem, 2005). The net gain was around 100 % in salmon, tilapia was reported due to genetic improvement programmes (Gjedrem, 2012).

8.7. Development and dissemination of improved rohu in India

The carp genetic research has been initiated in India by Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar of Indian Council of Agricultural Research (ICAR), New Delhi in collaboration with Norwegian Institute of Aquaculture Research (AKVAFORSK). The programme for the genetic improvement of rohu, particularly for better growth performance through selective breeding has been under operation at
CIFA since 1992 (CIFA, 2003). Since then 9 generations of the selection has been performed and improved rohu disseminated among wide range of farmers across the country. Over the period of development, the average response to selection per generation in final body weight was 17 per cent. (Mahapatra, K. D et al, 2007). The improved rohu, popularly named as “Jayanti”, as it was named in the year 1997 i.e. 50 years of Indian independence (Swarna Jayanti), has been released to several hatchery owners so that they can provide better quality seed to the fish farmers. In the subsequent years, the updated generations of the improved strains were released so as to replace the earlier generations.

Field-testing was done in different agro-climatic regions of India during the years 2000-2003. At Vijayawada (Andhra Pradesh) and Jallandhar (Punjab) in 2001, at Kausalyaganga State fishery department (Orissa) in 2000 and Rahara (West Bengal) in 2001 and 2002. In all the field testing centres, improved Rohu showed significantly higher growth efficiency compared to local hatchery stocks and control group. Field trial was also made in the farmers ponds of Orissa. In all the cases significantly higher growth of improved rohu was obtained over the normal / local rohu.

Initially, the seeds were directly given to the farmers but it is not feasible to supply seed across the country. Therefore, a three tier dissemination model was adopted involving Nucleus (CIFA), Multiplier units (recognized hatchery) and fish farmers. Now also a small quantities of seed given directly to the farmers from CIFA but mass scale seed production was expected from multiplier units. The state government farm of Kausalyaganga, Regional Center of CIFA and a private hatchery in Andhra Pradesh was developed as multiplier units to supply seed. The private hatchery has a production capacity to the tune of 2.07 billion spawn with a seasonal seed production capacity of over 828 million fry and 662 million fingerlings. Along with the supply of brood fish of improved Jayanti rohu, the multiplier units have been provided with procedures for rearing brood fish in respect of feeding type, quantity, feeding schedule and other management procedures. Culture procedures for grow-out farmers also have been provided through multiplier units. CIFA undertakes regular inspection to the multiplier units to ensure implementation of the guidelines in production of seed. The direct supply of seed has been made to the farmer of Uttar Pradesh, Maharashtra, Bihar, Punjab, Assam and the performance of these stocks was found to be very encouraging. Now efforts are being made to establish at least one multiplier unit in each state so that the awareness and exposure to the improved stain of the farmers increases. In the later stage the multiplier units will be increased to meet the demand in each state. Attempts are being made to create the multiplier units in government, private and NGO sector to provide a broad based institutional support to the dissemination of the improved strain in India.

8.8. Impact assessment of improved stain

A survey on the adopted and non adopted large farmers of Andhra Pradesh was conducted during 2006-07. The adopted farmers were used 2nd and 3rd generation of improved stock. The consumer surplus model was used to assess the economic benefits
of from the improved strain. The Fish Sector Model developed by WorldFish Centre was used to make projections. The assessment can be considered as ex-ante as actual adoption of the technology is at the preliminary stage.

8.8.1. Impact of improved strain

The impact of the improved strain was found to be positive primarily due to the efficiency in the feed conversion and faster growth. The Jayanti was able to attain the marketable size at 59 days lesser than the normal rohu. Such reduction has helped reduce cost of operation and more importantly the farmers were able to escape the culture in the water stress period. The production function model of both average and efficient farmers has given similar results as both categories of farmers were able to gain about 15% after separation of other effect in the model. In the production models separated the effects of improved strain from other inputs like feed, fertilization. The 15% of the gain was only due to improved strain using 2nd and 3rd generation of Jayanti rohu. Among the large farmers of Andhra Pradesh, the increase in the profits was found to be 50.0 %. Due to faster growth and efficient production has improved productivity in relation to in terms of time and space. At the household level, all consumers and carp farmers are expected to benefit from the adoption of improved carp strain. A typical farmer with an average of 5.8 ha polyculture pond, was gained Rs 53707/ year in 2006-07. The total economic gain estimated to be around 2.3 crores in same period.

There has been clear indication of the increase in the food efficiency as the feed conversion ratio has declined in the new strain which has been converted into the economic terms by reduction in the cost and so, increase in the profitability of the farmers. The profitability is most important criteria for the farmers to adopt the new strain. For the large commercial farming like that of Andhra Pradesh, there is a significant reduction in the period of growing so as to attain the preferred harvesting size. This reduction in time period has helped farmers to plan their crop in more efficient way and to avoid the water scarcity period of the year.

Carp being the low price and high volume product, it is consumed widely by the relatively poor people because the price is relatively low. Thus, most of the consumers’ benefit will go to the relatively poorer consumers. Moreover, the carp production system in India is primarily based on rohu, and widely adopted by low resource base small and marginal pond owners of the country. Therefore, the gain in the biological and economic efficiency is likely to benefit the poor consumers as well as small fish farming operator of the country.

8.9. Conclusion and policy implications

The genetic improvement programme is feasible and has the potential to generate wider socio-economic benefits in the country over a longer period. The programme is also economically a profitable enterprise and comparable with any other commercial investment. The investment of on the research is both socially desirable and economically profitable and hence required to be further invested to gain more in future. But, the
return to investment is conditional to the level of the dissemination and adoption in the farmers field, therefore, the investment in the extension and dissemination must go hand in hand with the research. The investment in this field is complementary to the investment made for the other activities of the aquaculture sector of the country. Therefore, the genetic research is required to be further encouraged in the country for furthering benefits of the aquaculture sector in the country.

The relevance of the development and dissemination of the improved strain increases in the context of a sudden raise of the cost of the fish feed in the country. In last two years, the cost of the feed ingredients increased by about 25 to 30% which add the cost of production by about 10 to 20 % depending on the level of dependence on feed. The fish production can be sustained economically by adopting more feed efficient fish like “Jayanti rohu” to reduce cost of production. It is particularly important for the efficient farmers who have exhausted all source of increasing efficiency in aquaculture. The only way out for them is to adopt the feed efficient species or strain. The reduction in the inputs level has implication on the economic and environmental sustainability the benefits of which is shared by the producers and consumers equally.

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9. Gamete quality in brood fish management

D. K. Verma and P. Routray


9.1. Introduction

The quality of male and female gametes is one of the limiting factors of the successful reproduction. The use of quality gametes from fish broodstock is most important for ensuring the production of viable larvae for aquaculture (Bromage and Roberts, 1994). Fish egg quality is defined as the ability of the egg to be fertilized and subsequently develop into normal embryo. Similarly, sperm quality is defined as its ability to successfully fertilize an egg and subsequently allow the development of a normal embryo. In aquaculture condition, the quality of fish gametes can be highly variable and influenced by a number of external factors including broodstock management practices. For these reasons, the gamete quality is most important aspects in brood fish management.

9.2. Fish semen or milt

Semen or milt of fish consists of seminal plasma (or seminal fluid) and spermatozoa. Seminal fluid is a secretary product of the testes and of the spermatic duct (Lahnsteiner et al., 1994). Seminal plasma creates optimal condition for storage of sperm in the

Table 9.1. Semen characteristics of six carp species

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mrigal</th>
<th>Catla</th>
<th>Rohu</th>
<th>Kalbasu</th>
<th>Silver carp</th>
<th>Grass carp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milt yield (ml/kg)</td>
<td>10.04±1.88 a</td>
<td>7.50±0.50 b</td>
<td>8.34±4.43 d</td>
<td>7.98±1.20 a</td>
<td>6.94±0.62 b</td>
<td>7.52±0.89 b</td>
</tr>
<tr>
<td>Spermatoctrit (%)</td>
<td>83.40±2.19 b</td>
<td>72.0±5.61 b</td>
<td>80.0±6.28 b</td>
<td>79.8±5.11 b</td>
<td>69.0±4.06 b</td>
<td>73.0±3.16 b</td>
</tr>
<tr>
<td>Sperm count (X 10⁹)/ml</td>
<td>33.52±2.73 b</td>
<td>28.02±4.55 b</td>
<td>27.2±3.07 b</td>
<td>35.22±2.36 b</td>
<td>26.76±1.84 b</td>
<td>29.6±2.60 b</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>92.2±2.66 b</td>
<td>90.0±3.80 b</td>
<td>89.8±4.14 b</td>
<td>88.0±6.32 b</td>
<td>91.8±2.89 b</td>
<td>89.0±4.47 b</td>
</tr>
<tr>
<td>Motility duration (Seconds)</td>
<td>110.0±5.83 a</td>
<td>80.0±5.47 b</td>
<td>90.0±8.60 b</td>
<td>100.±8.24 b</td>
<td>75.40±5.36 b</td>
<td>85.0±4.12 c</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg⁻¹)</td>
<td>284.0±8.60 b</td>
<td>278.0±5.83 b</td>
<td>269.0±4.47 d</td>
<td>289.0±5.0 a</td>
<td>276.0±4.06 c</td>
<td>269.0±4.06 c</td>
</tr>
<tr>
<td>pH</td>
<td>7.9 ± 0.1 b</td>
<td>7.80 ± 0.10 b</td>
<td>7.30 ± 0.07 c</td>
<td>8.14 ± 0.11 b</td>
<td>7.80 ± 0.15 b</td>
<td>7.9±0.07 p</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. (n=9). Values having different letters differ significantly in a row (P<0.05). (Source, Verma, et al., 2009)
reproductive system, where spermatozoa are kept in immotile state, their functional properties (motility and genomic packaging) are protected and metabolism is supported (Cosson et al., 1997). Numerous components of seminal plasma are involved in these functions. The milt quality parameter of carps has been reported earlier and the ranges for each parameter are shown in Table. 9.1.

9.3. Structure of mature fish spermatozoa (teleost)

Teleost spermatozoon is a relatively simple cell composed of a spherical head devoid of an acrosome, a short midpiece and an elongated tail. The head is large and contains a prominent nucleus surrounded by a thin layer of cytoplasm. The size of head varies in different males. The detailed study of ultrastructure of six carps (catla, *Catlacatla*; rohu, *Labeorohita*; mrigal, *Cirrhinusmrigala*, kalbasu, *Labeocalbasu*; grass carp, *Ctenopharyngodonidella* and silver carp, *Hypophthalmichthysmolitrix*) has been reported by Verma et al., (2009). The mean length of carp spermatozoa was in the range of 15±2.6 μm to 24±2.4 μm. Catla spermatozoa length was the lowest among the group (15 μm) and maximum 24 μm was observed in silver carp and rohu spermatozoa. The spermatozoa morphology as revealed by SEM is shown in Figure 9.1.

![Figure 9.1: Scanning electron micrograph showing the morphological structures of six carp species.](image)

The spermatozoa of these carps are uniflagellated and have circular to elliptical nucleus. A nucleus with electron dense granular chromatin occupied the head nuclear region. The centriolar apparatus was asymmetrically attached to the nucleus. The mid-piece was cylindrical, rich in cytoplasmic material, containing spherical mitochondria arranged around a post nuclear canal (Figure 9.2). The mid piece is joined with the posterior portion of the head, consists of a mitochondrial ring and centrioles (Figure 9.2). An axoneme with the typical pattern of two central microtubules surrounded by a ring of nine doublets originated from basal body of the distal centriole and pervaded the mid-piece. This pattern was found in all the six carps.

9.4. Sperm Quality

Quality of sperm is a measure of the ability of sperm to successfully fertilize an egg which such ability mostly depends on qualitative parameters of milts i.e. composition of seminal fluid, milt volume, sperm density and sperm motility (Rurangwa et al., 2004). Optimum sperm quality is important for effective broodstock management and is a criterion for the selection of male broodstock. Sperm is often inadequate both in terms of quantity and quality and doesn’t always give successful fertilization in the artificial insemination procedure commonly used for aquaculture species. The milt characteristics, which influence the fertilization success, have been found large individual variation in different parameter investigated. Therefore there has been limitation in the use of single sperm characteristics to define good sperm quality. Fish seminal fluid has a unique composition regarding the presence of the organic and inorganic components which support the viability of spermatozoa (Hajirezaee et al., 2010). Sperm motility and sperm density determine the fertilization capability of
spermatozoa and often are used to estimate milt quality (Krol et al., 2006) because of chemical properties of seminal fluid, fish spermatozoa are immotile in seminal fluid (Billard, 1986).

9.5. Sperm motility

Sperm motility has been reported as the simplest and most reliable biomarker of semen quality. This criterion is commonly used in the selection of milt, both for immediate insemination and for preservation by freezing. Sperm are usually immotile in the genital tract and are activated after dilution in the external medium. The quality of fresh semen is evaluated often based on an estimate of the percentage of motile spermatozoa after dilution of the sample. This is true for most of the external spawners as there is generally a positive relationship between motility and fertility, and motility is often used as an indicator of fertility in artificial insemination because it is easy to assess microscopically. The spermatozoa motility and its duration have great influence on successful fertilization. Spermatozoa acquire the motility during the transition from the testis to sperm duct. Their values were high in good quality sperm (Tekin et al., 2003).

9.6. Sperm density

Milt yield (ml/kg) from a milter is a measure of spermatozoa concentration, which determines the quantity of milt from a number of males required to fertilize a given number of eggs from a female fish. Hence, greater the volume of milt required, poorer the milt quality. Milt yield varies with collection frequency, season, age of milter, size and condition of milter, maintenance circumstances of milter, species (Buyukhatipoglu and Holtz, 1984; Piironen, 1985) and the inducing agents (Lin et al., 1996). Spermatozoa concentration may also influence the rate of fertilization (Aas et al., 1991). For this reason, determination of the spermatozoa concentration is important in fertilization studies.

9.7. Fertilizing assay

Fertilization assays evaluate the fertilizing capacity of spermatozoa and are therefore the most reliable quality markers. They directly reflect sperm fertilization capacity. It is currently used in most studies on artificial insemination and sperm preservation. The percentage fertilization, which usually refers to the percentage of eyed eggs or hatched larvae, is also critical, but evaluating the percentage of developing larvae to first feeding may be a better index of gamete quality.

9.8. Biochemical parameters as semen quality marker

Measurement of component with the sperm or seminal plasma also has been used to predict sperm quality. Lahnsteiner et al., (1996) listed an array of seminal plasma and sperm cell constituents (potential metabolic substrates, metabolic enzymes) used in their studies with rainbow trout and concluded that seminal plasma osmolality, pH and triglycerides were predictive of sperm quality. The detailed biochemical parameters
of seminal plasma of six carps i.e., catla, rohu, mrigal, kalbasu, grass carp and silver carp has been reported (Verma et al., 2010)

### 9.9. Factors affecting the quality of male gametes in fish

There are many other factors reported to be contributing to individual variation in sperm quality (Rurangwa et al., 2004); some of them are genetic variability among fish, rearing conditions, brood stress, and sperm collection methods, storage of milt and sperm activation conditions. The knowledge of the factors that affect sperm quality could be useful for adjustment and efficient management of artificial propagation.

#### 9.9.1. Biological characteristics of brood fish

The age of broodstock plays a significant influence on the sperm quality (Vuthiphandchai and Zohar, 1999). The 5+ years of age group of broodstock should be discarded in broodstock management. Gupta et al., (2006). In captive reared striped Bass (*Morones axatilis*), 3-year-old fish had higher sperm quality than the 1-or 12-year-old fish, based on higher sperm production and increased sperm longevity during short-term storage. (Gjerde, 1984) reported positive correlations between volume of milt and body size (weight and length) in Atlantic salmon (*Salmo salar*) and rainbow trout.

#### 9.9.2. Effect of rearing conditions on sperm quality

In aquaculture, various rearing condition plays a role in the gonadal development (Nash, 1999). However, the data about the role of photoperiod and temperature on quality of fish milt especially commercial species are rare. Sarkar et al., (2010) reported photoperiod-dependent changes of milt parameters in the Indian Major carps (*Catla catla, Labeo rohita*) exposed to different light periods (LP–AAT, SNP–AT and NP–AT)

#### Table 9.2. Milt characteristics in rohu under different photothermal regimes.

<table>
<thead>
<tr>
<th>Milt characteristics</th>
<th>Photoperiod regime (light : dark hours; 14L : 10 D) in combination with above ambient temperature at 28.6 ± 1.2°C. (LP–AAT, )</th>
<th>Natural pond condition (NP–AT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milt yield ml kg⁻¹</td>
<td>6</td>
<td>Nil</td>
</tr>
<tr>
<td>Spermatoцит value (%)</td>
<td>78</td>
<td>-</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>86</td>
<td>-</td>
</tr>
<tr>
<td>Sperm count no. X 10⁷ cu mm.</td>
<td>2.12</td>
<td>-</td>
</tr>
<tr>
<td>Fertilization (%)</td>
<td>85</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 9.2. In Indian major carp, *C. mrigala*, the quality of sperm varies across the spawning season (Verma et al., 2009) reported. In gold fish (*Carassius auratus*) (Iigo and Aida, 1995) and wolfish (*Anarhichas minor*) (Pavlov et al., 1997) photoperiod manipulation had no effect on sperm production. A few studies have shown that temperature can affect the milt parameters. It was observed that the sperm motility (percentage and duration) decrease coincident with the decline of water temperature during spawning season (Hajirezaee et al. 2010).

The gamete quality and seed production is mainly dependent on the broodstock nutrition and feeding. Labbe et al. (1995) reported diet with fish oil significantly increased milt volume, sperm concentration, survival rate of embryos and larvae in Sea bass (*Dicentrarchus labrax*) compared to those fed on a non-enriched diet. In another study, enrichment of diets with polyunsaturated fatty acid (PUFAs) enhanced reproductive efficiency of male sea bass (Astuarino et al., 2001). During artificial propagation, the contamination of milt with urine or fecal material has been reported poor fertilization in fishes (Linhart et al., 1999). Contamination of *Cyprinus carpio* milt with urine decreased the energetic stores of spermatozoa and motility (Perchec et al., 1998). The handling and transportation procedures used in aquaculture can be potentially stressful, and affects sperm quality. During brood husbandry practices, brood fish are exposed to various types of stress such as confinement, overcrowding, handling, biopsy, transportation and hormonally induced spawning. Stress has adverse effects on reproduction process and reduction of gamete and larval quality (Pankhurst and Van Der Kraak, 1997). In rainbow trout, repeated acute stress during reproductive development prior to spawning significantly delayed ovulation and reduced egg size, and significantly decreased sperm counts and most importantly significantly decreased survival rates for progeny from stressed fish compared to that from unstressed controls (Campbell et al., 1992).

### 9.9.3. Spawning induction

Hormonal induction of spawning is widely used in aquaculture to induce ovulation in species that do not spontaneously ovulate in captivity, or to synchronize ovulation for practical reasons in other species. It is revealed that hormone induction affect the sperm quality. In European catfish (*Silurus glanis*), both injected carp pituitary extract (CPE) and both implanted GnRHa (Gonadotropin releasing hormone analogue) increased the sperm density, although CPE had more effect (Linhart and Billard, 1994). The milt volume, sperm motility and seminal plasma pH were increased by GnRHa treatment in yellow tail flounder (*Pleuronectes ferrugineus*), (Clearwater and Crim, 1998).

### 9.9.4. Seasonal variations of milt quality

Seasonal variation of male gamete quality has been reported for carps (Billardet et al., 1995*; Linhart et al., 1995). Seasonal variation in milt yield has been recorded in some
species. Sperm quality of a milter varies with collection frequency, season, age, size and health of the brood (Wohlfarth, 1994; Lin et al., 1996). The sperm quality (motility percentage and duration), sperm count in carp, C. mrigala has been reported to be low in the beginning of season and recorded the peak during July and again started declining and reached to at the end of season (Verma et al., 2009).

9.9.5. Sperm quality evaluation (Prior to utilization)

Any quantifiable physical parameter that directly correlates with the fertilization capacity of sperm could be potentially used as a measure of sperm quality. Mere milting by pressure on the abdomen of the mature male does not speak about the efficiency of the male. The efficiency of the milter can be worked out by evaluating the quality as well as quantity of the milt produced by the individual. Milt can be evaluated by series of physical observation to the sample.

9.9.6. Milt volume

Each egg needs some thousand of spermatozoa; even though one successful sperm fertilizes the ovum. So quantity of the milt has a great deal during breeding. In captive breeding male needs hormonal induction to yield adequate quantity of free flowing milt and induced male of Indian major carp produces about 6-10 ml of milt/kg body weight within the first 6 hours of induction. A non-induced male yields on 0.5 to 1.0 ml milt/kg body weight.

9.9.7. Spermatocrit value

This is the unit to express the proportion of total solid and seminal plasma in a milt sample. The micro-haematocrit capillaries tubes (75mm length and 1.2 mm diameter) are filled with milt and one end of each tube was sealed for tube centrifugation in a microhaematocrit centrifuge at 10,000 x g (Hermle, USA) for 5 minutes and, the sediment in the capillary indicate the % of packed sperm cells in the milt sample when read in a haematocrit reader. Measurements are taken in triplicate for each sample and the average of the three measurements is used for the results immediately after semen collection to avoid abnormal reading.

9.9.8. Sperm cell count

The optimum spermatozoa population in a milt sample of Indian major carp varied 2.5 x 10^7 – 3.5 x 10^7/cumm. The sperm count become less during early pre-monsoon and post monsoon months. At the peak of the season (July) the sperm cells are at their optimum for which we get maximum fertilization rates during this time while breeding carps.

9.9.9. Sperm motility

9.9.9.1 Subjective methods

Unlike mammal, the teleostean spermatozoa are found immotile in the testis as well as in seminal fluid. Healthy carp spermatozoa on activation show a vigorous movement.
The motility of spermatozoa is based on the osmotic balance and K+ ion concentration in the milt. The motility of such activated spermatozoa is evaluated by six point (+ + + + + +) scale. This estimation is based on a subjective scale when observed under a microscope.

<table>
<thead>
<tr>
<th>Motility percentage</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>+</td>
</tr>
<tr>
<td>11-30</td>
<td>+ +</td>
</tr>
<tr>
<td>31-50</td>
<td>+ + +</td>
</tr>
<tr>
<td>51-70</td>
<td>+ + + +</td>
</tr>
<tr>
<td>71-90</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>91-100</td>
<td>+ + + + + +</td>
</tr>
</tbody>
</table>

Since all of these use arbitrary, nonlinear scales, they cannot be used for any statistical analysis. As expected such measurements have, in general, led to variable results between laboratories or observers and the value of such measurements of motility in predicting fertility is still questionable due to the subjectivity of the technique. Subjective estimation of the sperm quality is affected by various factors but in particular variation in the observers’ experience, the endpoints that are chosen and how these measures are interpreted.

### 9.9.9.2. Quantitative computer-assisted methods

Computer-aided sperm analysis (CASA) systems are the evolution of multiple photomicrography exposure and video-micrography techniques for spermatozoa track, using a computer equipped with imaging software. A CASA system refers to the physical equipment used to visualize and digitize static and dynamic sperm images, and to the methods used to process and analyze them. Computer-assisted sperm trackers comprise essentially a microscope coupled to a CCD camera which conveys a signal to a monitor, VCR recorder and computer (Boyer et al.,1989). Sperm movement is usually recorded onto videotape which is later analyzed by the Computer software. The CASA system is a useful tool to achieve reliable results to analyze sperm quality; because it takes several images of sperm samples and using an automatic process, analyzes the motility parameters that are essential to determine the quality of the studied sperm.

### 9.10. Egg quality

Egg quality may be defined as “those characteristics of the egg that determine its capacity to survive” (Bromage et al., 1994). Egg qualities includes “extrinsic” characteristics of the egg, i.e. chorion’s appearance, shape and size of the eggs, cell symmetry, egg
transparency, number and size of oil globules, and “intrinsic” characteristics as energy content and biochemical composition (genes and nutrients). The variance in these attributes might be used as quality indications. To be considered as an indicator of ‘good quality’ would imply associated with increased capacity of the egg producing a viable offspring (Nissling et al., 1998).

9.10.1. Fish eggs

In teleost fish, an egg is the final product of oocyte growth and development (Tyler and Sumpter, 1996). The all contents of an egg, genetic and nutritive determines its quality of egg. An egg needs all the necessary information to direct the development of a functional, free-swimming embryo and all the ‘building blocks’ to form the embryo; so, it needs all the amino acids, lipids and carbohydrates that make up an embryo, together with calcium, vitamins and metals for enzyme and other metabolic actions, and many other things. These specialized materials are derived from a number of maternal sources and must be incorporated during the growth of the oocyte in the ovary. If an egg does not contain a particular compound, or contains an inappropriate amount of a compound, it will not be able to sustain development of a viable embryo. Hence to understand egg quality, the role of the various constituents in eggs must be known.

9.10.2. Factor affecting egg quality

Egg quality is most important for the production of quality fish seed and economic utility of hatcheries. In the fish farming industry, good-quality eggs have been defined as those exhibiting low mortalities at fertilization, eyeing, hatching and first feeding (Bromage et al., 1992). Fish egg quality can be affected by so many factors such as maternal age and condition factor, the diet of the brood fish, the complement of nutrients deposited into the oocyte, the timing of the spawning cycle, overripening processes, genetic factors, and also by intrinsic properties of the egg itself (Kjørvik et al., 1990) and the physiochemical conditions of the water in which the eggs are subsequently incubated.

9.10.2.1. Hormonal effect on egg quality

Larval development is controlled by hormones and thus may affect egg quality. Hormones could be supplied to the egg before fertilization, in which case they must enter by maternal transfer, or they could be synthesized in situ at any time after fertilization. Studies looking at the ontogeny of the endocrine system (Tanaka et al., 1995) have so far indicated that fish larvae are physiologically immature, with little or no capacity to produce certain enzymes, growth factors and hormones, until at or around the end of yolk resorption. Thus, fish larvae appear to be dependent on exogenous
sources (mother or live food) for the supply of these regulatory factors, rather than their in situ synthesis in the egg and larvae (Lam, 1994). Maternal circulation allows hormone to pass into fish oocytes (Greenblatt et al., 1989). This store of maternal hormones fulfill the requirements of fish larvae for growth, development, osmoregulation, stress responses and other physiological functions prior to the functional development of their own endocrine glands (Lam, 1994). Thyroid hormones of maternal origin are deposited in egg yolk, and they may have significant effects on fish embryo development (Lam, 1994). Triiodothyronine is maintained in eggs in chum salmon (Oncorhynchus keta, Salmonidae) until hatching, and thereafter the amount in the egg gradually decreases, until completion of yolk absorption (Tagawa and Hirano, 1987), whereas the amount of thyroxine remains more or less constant throughout early development (Tagawa and Hirano, 1991). Several sex steroids have been reported to be present in fertilized eggs of tilapia (Rothbard et al., 1987). The functional significance of these hormones is unclear.

9.10.2.2. Effect of age and egg size of fish on egg quality

There are few reports available on the effect of age of fish on egg quality. The recent work in the rainbow trout has indicated that over their first two spawning seasons, females produce better quality eggs in the second season. Similarly, Bromage and Cumaranatunga (1988) found that the survival to eyeing of eggs from female rainbow trout ovulating for the second time (as 3-year-olds) was significantly higher compared with eggs from females spawning for the first time (as 2-year-olds; 75% versus 58% survival, respectively). In aquaculture, there is a perception that, in terms of quality, bigger eggs are better. Egg size may vary both within a species and between populations of the same species (West and Mason, 1987). Egg size in fish is also affected/modulated by the nutritional status of the female during ovarian recrudescence (Bromage et al., 1990; Tyler et al., 1994) and in asynchronous spawners, the size of eggs ovulated in the later batches is often smaller, a phenomenon associated with the diminishing resources of the female (Hsiao et al., 1994). Bromage et al., (1992) observed that egg size does not appear to be an important indicator of egg quality in cultured rainbow trout.

9.10.2.3. Environmental influences on egg quality

Environmental factors play an important role in spawning success and recruitment in the fish (Nissling et al., 1994). Environmental factors that may affect egg quality in fish include the diet of the brood fish and the physiochemical conditions of the water in which the eggs are incubated (temperature, pH of the water, etc.). In aquaculture, the photoperiod to which the brood fish have been exposed and the quality of the husbandry factors such as the level of stress to which the broodstock are exposed, the fertilization
procedures adopted, over ripening of eggs in the body cavity and bacterial colonization of fertilized eggs can all affect egg quality. In both wild and captive fisheries, exposure of maturing females, or exposure of the eggs or developing embryos to environmental pollutants may affect egg and fry survival (Matsui et al., 1992; Miller, 1993).

Broodstock nutrition is vital to producing high quality eggs and larvae, and dietary lipid and fatty acid contents and compositions are known to be important factors in determining the success of the developing embryos and larvae. Dietary components as diverse as polar and non-polar lipids (Watanabe et al., 1991a,b), fatty acids (Harel et al., 1994; Carrillo et al., 1995), protein (Harel et al., 1995) and ascorbic acid (Blom and Dabrowski, 1995) have all been shown to affect egg and embryo survival. Harel et al., (1995) reported dietary proteins and carbohydrate has been reported to appear to influence egg quality however these components have received less study than the lipids. In fish, carbohydrates are relatively poorly utilized, and the main sources of energy are protein and lipid (Walton and Cowey, 1982), in rainbow trout, broodstock fed on a diet low in carbohydrate had a reduced relative fecundity (they produced fewer eggs per kg body weight), and the eggs had a reduced survival to the eyeing stage and a reduced hatchability (Washburn et al., 1990). Successful embryonic development in fish has been shown to be dependent on the balance of amino acids present in the egg (Fyhn, 1989). Proteins act as a source of amino acids and as a reservoir of materials used during the many biosynthetic activities that are essential for the early stages of embryogenesis (Metcoff, 1986). It appears that different fish species may have different dietary requirements and that diets for broodstocks should be prepared species specific to ensure good egg quality.

9.10.2.4. Effect of photoperiod and temperature on egg quality

The environmental factors such as photoperiod, temperature, and pH of the water influences egg quality (Brown et al., 1995). Photoperiod manipulation is commonly used in aquaculture as a method for advancing or delaying spawning, to obtain a year-round supply of eggs of salmonids and other fish species. The photothermal manipulation accelerated sexual maturation in Indian major carps and induced bred during winter at least 4–5 months prior to their normal spawning season (Sarkar et al., 2010). Water temperatures during spawning and the incubation of the eggs are particularly important in affecting egg quality. Temperature may affect metabolism, activity and structure of the developing embryo (Brown et al. (1995) examined the effect of sea temperature on the spawning performance of Atlantic halibut over two spawning seasons, both in terms of egg morphology and viability of the subsequent embryos. The temperature at which the eggs are incubated can affect not only their quality, but also their growth rate and differentiation.
9.10.2.5. Effect of pollutants on egg quality

Environmental pollutants adversely affect the fish oocytes and eggs. Environmental contaminants, such as insecticides (van Leeuwen et al., 1986), organochlorinated biphenyls (Smith and Cole, 1973) and polychlorinated biphenyls (Matsui et al., 1992) has been reported Malformations and impaired development and viability. Miller, (1993) reported, accumulation of these pollutant in the oocyte/egg, when the oocytes are developing in the ovary. Gonads with high concentrations of chlorinated hydrocarbons yield eggs with lowerhatching success than less contaminated eggs (Von Westernhagenetal., 1989).

9.10.2.6. Effect of husbandry practices on egg quality

A poor husbandry practice resultspoor reproductive success of cultured fish. The husbandry practices have major effects on egg quality a (Bromage et al., 1992). During husbandry practices, over crowding of fish and stress may affect egg quality, which may lead to irregular spawning intervals, low fertilization rates and increased occurrence of abnormal embryos in Atlantic cod (Wilson et al., 1995). After fertilization, dead eggs become colonized with bacteria/fungus, and if these eggs are not removed quickly from the incubation trays, viable eggs may also be colonized. By incubating eggs in water of high quality, and removal of dead eggs at regular intervals, the egg survival (and, therefore, overall egg quality) can be enhanced considerably (Bromage et al., 1994).

9.11. Egg quality evaluation

The methods of egg quality assessment hasbeen found varying by different worker. The easiest and simplestmethod of egg quality has been reported is buoyancy of the eggs (Kjorsvik et al., 1990). Another easy and widely used assessment of egg quality reported is fertilization success. Fertilization checks are an easy way to screen dead and unfertilized eggs (Bromage, et al., 1994), several studies report a lack of correlation between fertilization rate and mortality rate in a batch of eggs, (Laine and Rajasilta, 1999), and fertilization success is not sufficient to determine egg quality. The assessment of fertilization success is easy, and both time and resource efficient. (Kjørsvik et al., 1990).

Egg size is the most common assessment of egg quality. Various worktedrepoted the egg size as female gamete quality indicator which was correleatedwith the size and viability of larvae hatching (Kamler, 1992, Knutsen and Tilseth, 1985). Other worker reported lack of relationship between egg size and ultimate egg quality, i.e. cod (Ouellet et al., 2001, Zhao et al., 2001). The bigger-is-better hypothesis may not always hold true as egg size might be related to spawning time and place, i.e. in herring (Hempel and Blaxter, 1967) and be an adaptation to local conditions, i.e. eggs from Baltic are larger (and more buoyant) than Atlantic cod eggs. (Nissling et al., 1994)
The biochemical composition of an egg is a potential way to assess egg quality. As an egg necessarily must contain all nutrients needed to ensure the embryos growth and development, the biochemical composition of an egg is a potential way to assess egg quality. The chemical composition of eggs should not be used as the sole criteria to determine egg quality (Izquierdo et al., 2001). Gene expression and RNA translation might be the key to egg quality (Brooks et al., 1997). Apart from these parameters the paternal origins and the environment it was released into also play a role in egg quality. These traits, as well as bacterial colonization (Hansen and Olafsen, 1999), over-ripening processes (Kjorsvik et al., 1990, Bromage et al., 1994) are also important to describe the gamete quality. Most of the factors affecting egg qualities are still not very clear (Brooks et al., 1997).

9.12. Conclusion

Good quality of gamete is of crucial importance for artificial insemination of eggs and storage of fish spermatozoa. The knowledge of biomarker of gamete quality in fish will be helpful to predict the suitability of eggs and milt, both for immediate insemination and for preservation by freezing. Ultrastructure of fresh and frozen spermatozoa as a biomarker can provide new strategies to improve the outcome using very recent technical innovations in the gamete preservation protocols.

References


10. Principles of short term and long term preservation of gametes for carp brood stock improvement programs

P. Routray and D.K. Verma


10.1. Introduction

Initial success in sperm cryopreservation occurred at about the same time for aquatic species and livestock. However, in the 50 plus years since then cryopreserved sperm of livestock has grown into a billion-dollar global industry, while cryopreserved sperm of aquatic species remained a research activity with little commercial application due to several reasons. Male gamete of fish is generally obtained in a specific period in some fish such as carps (Fig.10.1). Fish farmers are beginning to look more often to genetic improvement for gains in production; however, improving the genetics of fish species generally takes a longer time. It is difficult to keep track of individual males or females and thus the process of developing breeding stocks and improved lines could take a decade or more. At the same time, fish managers are looking for ways to protect endangered species. The availability of frozen sperm is a proven technique for developing, maintaining, and distributing genetic improvement in livestock, and provides great unexploited potential for fish breeding. In addition the availability of frozen sperm allows conservation programs to make a genetic bank of many males and increases the potential breeding population size to ensure that proper genetic combinations are produced in breeding of endangered species. First, cryopreservation can be used to improve existing hatchery operations by providing sperm on demand and simplifying the timing of induced spawning. Second, frozen sperm can enhance efficient use of facilities and create new opportunities in the hatchery by eliminating the need to maintain live males, potentially freeing resources for use with females and larvae. Third, valuable genetic lineages such as endangered species, research models or improved farmed strains can be protected by storage of frozen sperm. Fourth, cryopreservation opens the door for rapid genetic improvement. Frozen sperm can be used in breeding programs to create improved lines and shape the genetic resources available for aquaculture.

Finally, cryopreserved sperm of aquatic species will at some point become an entirely new industry itself. A successful industry will require integrated practices for sample collection, refrigerated storage, freezing, thawing, rules for use and disposal, transfer
agreements, and database development. In this way development of a single technology - cryopreservation - can assist two great needs: improved aquaculture and conservation of threatened and endangered fish species. Storage of male gametes and utilization is a new frontier for aquaculture research and development. In carp brood farming and seed production sector, storage of milt can facilitate some of this programmes viz. Selective breeding, hybridization and commercial seed production. Success of stored milt depends upon the quality of raw milt and standard process of preservation. To keep the selected sample alive for certain duration needs preservation. Preservation may be non-cryogenic (short-term) or cryogenic (long-term).

10.2. Short term preservation (Non-cryogenic)

One of the proven methods for best utilization of male gametes of fish during artificial insemination and controlled breeding programs is to use short duration preservation of milt for 1-2 days. The milt sample is diluted with extender and stored in a thermocol chamber with ice or in refrigerator. This is helpful for designing different sib breeding out of different male and female traits. Modified Extender C is found more easy and effective for such preservation. Milt samples are brought to room temperature before inseminating the egg sample for better result. The short term preservation method is very handy where availability of liquid nitrogen is a constraint. This method has been employed at CIFA to transport male gametes of catla from distant locations such as Andaman Nicobar Islands to Odisha by maintaining atemperature of 4 °C. The feasibility of transporting milt from one part of India to the other was carried out by transporting the back crossed variety milt from Andaman Islands to Bhubaneswar (CIFA campus). It was interesting to know that the viability of carp (Back cross variety; Catla X Rohu) milt could be successfully transported in cold chain (4 °C) over ice. Upon fertilization with eggs collected from a catla female 70% fertilization and 40% hatching could be obtained. The entire protocol is depicted in Fig. 10.2. It can be useful in artificial propagation and complement cryopreservation. Study conducted at CIFA revealed that that Indian major carp semen can be successfully preserved for 18 hours at 4 °C prior to artificial insemination. By this method milt from

Fig.10. 1. Male gametes of carp (rohu) obtained after spermatogenesis.
Andaman Nicobar Islands could be brought to CIFA and successful fertilization and seed production in catla was achieved. Similar short term preservation and utilization of carp gametes was observed in different locations of SAARC viz. Sri Lanka and Nepal (Table 10.1).

**Table 10.1. Utilization of short term preserved milt for stock upgradation at Nepal and Sri Lanka**

<table>
<thead>
<tr>
<th>Name of the Farm/place</th>
<th>Name of species</th>
<th>Average male weight Kg</th>
<th>Average female weight Kg</th>
<th>Duration after milt collection hours</th>
<th>Milt:Extend er-C dilution</th>
<th>Fertilization %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarahara, Nepal</td>
<td>Rohu</td>
<td>2.6</td>
<td>2.2</td>
<td>4</td>
<td>1:2</td>
<td>95</td>
</tr>
<tr>
<td>Tarahara, Nepal</td>
<td>Rohu</td>
<td>2.5</td>
<td>2.8</td>
<td>6</td>
<td>1:2</td>
<td>65</td>
</tr>
<tr>
<td>Tarahara, Nepal</td>
<td>Rohu</td>
<td>2.3</td>
<td>2.2</td>
<td>8</td>
<td>1:2</td>
<td>65</td>
</tr>
<tr>
<td>Janakpur, Nepal</td>
<td>Rohu</td>
<td>2.0</td>
<td>2.5</td>
<td>4</td>
<td>1:2</td>
<td>55</td>
</tr>
<tr>
<td>Janakpur, Nepal</td>
<td>Rohu</td>
<td>2.3</td>
<td>2.1</td>
<td>8</td>
<td>1:2</td>
<td>40</td>
</tr>
<tr>
<td>Tarahara, Nepal</td>
<td>Rohu</td>
<td>2.7</td>
<td>2.9</td>
<td>8</td>
<td>1:2</td>
<td>60</td>
</tr>
<tr>
<td>Dambulla, Sri Lanka</td>
<td>Catla</td>
<td>3.9</td>
<td>4.5</td>
<td>12</td>
<td>1:2</td>
<td>50</td>
</tr>
<tr>
<td>Dambulla, Sri Lanka</td>
<td>Catla</td>
<td>4.2</td>
<td>5.0</td>
<td>14</td>
<td>1:2</td>
<td>65</td>
</tr>
<tr>
<td>Dambulla, Sri Lanka</td>
<td>Catla</td>
<td>4.6</td>
<td>4.2</td>
<td>14</td>
<td>1:2</td>
<td>70</td>
</tr>
<tr>
<td>CIFA, India</td>
<td>Rohu</td>
<td>2.9</td>
<td>2.5</td>
<td>18</td>
<td>1:2</td>
<td>72</td>
</tr>
<tr>
<td>CIFA, India</td>
<td>Catla</td>
<td>3.2</td>
<td>3.5</td>
<td>13</td>
<td>1:2</td>
<td>70</td>
</tr>
</tbody>
</table>

Fig. 10.2. Protocol of transport and utilization of male gametes of catla from Andaman and Nicobar islands to CIFA, Bhubaneswar and successful utilization for seed production.
10.3. Cryogenic preservation (long term)

Spermatozoa can be stored for years together in liquid nitrogen (190 °C) where at this temperature all the biochemical activities of a living cell ceases. The milt is diluted in extender and mixed with cryoprotectant. Cryoprotectant is a chemical which protect that cell from chilling injury during the course of freezing.

10.3.1. Methods used for milt cryopreservation

Conventional methods

Conventional methods of cryopreservation, such as the use of a Styrofoam box filled with liquid nitrogen or a programmable freezer, rely on comparatively slow, controlled cooling during early ice formation. Such a method is being used at CIFA, Bhubaneswar and also being advocated for hatchery managers due to its convenience and cost effectiveness in cryopreserving carp spermatozoa. This method has been proven suitable for carp sperm cryopreservation in bulk.

For Indian major carps, milt samples were equilibrated for 30-40 minutes at 4 °C including filling and sealing of French straws / visotubes. These straws were kept over liquid nitrogen for 5 minutes over tripod stand in a manual cryofreezer (CIFACRYO) developed at CIFA. The liquid nitrogen vapour in the chamber provide a good enough freezing rate for freezing milt samples filled in 0.25-0.5 ml straws. Thus cryopreservaed spermatozoa have been utilized in hatcheries across India (Gupta et. al., 1995, Routray et al, 2007, Routray et al, 2008).

10.3.2. Vitrification

By following the glass transition principle liquid systems are rapidly cooled by avoiding ice crystal formation which is called as vitrification. In this process the solution forms an amorphous glass as a result of rapid cooling by direct immersion of the visotubes / straws containing the milt samples into liquid nitrogen. The glass retains the normal molecular / ionic distributions of a liquid but remains in an extremely viscous form. The glass is devoid of all ice crystals, and the spermatozoa are not subjected to the physical damage due to the ice crystal formation during freezing which is peculiar in conventional cryopreservation method. Vitrification is a simple and less expensive alternative to conventional freezing. Vitrification solutions are generally cryoprotectants of high concentrations which effectively dehydrate the cells prior to the initiation of the cooling process. Vitrification solutions can be of permeating or semi permeating type or a combination of both. Vitrification solutions share three common properties. First, these vitrification solutions contain a combination of low and high molecular weight cryoprotectants. Low molecular weight cryoprotectants penetrate cell membranes and protect the cytoplasm from damage during freezing. Higher molecular weight cryoprotectants do not pass across the cell membranes; however, they are effective extracellular dehydration agents. Secondly, the final overall concentration of the
cryoprotective agents in the mixture is high, so enhance vitrification and thus, avoiding lethal ice crystal formation. Finally, the standard vitrification solution contains an isotonic level of saline.

10.3.3. Cryoinjury

There are major cryoinjuries related to freezing and thawing processes during conventional cryopreservation as well as during vitrification (due to osmotic changes) within the temperature ranges of generalized cryopreservation procedures. Most cryoinjuries take place over the temperature range between 0 and -40 °C due to two major causes: heat removal and application of cryoprotectants. Other causes of cryoinjuries include cold shock, ice crystal formation, pH fluctuation, osmometric effect, and cryoprotectant toxicity.

10.3.4. Cold shock

Cold shock is caused by the change in membrane lipids from the liquid phase to the solid phase in the freezing process from -10 °C to -16 °C. Low temperature alone may not be sufficient for the phase change. At this temperature range, however, ice crystals spontaneously form, dehydrating the lipids and inducing change from lamellar liquid to the phase transition change. The increase in membrane tension resulting from temperature reduction results in cold shock. Cold resistant cells can face long periods of exposure to subzero temperatures.

10.3.5. Ice crystal formation

During cooling, intracellular as well as extra cellular ice crystal formation occurs over a broad range of subfreezing temperatures in a diverse array of biological cells (Mazur, 1977; Routray, 2003). Both ice crystal formation and solute concentration contribute to cell damage in the freezing process. It is essential to know the intracellular ice formation temperature of the cells to be cryopreserved to avoid fatal ice crystal formation.

10.3.6. Osmometric effect

Cell permeability plays a major role in cryoinjury. Less water is removed from cells that are less permeable in the freezing process. Cells that are more permeable demonstrate a greater tolerance to the cooling and freezing process, but they may become dehydrated. In an aqueous suspension of living cells, ice crystals are formed first in the solution surrounding the cells, resulting in increasing concentrations of solutes outside the cells. Because of the difference in osmotic pressure inside and outside the cells, water moves out until osmotic balance is restored. A typical cryoinjury due to osmometric effect in carp spermatozoa is shown in Fig. 10.3 (SEM). Due to ice crystal formation and osmometric effect the spermatozoon membrane gets injured and ultimately the spermatozoon becomes non-motile in carps and loses its viability (Routray, 2003).
10.3.7. pH fluctuation

Most biological salts are eutectic at a temperature range of 0 to -55°C. During the process of cryopreservation freezing and thawing occurs and by that the buffering function of these salts is destroyed and the pH of the biological solution changes. This pH fluctuation caused by freezing or addition of cryoprotectants may result in temporary or permanent cryoinjuries. Denaturation of proteins invariably takes place when their pH limits are exceeded.

10.3.8. Cryoprotectant toxicity

Low toxicity and high water solubility are essential considerations for a chemical to be cryoprotective. Cryoprotectants should be non- or minimally toxic, able to penetrate cell membranes easily, and exit easily. The apparent toxicity of cryoprotectants is dependent on type and concentration, the equilibration time, and the temperature during loading (Routray, 2003). It is necessary to determine the tolerance (toxicity) level of gametes to any particular CPA before use in cryopreservation.

10.3.9. Male gamete cryopreservation in carps

Carps are the mainstay of freshwater aquaculture in the Indian sub-continent. Long term preservation of teleost gametes and that of carps in particular could be highly beneficial for fish farming and wildlife preservation (Rall, 1993; Routray, 2003).
Successful freezing of sperm has been achieved in several species of carps which belong to the cyprinid family. Cryopreservation of common carp sperm has been extensively studied. In early trials fertility of thawed sperm was either not tested (Sneed and Clemens, 1956) or was low (Kossman, 1973; Mockzarski, 1977; Stein and Bayrle, 1978). The first practical results were reported by Kurokura et.al. (1984). Koi carp sperm were frozen in a methanol-dry ice bath in 0.5 ml straws. Freezing with 15% dimethyl sulfoxide (DMSO) resulted in 69% fertilization against a fresh sperm control of 83%.

Cryopreservation of carp milt has also been reported by Kumar (1988). Long term preservation of Indian major carp milt and their utilization in seed production have also been reported (Gupta & Rath, 1991, Gupta et al., 1995, Routray, 2003).

The general protocol of cryopreservation of carp spermatozoa is depicted in the Figure 10.4 and described below:

**1) Collection and quality assessment of milt**

Healthy milters of Indian major carps viz. catla, rohu and mrigal are required to be induced either carp pituitary extract @ 2-3 g/ Kg or orOvaprim @ 0.1-0.2 ml/kg body weight and stripping is done after 3-4 hours of induction. Neat milt should be collected in sterilized ice cold graduated centrifuge tubes and kept in refrigerator. Preferably the processing of sample should be done within an hour from the time of its collection.

A non-induced carp yields 0.5 to 1.0 ml milt per kg body weight whereas induced one gives 6-10 ml of milt. Milt volume is also an important aspect to be considered before cryopreservation as less amount of milt may give an indication of the status of male maturity. The carp milt sample which possesses spermatocrit value (% of packed sperm cells) of not less than 70, a suitable sperm count and a motility score of 4+ are considered as quality sample.

**2) Equilibration with extender and cryoprotectant**

The chemical components of extender-C mentioned earlier are weighed accurately and dissolved in double distilled water. The CPA used in this protocol is DMSO which gives consistent results. The CPA should not be mixed directly in the milt sample. It is mixed first in the desired ratio (DMSO 15 ml + Extender-C 65ml) and kept in the refrigerator due to its exothermal reaction. The resultant solution is called diluent. It is very important to maintain isothermal condition while mixing the milt with diluent. The time interval between mixing the milt with diluent and starting the freezing process is called the equilibration time. For major carp milt it is generally 30-45 minutes. During equilibration the CPAs generally enters into the cells and protects them from cryoinjury during freezing. This time varies from species to species as the CPA toxicity time depends on the equilibration time and temperature also (Routray et. al, 2002).

The highest gamete viability for any given DMSO concentration can be obtained with high concentrations and short times of equilibration rather than low concentrations.
and long impregnation times as it has been reported with medaka embryos (Routray et al., 2002). From this it can be said that longer duration of equilibration time is toxic and deleterious for the survival of fish gametes.

3) Freezing protocol

A rapid freezing protocol is advocated for carp sperm as it minimizes the thermal shock and intracellular ice formation. The sealed visotubes were required to be cooled at -15 °C/minute by programmable cooling chamber. Similarly, manual method of freezing is also possible by manual vaporization over liquid nitrogen in thermocol chamber described earlier. The frozen milt samples in straws/visotubes are quickly plunged into liquid nitrogen and kept undisturbed. The evaporation loss of liquid nitrogen is compensated by regular filling of the container.

4) Thawing / Warming and Fertilization

Thawing is an important step in cryoprotocol where if optimum conditions are not followed then it ice formation takes place due to recrystallization. In case of carp spermatozoa fast thawing is preferable, because slow thawing can recrystallize the small intercellular crystals which may damage the cells. In the present protocol the cryomilt samples are thawed in warm water 37 ± 1 °C. Visotubes and French straw takes approximately 65-70 seconds and 8-10 seconds respectively for slush formation. After the slush formation, it is important to keep the milt decanted from the visotubes in the fertilization plate for nearly 5 seconds before mixing it with the unfertilized eggs. This is done to bring both gametes to an isothermal condition at room temperature. The eggs fertilized with cryo-milt are washed thoroughly 5-10 times in freshwater and released into flow through incubation chambers for excess CPA to wash out.

Figure 10.4. Schematic diagram of the cryopreservation protocol for carp spermatozoa.
10.4. Protocol for cryopreservation

Preparation of extenders
Preparation of extenders: The chemical constituents are weighed accurately and dissolved in double distilled water. The desirable pH of the solution is 7.2 to 7.3. The solution kept under refrigeration can be used safely for a month.

Preparation of diluent
The cryoprotectants used in the present protocol are DMSO (dimethyl sulphoxide) and a combination of DMSO and glycerol. The cryoprotectant should not be mixed directly in the milt sample. It is mixed first with extender in the required ratio and kept in refrigerator due to its exothermal reaction. It is very important to maintain isothermal condition while mixing the milt with diluent. The ratio of extender and cryoprotectant in different diluents are as follows:

<table>
<thead>
<tr>
<th>Extender</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycerol 10 ml</td>
<td>Glycerol 10 ml</td>
<td>DMSO 15 ml</td>
</tr>
<tr>
<td></td>
<td>DMSO 5 ml</td>
<td>DMSO 8 ml</td>
<td>Extender 65 ml</td>
</tr>
<tr>
<td></td>
<td>Extender 90 ml</td>
<td>Extender 90 ml</td>
<td></td>
</tr>
</tbody>
</table>

The working ratio of milt and diluent is 1 : 4

Equilibration
It is a time interval between mixing the milt in the dilute and putting the mixture in to liquid nitrogen. Calibrated duration of equilibration time helps for efficient function of the cryoprotective agents. The longer the equilibration time the lower the fertility as because the cryoprotectant might be becoming toxic to cells. In the present protocol 45 minutes at 8-10°C was found to be the optimum equilibration time.

Ampouling
The diluted samples within the equilibration period is filled either in French straws (250ul) or visotubes (5ml) and plugged with sealing powder. While filling care should be taken to avoid any air gap in the visotubes which will not allow the tubes to be submerged in the liquid nitrogen.

Freezing
The freezing should be rapid so that thermal shock is minimal and not so fast as to allow the formation of intracellular ice crystals. The sealed visotubes required to cooled at 15°C/minute by programmable cooling chamber while straw freeing is possible by manual vaporization over liquid nitrogen in thermocol chamber. The success of cryopreservation depends on optimum concentration of cryoprotectant, freezing techniques and equilibration time.

Storing
The freezed milt straws/visotubes are immediately immersed in liquid nitrogen and kept undisturbed. The evaporation loss of liquid nitrogen compensated by regular filling to the container.
**Thawing**

In case of carp spermatozoa fast thawing is preferable, because slow thawing can recrystallize the small intercellular crystals which may damage the cells. In the present protocol, the cryomilt samples are thawed in warm water 38±1 °C. Visotube and French straw take approximately 65-70 seconds and 8-10 seconds respectively for slush formation.

**Insemination and Post-insemination Management**

One visotube containing 3 ml thawed diluted milt is sufficient to fertilize 1.0 lakh of eggs. The thawed milt is mixed properly to the stripped eggs and activated in drop of water. The fertilized eggs are to be incubated in flow through system so as to remove the cryoprotectant trace from the eggs as it is toxic to developing embryo.

10.5. Utilization of cryopreserved milt for stock upgradation

Sperm cryobanking could be a good alternative to breeding in captivity in order to preserve genetic diversity. Cryobanking of sperm has considerable advantages over breeding in captivity in terms of costs, labour and security; since thousands of samples from different generations can be maintained in a minimum space, without the risk of loss caused by disease or genetic drift over time. Moreover, transportation and management of frozen samples are relatively simple, allowing greater flexibility in designing recovery programs. Routine technologies for cryopreservation of sperm are presently in use in several sperm banks in Russia (Ananiev, 1998) and in Europe as well as in North and South America (Harvey, 1998) for the purpose of aquaculture and conservation. Recently, during 2009-10 four fish semen cryobanks were established in India. These cryobanks (2 in Andhra Pradesh and 2 in Orissa) are being used for the purpose of stock upgradation and raising of good quality brood fish of Indian major carps (Catla catla, Labeo rohita, Cirrhinus mrigala). Cryosemen from these banks were used for stock upgradation in ten hatcheries during 2009-10. More than 40% hatchlings were recovered by using the cryomilts from good milters and local females in carp hatcheries where inbreeding and slow growth of carps has been reported. By applying this technology it has been possible to increase the brood fish status in many hatcheries. The ease of carrying the cryomilt of carps to hatcheries and producing brooder seed has been successful and more over this has changed the germplasm of the farms for future brood stock development. An ambitious programme of cryomilt utilization of carps is being pursued in these states at present through these fish semen cryobanks. Similarly with CIFA’s technical assistance a Fish Semen Cryobank has been established in Dambulla under the National Aquacultural Development Authority of Sri Lanka.

10.6. Conclusion

It has been well demonstrated that carp seed produced by stored milt is no way inferior to the seed produced by fresh milt. Growth, breeding response and fecundity of the stock developed by preserved milt are comparable with that of fresh milt.

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11. Minor carps and barbs in aquaculture: Importance and technology for their seed production and grow-out culture

Pratap Chandra Das


11.1. Introduction

The freshwater aquaculture system has evolved itself as one of the most promising and profit making venture among the various agriculture enterprises in recent years. This has been realised with a series of research and development in all fronts of aquaculture including domestication of species, development of their artificial propagation methods, standardisation of seed rearing and grow-out technology, health management, nutrition, etc. Utilisation of the locally available material including the feed ingredients and organic manures as the critical inputs for aquaculture has also helped in keeping the operational cost low for freshwater farming. Though such carp farming is considered a profitable venture, the margin of profit is lower than the other species cultured in freshwater elsewhere in the globe, mainly because of the low value of the carp species in the international market. Besides, the sector has also been posed with several other challenges that include (i) the ever increasing fish demand by the phenomenal population growth and urbanization (ii) increased purchasing power and changed preference of consumer for varied fish protein, (iii) need to increase fish production per unit use of land, water and other critical natural resources such as feed ingredients and manures, (iv) increase the farm income against the low market value of carps, (v) preserve the species diversity against their dwindling stock in the nature, (vi) produce fish in a sustainable and environment friendly manner, etc. Besides, adoption of the scientific farming technology has not yet been wide spread leading to ineffective utilisation of the aquaculture resource and low unit area productivity.

India is one among the very view countries in the world having a rich fish biodiversity in the natural waters. Out of the 27800 fish species reported globally, 2358 indigenous species consisting of 877 freshwater, 113 brackishwater and 1,368 marine species; and 291 exotic species have been documented in the country (Gopalakrishnan et al., 2011). At present, more than 35 species are cultured in the freshwater aquaculture sector. Among these, carps form the main species for the aqua-farming i.e. the three Indian major carps (catla, rohu and mrigal) and the three exotic carps,( silver carp, grass carp and common carp) is primarily farmed in India. Besides, few other cyprinids also form part of the species composition on regional basis.
In recent years, increasing species spectrum has been emphasized in the culture system mainly for increasing the production and farm income, with increased consumer’s choice and species conservation as the secondary objectives. However, substituting the entire major carp component from the culture system is a remote possibility in India. In such situation, successful introduction of any new species largely depends on its compatibility with the major carps and performance in the grow-out system since polyculture with carp is the most feasible option. Development and standardization of the mass scale seed production and rearing technologies are also prerequisite for promoting culture of any such species. Efforts are made in recent years to bring some new candidates in to the culture system and such efforts included seed production as an integral component besides species evaluation for their compatibility and performances.

11.2. Minor carps and barbs: potential candidate for freshwater aquaculture

India is blessed with rich diversity of potential cultivable fish fauna which includes many indigenous minor carps, barbs and others. A wide variety of minor carps and barbs are available for culture in India as given in the Table 11.1.

Table 11.1. Minor carps and barbs and their distribution in India

<table>
<thead>
<tr>
<th>Species</th>
<th>Parts of the country where cultured</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minor carps</strong></td>
<td></td>
</tr>
<tr>
<td><em>Labeo calbasu</em> (Kalbasu)</td>
<td>Eastern Indian states</td>
</tr>
<tr>
<td><em>L. fimbriatus</em> (Fringe lipped carp)</td>
<td>Karnataka, Odisha</td>
</tr>
<tr>
<td><em>L. gonius</em> (Kuria labeo)</td>
<td>North eastern states</td>
</tr>
<tr>
<td><em>L. bata</em> (Bata)</td>
<td>West Bengal, Assam, Odisha</td>
</tr>
<tr>
<td><em>Cirrhinus reba</em> (Reba)</td>
<td>West Bengal, Assam, Odisha</td>
</tr>
<tr>
<td><strong>Barbs</strong></td>
<td></td>
</tr>
<tr>
<td><em>Puntius sarana</em> (Olive barb)</td>
<td>Odisha, Bihar</td>
</tr>
<tr>
<td><em>P. gonionotus</em> (Silver barb)</td>
<td>Odisha, West Bengal, Assam</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
</tr>
<tr>
<td><em>Osteobrama belangiri</em></td>
<td>Manipur</td>
</tr>
<tr>
<td><em>Semiplotus semiplotus</em></td>
<td>Arunachal Pradesh</td>
</tr>
</tbody>
</table>

11.3. Minor carps for intercropping in polyculture environment

The minor carps and barbs enjoy high consumer preference on regional basis and even at times fetch higher market price than the major carps. Breeding and seed production of these species are easy and almost similar to the conventional carp breeding technique except in few species like *P. sarana* which needs substrate. The growth in many of these species is almost similar to the major carp during the initial 4-6 months. Since these species have smaller marketable size, they can be cultured in seasonal ponds. Series of
grow-out studies at CIFAof these species with the major carps have revealed their compatibility in certain species while some do not significantly affect the total biomass production despite having certain degree of competition with one or two major carps. Rearing these species on a supplementary feed similar to that of major carps has made possible to culture them along with the major carps. This has led to development of the concept of intercropping of these species in the major carp based culture system. Since, standing crop of carp pond remains much below the carrying capacity during initial six months, intercropping of these species without altering the stocking density of major carps does not significantly affect the normal growth process of the latter group. In turn, an interim production of the minor carps/barbs after six months of culture gives an additional fish production and income to the farmer and increases the total fish productivity of the unit culture area, besides ensuring effective utilisation of pond productivity.

11.4. Induced breeding

The breeding protocols for *L. calbasu*, *L. fimbriatus* and *L. gonius* are almost similar to that of major carps. The species have been successfully bred using Chinese eco-hatchery and FRP hatchery models. Both the species are induced to breed using single injection of Ovaprim at a rate of 0.3 and 0.15 ml/kg body weight of female and male brooder, respectively. The response time is between 8-11 hours at water temperature 27-28°C. The eggs in both the species are non-adhesive and sinking types and need incubation process similar to the one followed for major carps in these hatchery systems. The larvae of *L. fimbriatus* and *L. gonius* usually hatch out after an incubation period of 15 hours at 27-30°C temperature and measure about 4-5 mm in length. The incubation time for *L. gonius* is reported to extend at lower water temperature. Spawn recovery was in the range of 72,000-77,000 per kg female.

The breeding habit of *P. sarana* is different from the above two species. The species is a batch spawner. It releases adhesive eggs which, unlike having an adhesive egg cell as in common carp, is provided with a stalk that attaches to the submerged substratum. Sometimes natural breeding of the species takes place in the confined pond system if the water depth is maintained at more than 1.0-1.5 m and pond having marginal vegetation. Female and male are induced to breed with Ovaprim at a rate of 0.3 and 0.15 ml/kg body weight, respectively. Provision of bunches of aquatic weed, preferably with rich foliage, at the top one feet layer of the water in the breeding tank facilitates spawning of the species. Female lays egg in the weed masses which are later collected and kept in the incubation tank. The response time is usually 6-8 hours and the spawning activity continues for 2-4 hours during a mass breeding programme. Like the other two
minor carp species, the larvae take 15-16 hour to hatch out which are further kept in incubation tank for 60-62 hours before they are transferred to nursery. The spawn measures about 5 mm in length. Though it is difficult to measure the actual fertilisation in the egg of \textit{P. sarana}, usually the rate remains higher.

The exotic silver barb is also a natural spawner in confined pond system with continuous water depth of more than 1.0-1.5 m. The species can breed two times in the pond system during a year. Induced breeding of the species in the eco-hatchery system is also carried out with use of Ovaprim as inducing agent at a rate of 0.3 and 0.15 ml/kg body weight of female and male brooder, respectively. The response time in this species is about 6-7 hours. The eggs are small, unattached and sinking type. The water hardened egg measures about 2-3 mm in diameter. The hatchlings of this species are thin and small measuring only 3 mm in length and thus a finer mesh cloth is required to prevent escape through the central screen of the incubation tank. Any attempt to filter the spawn out of water leads to large-scale mortality and therefore, extreme precaution should be maintained during spawn harvest and transferring from incubation tank. Channelizing water from incubation tank directly to the nursery pond improves spawn survival.

11.5. Seed rearing in nursery

Use of concrete tanks has been recommended for nursery rearing of the minor car/barb species which ensures better survival. However, earthen ponds can be used for the same. Culture of single species is recommended for the minor carps. Species like \textit{P. sarana} is highly carnivorous during nursing phase and some soot fry from the same population may cause extensive damage to the population of other carps/barbs stocked together.

The nursery phase for \textit{L. fimbriatus, L. gonius and P. sarana} usually extend up to 25-30 days while in \textit{P. gonionotus}, the nursing phase extends up to 45 days. During this nursing phase, the fry of these minor carps reach to a size of 15-20 mm. Pond preparation method followed for minor carps is similar to the one followed for major carps. Phased manuring with a mixture of 750 kg/ha groundnut/mustard oil cake, 200 kg/ha raw cattle dung and 50 kg/ha single super phosphate have shown to be effective in production of desired plankton in nursery (Jena et al., 2009). Half of the above amounts, made into thick paste by addition of sufficient water, are applied as basal dose 5 days prior to spawn stocking and the remaining amount is applied later in 2-3 splits depending on the plankton population of the ponds.

During the last decades, several studies have been undertaken at CIFA to standardize the nursery rearing technique of these minor carps and barbs with regard to stocking density and supplementary feeding which are presented below.
Table 11.2 Stocking density for nursery

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended density for concrete nursery</th>
<th>Nursing phase (days)</th>
<th>Feed</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L. calbasu</strong></td>
<td>1000 spawn/ m²</td>
<td>25-30</td>
<td>GNOC+rice polish</td>
<td>31-51</td>
</tr>
<tr>
<td><strong>L. finmbriatus</strong></td>
<td>1600 spawn/m²</td>
<td>25-30</td>
<td>GNOC+rice polish</td>
<td>50-60</td>
</tr>
<tr>
<td><strong>L. gonius</strong></td>
<td>800 spawn/m²</td>
<td>25-30</td>
<td>GNOC+rice polish + 20% dry fish mill</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Soya milk+ egg custard</td>
<td>43</td>
</tr>
<tr>
<td><strong>P. sarana</strong></td>
<td>1000 spawn/ m²</td>
<td>25-30</td>
<td>Soya milk+ egg custard</td>
<td>-</td>
</tr>
<tr>
<td><strong>P. gonionotus</strong></td>
<td>Based on the count of fertilised egg and hatching percentage</td>
<td>45</td>
<td>Soya milk+ egg custard</td>
<td>-</td>
</tr>
</tbody>
</table>

An intermediate nursing period of 10-15 days is recommended prior to the nursing phase as it is difficult to count the tiny spawn

(GNOC: Groundnut oil cake)

The most commonly used supplementary feed in carp nursery has been the powdered mixture of groundnut oil cake and rice bran at 1:1 ratio by weight. Incorporation of 5-10% fish meal in the diet increases the seed survival in all the species. The dry feed mixture is normally supplied in nurseries @ 6 kg/million/day for the first 5 days and 12 kg/million/day for the subsequent days. The daily ration may be supplied in two equal meals during morning and evening hours.

In nursery rearing of *L. finmbriatus* from spawn to fry in big concrete nursery tanks (50 m²) at 16 million spawn/ha density, provision of soil base in the tank significantly increased the fry survival to 50.9% compared to 31.4% in tank without soil base. After rearing the species at 8-24 million spawn/ha densities in concrete seed rearing system, stocking density of 16 million spawn/ha has been recommended (CIFA, 2008). Nursery rearing of *L. gonius* spawn at 8-24 million/ha densities revealed 8 million/ha to be ideal (CIFA, 2008). Similarly, in *L. calbasu* spawn reared in 50 m² concrete nursery tank at 5-15 million/ha densities showed 10 million/ha density to be ideal considering the outcome in terms of number harvested and growth of the fry (Sahu et al., 2007a). Stocking density of 10 million/ha is also recommended for spawn rearing of *P. sarana* in concrete nursery (CIFA, 2007). Since fry of this species shows highly carnivorous feeding habit, addition of 20% fish meal to the groundnut oil cake and rice bran mixture improves the fry survival of this species. Study has also revealed feasibility of its fry rearing with soya milk and egg custard combination as supplementary diet with 43.2% seed survival. Fry of *P. sarana* has proven to serve as an *in situ* biological agent for controlling aquatic insect population during rearing of carp fry to fingerling (Das et al., 2011).
11.6. Rearing fry to fingerlings

Several studies have been conducted on rearing fry of the minor carp/barb species in monoculture as well polyculture system using varied inputs and varied incorporation levels. *L. calbasu* incorporated at 10, 20 and 30% species composition with the three Indian major carps based polyculture rearing system revealed incorporation at 10% level to be ideal. In a fingerling rearing trial of *L. calbasu* along with catla, rohu and mrigal at 0.5 million fry/ha density, provision of aeration, apart from supplementary feed has been found to be important for achieving higher growth in catla and rohu, while aeration did not have significant influence on mrigal and *L. calbasu* rearing.

Fry of *P. sarana* were reared with rohu and mrigal at 0.25 million fry/ha for 75 days in concrete seed rearing tanks with provision of feed (rice bran, groundnut oil cake and fish meal at 47.5, 47.5 and 5% on dry weight basis) at 10, 8 and 6% of body weight per day during 1st, 2nd and 3rd months (Jena et al., 2007a). The study revealed *P. sarana* to be more compatible with rohu than mrigal during fingerling rearing. Better growth performance of *P. sarana* than both rohu and mrigal in that study indicated higher growth potential of the species at the initial phase.

Rearing of fry *L. fimbriatus, L. calbasu* and *P. gonionotus* along with rohu to fingerling size in outdoor concrete seed rearing system at 10-40/m² under polyculture showed recommended rearing density up to 40/m² during rearing fry to fingerling in concrete tank. Provision of night-time aeration during high density carp fingerlings raising at

| Table 11.3. Compatibility of minor carps and barbs with IMC in rearing and grow-out culture |
|-------------------------------------------------|-----------------|-----------------|-----------------|----------------|
| L. fimbriatus | Compatible | Compatible | Competition | Can partially replace mrigal |
| L. gonius | Compatible | Compatible | Strong Competition | *L. gonius* with 33% incorporation level can be alternative species to mrigal for polyculture |
| P. sarana | Compatible | Compatible | Competition | *P. sarana* with 33% incorporation level can replace mrigal in grow-out carp polyculture |
| P. gonionotus | Compatible | Strong competition | Compatible | Strong competition with rohu. However, no influence on survival/biomass production of any species |
0.5 million fry/ha (50/m²) in concrete tanks revealed 8-12 hour night time aeration to be advantageous for catla and rohu due to their higher oxygen requirement, whereas aeration for 4 h was adequate for *L. fimbriatus* and *P. sarana* (Pawar et al., 2009). Similar study on aeration in rearing fry of *P. gonionotus* to fingerling size recommended rearing density of 75/m² with aeration, whereas, 50/m² was recommended under non-aerated condition (Das et al., 2012).

### 11.7. Grow-out culture

With emphasis on diversification of culture systems in recent years, attempts have been made for incorporation of many potential species into carp culture systems. Silver barb has been identified as one such important candidate species for culture in many south-east Asian countries (Haroon & Pittman 1998; Rothuis et al., 1998; Vromant et al., 2002; Alam et al., 2004). Kohinoor et al. (1993) had reported a production of 2384 kg/ha/6 months from monoculture of silver barb in Bangladesh while Hossain et al. (1998) reported 3125-3582 kg/ha through polyculture of the species with *Mystus cavasius* and silver carp in Bangladesh. Although the species was introduced in 1972 in India (Das et al., 1994), it has not attracted the attention of the fish farmer prior to the move for diversification in the aquaculture system. However in recent years, popularity of this species has increased in the culture system. Several studies have been conducted on stocking density and incorporation level in the carp polyculture. Monoculture of *P. gonionotus* at 10,000/ha has realized production of 1.56-2.13 t/ha/8 month. The species incorporated up to 30% of the species composition in carp polyculture has been found productive. In a grow-out study on incorporating this species along with catla, rohu and mrigal showed its strong inter-specific competition with rohu compared to mrigal while competition with catla was not perceptible (Jena et al., 2007b). However, the species neither affected the survival of any carp nor yielded any significant changes in the total biomass production indicating its feasibility of polyculture with major carps. In a multispecies grow-out culture study, the *P. gonionotus* showed a growth almost similar to catla during the initial six months.

Kalbasu is another promising candidate species for inclusion in carp polyculture systems. Although this bottom dweller has slow growth rate compared to the major carps, it fetches very high market demand. Though the species formed a component in certain experimental grow-out trials (Aravindakshan et al., 1999; Wahab et al., 1999; Tripathi et al., 2000), inadequacy in its seed availability and information on culture probably have restricted its incorporation into the commercial carp polyculture system in the country. Efforts have been made in recent years to develop the mass scale seed production and rearing of this species. In grow-out culture *L. calbasu* up to 15% incorporation is found to improve the biomass production. Provision of periphytic substrate could significantly increase the growth of rohu and kalbasu by 33% and 28%, respectively which together registered 13% increase in net biomass production (Sahu et al., 2007b).
**Puntius sarana** is another important species for grow-out culture. The high consumer preference, even at smaller size of 100–200 g, makes the species a suitable candidate for diversifying the carp culture (Gopakumar et al., 1999; Chakraborty et al., 2003) and also for short-term culture in seasonal water bodies. In an agrow-out study in Bangladesh, Chakraborty et al. (2005) reported production of 4200-4819 kg/ha from polyculture using olive barb at 30-35% of the stocked density with four other major carps. Jena et al. (2008) reported production of 3155-3418 kg/ha/year through polyculture when *P. sarana* constituted 33% of species composition with one third of surface feeder (catla and silver carp) and the rest as rohu/mrigal. In this study, they found the species to have inter-specific competition with rohu while the same was minimal with mrigal. However, they found higher biomass production when the species was cultured along with catla and rohu compared to that with catla and mrigal which suggested feasibility of replacing mrigal with *P. sarana* in the grow-out carp polyculture system. Compatibility study of *L. gonius* with catla, silver carp, rohu and mrigal in a grow-out polyculture revealed the species to be compatible with rohu, while its inter-specific competition was observed against mrigal (Jena and Das, 2011). The study further revealed feasibility of using this species as an alternative to mrigal in the major carp polyculture system. *L. fimbriatus* incorporated at 33% of the species composition in grow-out trial at combined density of 7,500 fingerlings/ha with major carps revealed certain degree of inter-specific competition between *L. fimbriatus* and mrigal, it also suggested feasibility of using the former in place of the latter for higher biomass yield in the grow-out carp culture system. The compatibility of minor carps and barbs with IMC in rearing and grow-out culture is shown in Table 11.3.

Species like *Labeo bata* and *Cirrhinus reba* have already become popular in the culture systems of certain states like Assam, West Bengal and Odisha. Monoculture of these species is rare. These species are mostly cultured as a component species in the major carp polyculture system. In West Bengal, culture of these species in the sewage fed system along with carpsin varied species and input combination with impressive production levels varying between 3.7-5.1 tons was observed (Sahu et al, 2012).

### 11.8. Epilogue

All these minor carp and barb species have initial higher growth rate and market acceptability at smaller size of 300-400 g which qualifies them as ideal species for intercropping in the major carp culture system particularly during the initial six months of culture. Such practice would also help in more effective utilization of pond productivity during the initial culture months when the biomass of major carp in the pond is low. Further, these species look promising for utilization of seasonal ponds which have 5-6 months of water retention. The technology developed at CIFA can be used to bring these new spectrum of fish species for diversification of Indian aquaculture.

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pp. 126.


12. Pond and hatchery environment management

S. Adhikari


12.1. Introduction

Specific quality of water is required for successful pond aquaculture. Some of the problems in water qualities are related to site characteristics. Bottom soils may have undesirable properties such as potential acid sulfate, high organic matter content or excessive porosity. The water may be of poor quality, viz. highly acidic, rich in nutrients and organic matter, high in suspended solids or polluted with industrial or agricultural chemicals. Addition of large inputs of nutrients and organic matter due to feeding and fertilization very often lead to poor water and bottom soil conditions. The water quality problems are common in hatchery and aquaculture ponds, and protocols are being developed for the purpose of improving pond water quality. The present paper summaries the problems in water qualities and means to mitigate them for sustainable aquaculture management in tropical conditions.

12.2. Water quality management

Fishes are in equilibrium between potential disease organisms and their environment. Changes in this equilibrium such as deterioration in water quality (environment) can result in fish becoming “stressed” and vulnerable to disease. It is, therefore, very important to know about the water quality parameters and their management that have influence on growth and survival of aquatic organisms.

12.3. Dissolved oxygen

The optimum dissolved oxygen (DO) content of pond waters should be in the range of 5 mg/L to saturation level for good growth of fish. Fluctuations and low levels of oxygen lower fish immunity.

12.3.1. Dissolved oxygen ranges for fish production

- 5.0 mg/L - optimum for normal growth and reproduction in tropical waters;
- 1.0–5.0 mg/L - may have sub-lethal effects on growth, feed conversion and tolerance to disease;
- 0.3–0.8 mg/L - lethal to many species if sustained for a long period.
12.3.2. Measures to rectify oxygen depletion in water:

- **Manual aeration:** In this method, water surface is splashed with bamboo sticks. This helps in dissolving atmospheric oxygen in water.

- **Mechanical aeration:** A diesel water pump is operated through this method. Water is pumped out and simultaneously sprayed again into the water body. This helps in dissolution of atmospheric oxygen.

- **Use of Aerator:** Aerators are mechanical floating devices. Their rotating blades churn the water helping in dissolution of atmospheric oxygen in water. Depending upon the concentration of oxygen in waters, the number and placement of such aerators are determined.

**Other steps taken to control the oxygen level are:**

- Care should be taken to feed fish in the afternoon or evening in heavily stocked pond systems as oxygen requirement in fish after feeding increases and dissolved oxygen is low in pond during early morning.

- Organic manure application in a water area should be done carefully as organic material consumes oxygen during decomposition. Therefore, the quality of manure to be applied without the risk of oxygen depletion can be calculated taking into consideration the availability of dissolved oxygen during the 24 h period.

- During collapse of phytoplankton bloom, decomposition occurs and in the process oxygen requirements of microorganisms increase. Thus, special care has to be taken during that time.

- When temperature rises special care has to be taken as fish require more oxygen with increasing temperature.

12.4. Temperature

In an established system, the water temperature controls the rate of all chemical reactions and affects fish growth, reproduction and immunity. Drastic temperature changes can be fatal to fish. Temperature sets the pace of metabolism by controlling molecular dynamics (diffusibility, solubility, fluidity) and biochemical reaction rates. Under favourable conditions, the optimum temperature range for many coldwater and warm water fishes are 14–18 °C and 24–30 °C, respectively. Water temperatures can be adjusted to optimum levels in controlled system such as hatcheries. It is difficult to adjust water temperature in large water bodies. Operation of aerator during calm and warm afternoon helps to break thermal stratification by mixing warm surface water with cool subsurface water. Planting of trees on pond banks to give shade will reduce stratification but at the same time, reduces the beneficial effects of wind mixing and restricts solar energy for photosynthesis.
12.5. Turbidity

Turbidity in culturable water is the resultant effect of several factors like suspended soil particles, planktonic organisms, humus substances produced through decomposition of organic matter, etc. Turbidity is measured by Secchi disk visibility. Optimum Secchi disk visibility in fishponds is considered to be 40–60 cm. Turbidity resulting from plankton is generally desirable.

12.5.1. Suspended soil particles:

- Up to 10,000 mg/L Freshwater carps, *Tilapia* sp. and catfishes are tolerant, however, the effect will depend upon the nature of the suspended particles.
- Pond waters turbid with suspended soil particles can be controlled by application of 500–1,000 kg/ha organic manure, 250–500 kg/ha gypsum or 25–50 kg/ha alum.

12.6. Carbon dioxide

Carbon dioxide is present in the atmosphere in very small quantity. For this reason, in spite of its high solubility in water, its concentration in most water bodies is low. It occurs in water in three closely related forms, i.e. free carbon dioxide, bicarbonate ion (HCO₃⁻), and carbonate ion (CO₃²⁻). The amount of each form depends on the pH of water. For example, in neutral or acidic waters high concentrations of free carbon dioxide up to level of toxic form is found.

12.6.1. Carbon dioxide value for fish ponds

- 12–50 mg/L - sub-lethal effects include respiratory stress and development of kidney stones (nephrocalcinosis) in some species;
- 50–60 mg/L - lethal to many fish species with prolonged exposure.

12.6.2. Measures for controlling high carbon dioxide

- Repeated aeration of water;
- Increasing the pH of water by hydrated lime can control high carbon-dioxide concentration. Experiments have shown that 1.0 mg/L of hydrated lime can remove 1.68 mg/L of free CO₂; and
- Correct stocking, feeding and fertilization should regulate phytoplankton population and the organic loading in a water body.

12.7. pH

pH is a measure of the hydrogen ion concentration in water and indicates level of acidity in water (Table 12.1). Water pH affects metabolism and physiological process of fish. pH also exerts considerable influence on toxicity of ammonia and hydrogen sulphide as well as solubility of nutrients and thereby water fertility.
12.7.1. pH for fish production

Table 12.1. Effect of pH on fish growth

<table>
<thead>
<tr>
<th>pH range</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Acid death point</td>
</tr>
<tr>
<td>4-6</td>
<td>Slow growth</td>
</tr>
<tr>
<td>6-9</td>
<td>Best for growth</td>
</tr>
<tr>
<td>9-11</td>
<td>Slow growth, lethal to fish over long period of time</td>
</tr>
<tr>
<td>11+</td>
<td>Alkaline death point</td>
</tr>
</tbody>
</table>

12.7.2. Measures to rectify alkalinity and acidity

Alkaline waters

- Ensuring good water management may rectify rapid fluctuations in pH caused by excessive phytoplankton populations. Water body should have an alkalinity of more than 60 mg/L as CaCO$_3$.
- Application of acid forming fertilizers.
- Acidic waters
  - Calcium carbonate (CaCO$_3$), calcium hydroxide [Ca (OH)$_2$], calcium oxide (CaO) or dolomite is used to rectify the acidic water bodies depending upon the pH.
  - Salt water like seawater may be flushed through water bodies of coastal farms to neutralize acidity.

12.8. Total alkalinity

Alkalinity refers to the concentration of bases in water and the capacity of water to accept acidity, i.e. the buffering capacity. In most waters, bicarbonates and carbonates are the predominant bases. Alkalinity is an important criterion for determining the effect and concentration of water quality constituents and criteria; and therefore, the general suitability of a water source for the culture of fish and prawns. Water of low alkalinity (<20 mg/L CaCO$_3$) is considered to be less suitable for fish and prawn culture due to the associated unstable water chemistry. The upper limit of alkalinity is largely defined by individual species requirements and the magnitude of the concomitant increase in pH value. In general, freshwater fish in hard water (100-150 mg/L CaCO$_3$) tend to spend less energy on osmoregulation, resulting in a better growth. The upper limit of alkalinity may also be related to its effect on osmoregulation at high ion concentrations.
12.8.1. Alkalinity for fish growth

- 300 mg/L - create stress to fish;
- 75–200 mg/L - ideal for fish;
- <75 mg/L - create stress to fish.

12.8.2. Measures to maintain proper level of alkalinity

For hatchery use, the water should have less than 100 mg/l of alkalinity level. If alkalinity is more than this, then the water should be treated with alum \([\text{Al}_2 (\text{SO}_4)_3 14.3 \text{H}_2\text{O}]\). To reduce 1.5 mg/l alkalinity, 1.0 mg/l alum is required. As alum is solid material, it should be boiled with water and then cooled before using. If other water sources with low alkalinity are available, then such waters should be used fully or partially so that the resultant water alkalinity could be less than 100 mg/l. In general, at the starting of monsoon, the water may have higher alkalinity, but after continuous rain, the water could have desirable alkalinity level for its hatchery use. The stocking pond with lime application also shows higher alkalinity of water. This water needs one to two months rain for its reduction of alkalinity or this water should be mixed with low alkalinity water, if available for its hatchery use.

Survival and growth study of *M. rosenbergii* juvenile (0.12±0.03 g, 60 days old) was investigated under different bicarbonate levels from 100 to 370 mg/L CaCO\(_3\) for 60 days (Adhikari et al. 2007). Survival rate of the prawn was highly impaired at the highest bicarbonate levels tested while maximum survival rate (90%) was recorded at bicarbonate level of 100 mg/L CaCO\(_3\). The highest growth (4.92±0.13 mg/day) of the prawn was recorded at lowest bicarbonate level of 100 mg/L CaCO\(_3\) tested and the growth significantly (P<0.05) declined at 205 mg/L CaCO\(_3\) bicarbonate alkalinity and above.

12.9. Total hardness

Contents of alkali earth metals, mainly calcium and magnesium constitute the total hardness of a water body. There are several units of measurement currently used to determine water hardness. The most commonly used method measures both alkalinity and general hardness as mg /litre of calcium carbonate (CaCO\(_3\)). Each fish species has its preferred range of water hardness.

Hardness influences egg maturation, embryo hatching and health of the fry. Fishes can successfully adapt to local hardness conditions, but breeding may be impaired and incorrect hardness can affect egg fertility. Bailey (1975) and Germann and Reeves (1975) have suggested that hardness has no effect on the survival of striped bass larvae and that brood-stock differences would more likely account for variability in survival among
Experimental groups exposed to different hardness. However, elevated hardness has been reported to enhance larval survival in fresh water. Hazel et al. (1971) could not use low hardness (25-30 mg/l) river water for bioassays due to low survival of control fish. This problem was eliminated by either adding salts or using different river water with higher hardness (150-200 mg/l). Murray-Brown (1987) observed enhanced larval survival in elevated (60-240 mg/l) hardness treatments. Exposure of embryos and larvae in water having hardness of 2 mg/l CaCO₃ was associated with mortality, even in the absence of silver (Morgan et al., 2005). Fecundity of fish *Hemicromis bimaculatus* increased 12.0% and 19.4% from aquaria maintained with additional 100 and 300 ppm total hardness. Fishes from three sets of aquaria [with bore well water (250 ppm hardness) of additional 100 and 300 ppm] bred timely and with gradual increase in fecundity (Mehta et al. 2012). The presence of Ca²⁺ in the water decreases Na⁺ and Cl⁻ effluxes in the gills of adult goldfish and brown trout through its effects on the permeability of the gills to these ions (Hunn, 1985). Reader et al. (1988) noted that skeletal Ca²⁺ deposition was impaired at a low water Ca²⁺ concentration (1 mg/L), although survival was not affected.

Calcium and magnesium are very important to freshwater prawns in keeping exoskeletons strong. When hardness is too low prawns will take longer time after a molt for the exoskeleton to harden. While soft prawns are vulnerable to mechanical damage and predator. Very hard water can also be a problem. Ideal ranges of water hardness could be 50 to 200 mg/L CaCO₃ for optimum growth of prawns (Wetzel, 2001). Wickins (1972) suggested a hardness range of 65 to 200 mg/L CaCO₃ for satisfactory culture of *M. rosenbergii* while New and Singholka (1985) recommended hardness levels of 40 to 100 mg/L CaCO₃. A greater inhibition of growth at hardness levels above 65 mg/L CaCO₃ was reported by Cripps and Nakamura (1979). Brown et al. (1991) also reported that the growth of juvenile prawn was maximum at <53 mg/L CaCO₃ and did not change significantly at lower hardness levels but decreased at higher levels. Hardness levels between 940 and 1060 mg/L CaCO₃ did not adversely affect the growth, though the water had relatively low alkalinity between 58 and 86 mg/L CaCO₃ (Bartlett and Enkerlin, 1983). However, Howlader and Turjoman (1984) also reported an adversely affected growth at high hardness levels of 688 to 987 mg/L CaCO₃.

Survival and growth of *Macrobrachium rosenbergii* juvenile (0.045±0.007 g, 25 days old) were investigated by Adhikari et al. (2007) under different calcium hardness levels from 92 to 384 mg/L CaCO₃ for 42 days and reported that the maximum survival rate (100%) was observed at a calcium hardness level of 92 mg/L CaCO₃, while the lowest survival rate (60%) was recorded at the highest calcium hardness level of 384 mg/L CaCO₃. Maximum growth of the prawn (11.6±2.7 mg/day) was observed at 132 mg/L CaCO₃. However, there were not significant (P>0.05) changes in growth at calcium
hardness levels of 92 and 192 mg/L CaCO₃. The growth of the prawn declined significantly (P<0.05) at calcium hardness level of 228 mg/L CaCO₃ and above.

Water hardness affects fish health because it influences osmoregulation. Being open systems, fish are affected by the makeup of the surrounding water. As a consequence of osmosis, freshwater fish are subject to a continuous influx of water, while marine fish have to live with a continuous outflow of water.

Against this continuous movement of water into or out of the body, fish have to maintain a constant internal body fluid concentration – a process called osmoregulation. The greater the difference in concentration between the fish’s body fluids and the surrounding water – the greater the osmotic effect. As hard water is more concentrated than soft, there will be less difference and therefore less water influx and consequently the fish will not have to work so hard at osmoregulation. This is particularly important in cases of bacterial ulceration where water can flood into open tissues.

12.10. Hardness value for fish growth

- 60 mg/L - satisfactory for pond productivity and helps protect fish against harmful effects of pH fluctuations and metal ions;
- <60 mg/L - creates stress to fish.

Ponds with low hardness can be treated with lime for rectification.

12.11. Ammonia

The total ammonia concentration in water available in two forms, i.e NH₃ (unionized ammonia or Free ammonia) and NH₄⁺ (Ionized ammonia).

They maintain equilibrium as per the equation

\[ \text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{OH}^- \]

The low levels of un-ionized ammonia may affect the fish’s central nervous system, reduce its ability to obtain oxygen and lowers resistance to disease. The un-ionized ammonia fraction is more toxic to fish and the amount of the total ammonia in this form depends on the pH and temperature of the water. As a general rule, the higher the pH and temperature, the higher is the percentage of the total ammonia present in the toxic un-ionized form.

The survival rate, growth and feed intake were determined by Naqvi et al. (2007) for late juveniles (4.31 ± 0.18 g) of river prawn Macrobrachium rosenbergii in freshwater with total ammonia-N (NH₃-N+NH₄-N) concentrations of 0.015 (control), 0.5, 1.0 and 1.5 mg L⁻¹ for 60 days at pH 7.53 ± 0.04 and temperature 24.0 ± 2.5°C. The authors reported that survival rate was significantly (P<0.05) lower (54 ± 4.2–70 ± 5.4%) for total ammonia concentrations from 0.5 to 1.5 mg L⁻¹ [0.0139–0.0419 mg L⁻¹ of unionized
ammonia (NH$_3$)]. Growth (0.026–0.030 g day$^{-1}$ range) of the prawns did not differ for the different NH$_3$ levels but were significantly ($P<0.05$) lower compared with control (0.056 g day$^{-1}$). Feed intake rates also diminished significantly ($P<0.05$) from 77.60 ± 2.45% at control (0.015 mg L$^{-1}$ NH$_3$-N) to 48.69 ± 2.13% at 1.5 mg L$^{-1}$ NH$_3$-N (0.0419 mg L$^{-1}$ of unionized NH$_3$).

12.11.1. Un-ionized ammonia level for fish growth
- 0.02–0.05 mg/L - safe concentration for many tropical fish species;
- 0.05–0.4 mg/L - sub-lethal effects depending on the species; and
- 0.4–2.5 mg/L - lethal to many fish species.

12.10.2. Measures to maintain safe ammonia level
There are number of measures to maintain safe ammonia concentration in pond water. Generally at high dissolved oxygen and high carbon dioxide concentration, the toxicity of ammonia is reduced. Some recommended measures to reduce the effects of ammonia are as below:
- Aeration will increase the dissolved oxygen concentration and decrease the increasing pH thereby reducing toxicity.
- Healthy phytoplankton populations remove ammonia from water. Care should be taken while using fresh manure with high ammonia content. The manure should be dried to allow ammonia gas to escape before application to the pond.
- Biological filters may be used to treat water for converting ammonia to nitrite and then to harmless nitrate through nitrification process.
- A high quality feed that contains no more nitrogen (crude protein) and phosphorus than actually needed by fish should be used in ponds and also over-feeding should be avoided.
- Excessive liming should be avoided as it raises pH and high pH favours ammonia toxicity to aquatic animals.
- Water exchange can reduce ammonia concentrations in fish and prawn ponds. From both economic and environmental perspectives, water exchange should only be used when necessary.

12.12. Nitrite
Nitrite is an intermediate product in the biological oxidation of ammonia to nitrate, a process called nitrification. In most natural water bodies and in well maintained ponds, nitrite concentration is low. In water bodies with high organic pollution and low oxygen concentration, nitrite concentration may increase. High nitrite levels, combined with
low chloride and dissolved oxygen concentrations, may result in methemoglobinemia, better known as brown blood disease. High nitrite levels also affect fish growth and immunity.

12.12.1. Nitrite value for fish growth

- 0.02–1.0 mg/L - sub-lethal level for many fish species;
- 1.0–10 mg/L - lethal level for many warm water fish species.

12.12.2. Measures to maintain safe nitrite level

- Correct stocking, feeding and fertilization practices should be maintained. The ponds should be kept well oxygenated.
- Bio filtration can be done through special filters for biological conversion of nitrite to harmless nitrate.

12.13. Hydrogen Sulphide

Freshwater fish ponds should be free from hydrogen sulphide (H₂S). Hydrogen sulphide is produced by chemical reduction of organic matter that accumulates and forms a thick layer of organic deposit at the bottom. Unionized hydrogen sulphide is toxic to fish, but the ions resulting from its dissociation are not very toxic. A higher level of hydrogen sulphide lowers the immunity of fish.

12.13.1. Hydrogen sulphide value for fish growth

- 0.01–0.5 mg/L - lethal to fish and any detectable concentration of hydrogen sulphide in water creates stress to fish.
- 0.1–0.2 mg/L - prawn lose their equilibrium and create sub-lethal stress.
- 3 mg/L - prawn die instantly.

12.13.2. Measures to rectify increase in hydrogen sulphide levels

- Frequent water exchange to prevent building up of hydrogen sulphide in the water bodies.
- With liming pH of water increases which helps to reduce toxicity of hydrogen sulphide.


In surface waters, iron occurs in ferrous state (soluble compounds) or ferric state (mostly insoluble compounds). The ratio of these two forms of iron depends on the oxygen concentration in the water, pH and other chemical properties of the water. Iron compounds in poorly oxygenated waters with a low pH may harm fish where the iron is present mainly in the form of soluble compounds. As the gill surface of the fish tends
to be alkaline, soluble ferrous iron can be oxidized to insoluble ferric compounds, which then cover the gill lamellae and inhibit respiration. At a low water temperature and in the presence of iron, iron-depositing bacteria will multiply rapidly on the gills and further contribute to the oxidation of ferrous iron compounds. Their filamentous colonies cover the gills; at first they are colourless but later the precipitated iron gives them a brown colour. The precipitated iron compounds and tufts of the iron bacteria reduce the gill area available for respiration, damage the respiratory epithelium and may thus suffocate the fish. In a similar toxic action, iron compounds can precipitate on the surface of fish eggs, which then die due to a lack of oxygen.

The lethal concentration of iron for fish is not easy to measure because it depends to a large extent on the physico-chemical properties of the water. In cyprinid culture, it is generally accepted that the concentration of the soluble ionized forms of iron should not exceed 0.2 mg per litre, for salmonids this limit is 0.1 mg per litre.

In hatchery operation, sometimes, ground water is used. In that case, iron toxicity is very common. To remove iron toxicity, the water should be passed through some hays or straw so that the ferrous iron could be oxidized to ferric iron which is comparatively less toxic. Also, the ground water could be kept in a storage tank for a week and then it could be used for hatchery purposes. To oxidize the ferrous iron aerator could also be used.

The effect of iron on survival, growth and feeding of giant river prawn *Macrobrachium rosenbergii* (De-Man) juveniles was studied at the Central Institute of Freshwater Aquaculture (Adhikari *et al*. 2007). The results showed that the survival rates of *M. rosenbergii* juveniles following 60 days exposure were 100%, 83.3±3.6%, 73.3±3.3% and 63.3±4.7%, respectively at the total iron levels of 0.02(control), 0.32, 0.65 and 1.2mg/L. Average growth of the prawn exposed to 0.65 and 1.2 mg/L total iron were significantly lower (P<0.05) than in control (0.02 mg/L iron) and 0.32 mg/L treatment. Feed utilization was significantly (P<0.05) reduced in the prawns at all the iron treatments compared to control (0.02 mg/L iron). The accumulation of iron was minimum in the muscle and maximum in the hepatopancreas of the prawns.

### 12.15. Water exchange

The primary reasons for exchange of water are to reduce salinity, flush out excessive nutrients and plankton and reduce ammonia concentrations. However, daily water exchange usually does not improve water quality in ponds, and pumping costs increases. Ponds are highly efficient in assimilating carbon, nitrogen and phosphorous inputs, but if water exchange is high, these substances are discharged from ponds before they can be assimilated. Moreover, the pollution effect of aquaculture ponds increases due to water disposal. From both economic and environmental reasons, water exchange should only be used sparingly when necessary.
12.16. Conclusion

The best method for preventing water quality problems in hatchery and in aquaculture ponds is to select a site with good soils, adequate supply of high quality water and maintenance of moderate levels of prawn and fish production. If this is done, liming, fertilization and aeration will reduce negative effect of water quality imbalances. However, in some instances, sedimentation basin may be needed to prevent ponds from filling in and water exchange may be required periodically. In intensive aquaculture ponds, bottom soil treatment such as drying and liming between crops, phosphorous precipitation, turbidity removal and oxidation of bottom soils with sodium nitrate may be beneficial. Some treatments are either ineffective or potentially hazardous to the stock. Therefore, proper pond management is the key to sustainability in aquaculture, and by enhancing sustainability of pond aquaculture can improve soil and water quality in ponds and reduce the volume and pollution potential of pond effluents. Proper procedures for pond management will improve environmental conditions, sustainability and profits.

References


of the Annual Conference Southeastern Association of Game and Fish Commissioners 28:199-208.


13. Nutritional requirements and feed management for carp brood and larvae

S. C. Rath


13.1. Introduction

Carps are grown in semi-intensive polyculture system in most of the Asian countries. In this culture system catla (Catla catla), rohu, (Labeo rohita), mrigal (Cirrhinus mrigala), grass carp (Ctenopharyngodon idella), silver carp (Hypophthalmichthys molitrix) and common carp (Cyprinus carpio) are cultivated together or as monoculture. Natural food such as plankton, periphyton, benthic organisms, aquatic plants provides a part of their nutrition and supplementary feed is given to meet the rest. Carps eat both living and nonliving matters as their food. In wild culture or extensive culture, fish production entirely depends upon the live food available in that water body. In semi-intensive and intensive culture live food serves a part of the diet whereas prepared feed compensate the rest. Prepared feed or artificial diets may be either complete or supplemental. Complete diets provide all the required nutrient viz. protein, carbohydrates, fats, vitamins, and minerals for their optimal growth, health and reproduction. In contrast, supplemental (complete or compensatory) diets are intended only to help and support the live food available in pond environment. Tacon et al., (2011) reported that only farm made feeds are used for carp production in Asian countries. Ayyapan and Ali (2007) reported that 97 % of carp production in India is due to the farm made feeds supplementation. The research in fish nutrition walked along the development of seed production and husbandry as the nutrition is fundamental to the development of aquaculture. The concept of nutrition in fish encompasses food, feeds, feeding system and biological phenomena of their acceptance and utilization. Raising of brood as well as nursery rearing of larvae are two essential component in carp aquaculture where nutrition plays important role.

This paper presents a holistic overview of the fish nutrition with special reference to broodstock development in freshwater aquaculture. The concepts, principles and mechanism of the fish nutrition is described in the paper.
13.2. Appetite and satiation

Appetite is the desire for food and satiation is the fulfillment of such desire (Fig. 13.1). Appetite is a physiological phenomenon controlled by metabolic and hormonal mechanism regulated by specific hypothalamic centers. Feeding regimes and ration size depends on the appetite and satiation. As it is understood by the given schematic expression, the evacuation of gut is one of the factors for the food ingestion. So there is direct relation between length of digestive tract and the frequency of food intake. The digestive tract of any adult carp is a long tubular and coiled structure. The food after ingestion through mouth takes considerable time to pass through the anal aperture. So feeding to the brood carp once or twice a day solves the purpose. In case of larvae or juveniles the length of gut is relatively small and less coiled, therefore the frequency of feed application is more in initial life stages and needs to be manipulated as fish grows from larvae to juveniles and to adult. Water quality parameters and environmental temperature influence the appetite, feed intake and satiation.

13.3. Gustatory stimulant

Larvae of all cultivable carp species feed on the plankton as well as fine particle of formulated feed at their early stages. As they grow bigger their individual food choice is varied from each other. This diversification on food choice is prominent at family level of the individuals. Although the cultivable carps belong to family cyprinidae the food diversification exists among species. For example the preference of food of grass carp post-larvae and adult is different than that of other carps. This is because of the chemical stimulant known as gustatory stimulant. Such chemical stimulant for one species may not be same for other species. Therefore, there is a relation between the gustatory stimulant and food habit of the species. Accordingly, the feed is formulated for different life stages of the cultivable carp species.

13.4. Nutritional requirement

Several studies have been conducted on nutrient requirement and diet development of cultivable major carps (Sen et al., 1978; Renukaradhya and Varghese 1986; Mohanty...
et al., 1990; De Silva and Ganasekara, 1991; Jena et al., 1999). In grow out culture, brood raising and nursery rearing system, fish may get *ad libitum* food, but it is of no use if the food does not satisfy their nutritional requirement. Protein, lipids, carbohydrates, minerals and vitamins are the chief constituents of the fish feed, which are required to improve the spawning efficiency and breeding response of brood in hatcheries as well as survival and growth of the larvae in the nursery ponds.

### 13.5. Proteins

Protein is an essential component in the fish feed which is directly responsible for tissue building and growth. The dietary protein requirement of the carp broods is 30-35%. The formulated feed is superior than the traditional cake-bran mixture (groundnut oil cake and rice bran at 1:1 by weight). Protein requirement of carp spawn is 35-40%. The fish does not have true protein requirement but need a well-balanced mixture of essential and non-essential amino acids. About 10 amino acids viz. Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, and Phenylalanine Threonine, Tryptophan and Valine are considered as essential amino acids which cannot be synthesized in the body of fish. So incorporation of these amino acids through dietary ingredients makes the feed more useful. Requirement of Methionine and Phenyleanalyne, depends upon the amount of cysteines and Tyrosines present in the diet, respectively. The level of incorporation of such amino acids varies in different species of Asian carps and in different stages of their life such as larvae, postlarvae, juveniles and adults. However the common range of requirement is given in Table 13.1.

#### Table 13.1. Essential amino acids requirement range in cultivable carps

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Requirement range (g / 100g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>4.80-5.75</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.13-2.45</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.35-3.00</td>
</tr>
<tr>
<td>Leucine</td>
<td>3.70-4.63</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.58-6.23</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.88-3.55</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.70-4.00</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.13-4.95</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.951.13</td>
</tr>
<tr>
<td>Valine</td>
<td>3.50-3.75</td>
</tr>
</tbody>
</table>

### 13.6. Lipids

Lipids are the source of energy, essential fatty acids, and phospholipids. It provides a vehicle for absorption of fat-soluble sterols and vitamins. It also plays vital role in the formation of cell membranes and serve as the precursors of several hormones in addition to their role in prostaglandin synthesis. Lipids are classified as fats, phospholipids, spingomyelins, waxes and steroids. It also provides essential fatty acids.
As in other vertebrates, fish cannot synthesize n-3 (linolenic) and n-6 (linoleic) polyunsaturated fatty acids (PUFAs) but fish have a requirement of these 2 essential fatty acids that are to be provided from exogenous sources. About 8-10% and 4-6% of lipids is required in the carp brood and larval diets respectively (Mohanty et al. 2011). The Indian major carps have requirement of phospholipids to the tune of 4% for enhancing larval growth and survival rates. Supplementation of n-3 and n-6 PUFA is also required for brood stock diet. Incorporation of vegetable oil and fish oil of marine source in carp brood diet greatly influences gonadal maturation, breeding efficiency and spawn recovery of Indian major carps (Nandi et al. 2007).

13.7. Carbohydrate

Unlike terrestrial animals, fish require less amount of carbohydrate but carbohydrates are always included in fish diets as they are inexpensive energy source and act as pellet binder. Balanced dietary carbohydrates would enable fibre to move other nutrients in gastrointestinal tracts for proper digestion. The incorporation of carbohydrate in feed reduces the protein flow for energy purposes and thus reduces the cost of the feed (Mukhopadhyya et al., 1999). Several species of carp are considered herbivorous in nature and hence use of cheap sources of digestible energy becomes an important consideration for their growth. The requirement of carbohydrate in Indian major carp feed is in the range of 22-26% in different species and in different life cycle stages. Excess incorporation of carbohydrate as food not only reduces the feed efficacy but also affects the digestibility. More carbohydrate supplementation causes deposition of glycogen in liver and muscle and accumulation of lipid in the viscera, which in turn results the poor gonadal development and breeding response. Grass carp and common carp brood perform considerably less under the influence of carbohydrate rich feed (Rath et al 1999).

13.8. Energy

Energy is not a nutrient, but is the property of nutrients, which is released during metabolic oxidation of proteins, lipids and carbohydrates. Fish do have a low energy requirement because no energy expenditure is involved for maintenance of body temperature and due to its neutral buoyancy. Other explanations for low energy requirement are less muscle activity to maintain their position in aquatic ecosystem as many fishes have swim bladders, and less energy expenditure for excretion of ammonia, which is 85% of metabolic wastes that are excreted directly through gills into surrounding water. Physical activities like swimming, escaping from predators and stress, temperature, size, growth rate, species and food are some of the factors that affect energy requirements of fish. Proteins, lipid and carbohydrates contain 5.6, 9.4 and 4.1 kcal of GE/g respectively. Carp brood requires gross energy of 4,000Kcal/kg feed.

13.9. Protein-energy ratio

The condition where energy supply is inadequate, energy is drawn from protein sources. Excess protein is not only wasteful and uneconomical but also causes stress to fish and
aquatic pollution as well. Diets containing excess energy leads to lipid accumulation resulting in fatty fish. Therefore, a balance between protein and energy is considered important in fish diet, so that energy spares protein for growth. The optimum protein and energy (P:E) ratio is known to be size-related and is higher in small fishes. Few information is available for P:E ratio in carps are given in Table 13.2.

**Table 13.2. Dietary P:E ratio for optimum growth of carps in different species and life cycle**

<table>
<thead>
<tr>
<th>Species</th>
<th>P:E ratio (mg/kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catla fingerlings</td>
<td>97.3 (40% dietary protein)</td>
</tr>
<tr>
<td></td>
<td>109 (45% dietary protein)</td>
</tr>
<tr>
<td>Rohu fry</td>
<td>113 (40% dietary protein)</td>
</tr>
<tr>
<td>Rohu fingerlings</td>
<td>95 (38% dietary protein)</td>
</tr>
<tr>
<td>Common carp</td>
<td>108</td>
</tr>
<tr>
<td>Grass carp</td>
<td>110</td>
</tr>
</tbody>
</table>

**13.10. Minerals**

Certain minerals like calcium and phosphorus are required in greater amount, as these are required for scale and bone formation. Calcium is also needed for other physiological process such as blood clotting; muscle functioning and nerve impulse transmission etc. phosphorus is involved in energy transformation, membrane permeability, genetic coding etc. Apart from these two other minerals are chlorine, magnesium, sodium, potassium, cobalt, copper, selenium, zinc, aluminum, chromium and vanadium, required in varied quantity.

**13.11. Vitamins**

Vitamins plays important role in body metabolism. Fish requires at least eleven water soluble vitamin such as thiamin, riboflavin, pyridoxine, niacin, pantothenic acid, inositol, folic acid, choline, biotin, ascorbic acid and cyanocobalmin. It also required at least four fat soluble vitamins such as A, D, E and K. The vitamin requirements of carps per kg of diet are Riboflavin (4-10 mg), pyridoxine (4mg), pantothenic acid (25mg), biotin (1-1.5mg), inositol (200-440mg), ascorbic acid (30-50mg), vit-A or retinol (1000-2000 IU) and vitamin E (80-300mg).

**13.12. Ration size**

Ration size or the feeding rate defines the amount of feed or diet applicable to the cultivated species. The optimum ration gives best growth, health and breeding performance in brood and better growth and survival in case of nursery rearing. Underfeeding causes poor gonadal growth, disease and poor survival. Whereas, overfeeding causes water quality deterioration and increases cost of production. Ration size is different for fry, fingerling, yearling and brood carps. The fry, fingerling, yearling...
requires more energy for metabolism per unit weight. They have the potential to grow faster than the adult fish therefore they need higher ration than that of the grow-out and brood fish. Again the fry, fingerling, and yearling have their own ration size depending upon duration of culture and marketing strategy. In general the ration size of carp nursery is 400 % of the initial body weight of the spawn for first 5 days and 800 % for subsequent days (Jena and Das, 2005). For an example one lakh spawn weigh about 150-160g. So one million spawn need about 6 kg feed for first 5 days and 12 kg for subsequent days. The spawn are reared in the nurseries for 15-20 days to get fry of about 25 mm in length. The nutritional requirement of carp spawn have been evaluated over the years as 35-40% protein, 4-6 % fat, 22-26 % carbohydrate, 0.1 % vitamin B complex, 600 mg/kg vitamin C and 200 IU vitamin -A per kg feed. Feeding rate of 5-10 % and 3-5% of the bodyweight are recommended for fingerling and juvenile rearing respectively. In brood rearing system feed at 1-2% of the biomass is recommended. The ration size also depend open the water temperature as ration size of a given crop is not same during winter and summer.

13.13. Ration type

Ration can be applied in different forms viz. powder, crumble, granule, or pellet in different life cycle stages of the carps. The different types of the pellet such as floating, semi-floating and sinking etc. can be decided as per the requirement of the crop. Pelletization reduces the wastage (qualitative and quantitative) of feed in the pond. Powdered feed to larvae, crumbles for fry or early fingerling and pellet of desirable size are always preferred for brood and grow out carps.

The supplementary feed application for brood rearing is also species specific. For catla the powdered feed or floating pellets are broadcasted, for rohu semi-soaked mash feed or slow sinking pellets are suspended in the water column, whereas soaked feed ball or sinking pellets are provided in mrigal dominated ponds (Rath, 1999).


In carp culture system weaning means the transformation of food habit of spawn from natural food (plankton) to the formulated external food (supplementary feed). Spawn and early fry stages of the carp are crucial as there is no parental care. At this stage high mortality is notices due to several factors. The yolk reserve of the carp larvae exists for a limited period. Neither mouth nor the digestive system is well developed to accept and digest the external food particles. At the early spawn stage the digestive enzymes are not all operative. The most important factor is particle size, texture and palatability of the food and feed. Spawn and early fry prefer small food in relation to their mouth size and ingesting capabilities. Keeping this in view the pond fertilization programme should be undertaken to get suitable size of livefood and care should be taken to formulate and develop preferable diets for carp larvae and post larvae. The information on feed particle and pellet size is given in the Table 13.3.
13.15. Practical diets

The traditional supplementary feed used in carp culture generally consists of rice-bran and groundnut oilcake which are compounded in 1:1. Some other ingredients like mustard oilcake, sunflower oilcake, til oilcake and soybean cake are used in place of groundnut oilcake. Cake-bran mixture in conjunction with natural fish-food organisms is a common practice for brood rearing in many Asian countries as farm made feeds. This mixture is not nutritionally balanced, and is normally used to supplement protein and energy. Several diets have been developed for carp spawn, fry and brood. Some of them are given in Tables (13.4 to 13.5).

<table>
<thead>
<tr>
<th>Crop</th>
<th>Particle/Pellet size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spawn</td>
<td>&lt; 50-80 μm</td>
</tr>
<tr>
<td>Fry</td>
<td>0.5 mm dia crumbled pellet</td>
</tr>
<tr>
<td>Fingerlings (3-4g)</td>
<td>up to 2.0 mm dia crumbles</td>
</tr>
<tr>
<td>Growout / brood</td>
<td>up to 3.0 mm dia pellet</td>
</tr>
</tbody>
</table>

### Table 13.4.

<table>
<thead>
<tr>
<th>Feed formulations for IMC larvae (spawn)</th>
<th>Incorporation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Finely powered soybean meal</td>
<td>10</td>
</tr>
<tr>
<td>2 Finely powdered groundnut oilcake</td>
<td>32</td>
</tr>
<tr>
<td>3 Finely powdered fish meal</td>
<td>20</td>
</tr>
<tr>
<td>4 Finely powdered rice-bran</td>
<td>30</td>
</tr>
<tr>
<td>5 Vitamin and mineral premix</td>
<td>2</td>
</tr>
<tr>
<td>6 Phospholipid (as soya lecithin)</td>
<td>4</td>
</tr>
<tr>
<td>7 Veg. Oil : fish oil (1:1)</td>
<td>2</td>
</tr>
</tbody>
</table>

### Feed formulations for fry and fingerlings of IMC

<table>
<thead>
<tr>
<th>Feed formulations</th>
<th>Incorporation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Azolla powder</td>
<td>60.0</td>
</tr>
<tr>
<td>2 Soybean meal</td>
<td>19.0</td>
</tr>
<tr>
<td>3 Groundnut oilcake</td>
<td>13.9</td>
</tr>
<tr>
<td>4 Sesame oilcake</td>
<td>4.0</td>
</tr>
<tr>
<td>5 Rice-bran</td>
<td>2.0</td>
</tr>
<tr>
<td>6 Vitamin mineral mix</td>
<td>1.0</td>
</tr>
<tr>
<td>7 Attractant (seeds of Trigonella and Murraya)</td>
<td>0.1</td>
</tr>
</tbody>
</table>
### Feed Formulations for Fingerlings and Grow-out Culture of Carps

<table>
<thead>
<tr>
<th></th>
<th>Groundnut oilcake</th>
<th>Soybean meal</th>
<th>Rice-bran</th>
<th>Fish meal</th>
<th>Vitamin mineral mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td>26.0</td>
<td>23.0</td>
<td>33.0</td>
<td>16.0</td>
</tr>
<tr>
<td>3</td>
<td>Groundnut oilcake</td>
<td>28.0</td>
<td>20.0</td>
<td>30.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Source: Mohanty et al. (2011)
13.16. Formulated non-foliage diet for grass carp brood (Rath et al. 1999)

Grass carp brood when reared with foliage feed shows very poor gonadal development and breeding performance. In contrast those fed with a formulated diet bred luxuriantly. The suggested formulated diet contains soybean 50 kg, ground nut oil cake 25 kg, rice bran 20 kg and fish meal 5 kg in 100 kg feed. The proximate composition of the above feed was evaluated as crude protein 36.75 %; crude fat 6.80 %; crude fiber 14.25 %, total ash 13. %, nitrogen free extracts (NFE) 29.20 % and energy was 3.252 K cal/ g.

Table 13.5. Carp brood feed (incorporation of ingredients in 100 kg feed)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundnut oil cake</td>
<td>70.0 kg</td>
</tr>
<tr>
<td>Rice bran</td>
<td>28.4 kg</td>
</tr>
<tr>
<td>Common salt</td>
<td>01.5 kg</td>
</tr>
<tr>
<td>*Trace element</td>
<td>00.1 kg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>03.0 g</td>
</tr>
</tbody>
</table>

* (Trace element: ferrous sulphate 50 g, copper sulphate 8g, zinc oxide 6.7g, manganese sulphate 15.4g, potassium iodide 4.2g, cobalt chloride 2 g, calcium carbonate 13.7g). Source (Gupta et al. 1995)

13.17. Conclusion

Nutrition is one of the most important aspects of aquaculture. Each pond has its own carrying capacity so far as the natural productivity is concerned. Intensification of aquacrop certainly depends upon the exogenous feed supply. Exogenous feeds or the supplementary feeds should be cost effective, eco-friendly and nutritious to support the crop. Feeds for different brood carps are different as per their food habit and physiological need. Spawning performance and quality of seed is a function of formulated feed. Hence there is no compromise on feed formulation and diet development for brood and larvae for every success of seed production. Good seeds and good feeds make the aquaculture profitable and sustainable.

References


14. Disease management in broodstock and carp seed rearing

P.K. Sahoo and S. Das


14.1. Introduction

Aquaculture is one of the fastest-growing animal-food-producing sectors in the world and an average annual growth rate of 7.1% has been recorded in India in recent times (FAO, 2010). Although the number of fishers and fish farmers in India has increased during last years, still the average annual production has been only 2 tonnes which is really low compared to other countries like Norway (172 tonnes), Chile (72 tonnes) and China (6 tonnes). Fish seed hatcheries have grown extensively; however, there is a major constraint for getting quality seed and timely supply of seed. Further, fish disease is a major constraint for the development and sustainability of these industries which in turn influence the socio-economic status of the country. Infected broodstock and seed affect aquaculture through direct loss in production, growth reduction in fish, emergence of newer diseases and resistant pathogens, water pollution and most importantly increase the biosecurity risk. Though the economic loss due to diseases is not properly estimated, however, diseases account for 10-15% towards the production cost world-wide. For an example, in India a loss to the tune of 29524.40 INR per hectare per year was recorded due to argulosis from semi-intensive carp culture ponds (P.K. Sahoo, , 2012). There are several disease management strategies which can improve the production efficiency to a great extent. For proper management techniques, identification of pathogens causing the disease, their pathogenesis, availability of diagnostics plays major roles. This chapter deals with major pathogens prevalent in India in brood fish and seed rearing systems and also emphasizes upon the management strategies being followed to control those diseases.

14.2. Diseases in broodfish

Like any stage of development, brood fish also suffer from various types of pathogen attack including bacterial, parasitic, fungal, viral and other miscellaneous reasons. An earlier study in carps indicated that out of the total disease incidences, parasitic infections account for 70%, whereas bacterial and fungal infections being 27.5% and 2.5%, respectively (Gopal Rao et al., 1992). Some of the frequently encountered diseases in carp broods in India are given hereunder.
14.2.1. Bacterial diseases

14.2.1.1. Aeromoniasis

*Aeromonas hydrophila*, one of the motile aeromonads is an opportunistic pathogen causing a wide range of acute, chronic and covert infections in fish such as dermal ulceration, fin rot and tail rot, ocular ulcerations, erythrodematitis, hemorrhagic septicaemia, red sore disease, red rot disease and scale protrusion disease. The disease, due to *A. hydrophila*, can be of four categories; an acute rapidly fatal septicemia showing a few gross symptoms; an acute form showing symptoms of dropsy, blisters, abscesses and scale protrusion; chronic ulcerous form with furuncles and abscesses; and latent form with no symptoms (Snieszko and Axelrod, 1971). *A. hydrophila* is frequently associated with diseases in several freshwater fishes such as Nile tilapia, channel catfish, rainbow trout, catla, rohu, mrigal, catfish, eel, goldfish, *Puntius* sp., Asian catfish, gizzard shad, few brackish water species and also some marine fishes (Austin and Austin, 1993; Sahoo et al., 1998). Though, it’s a part of normal intestinal flora of fish, but it can cause severe disease under stress (Trust et al., 1974). The disease caused by this bacterium called Motile Aeromonad Septicaemia (MAS) is considered as a principal microbial disease these days. The spread of this type of septicaemia is also very fast and can cause in high rate of mortality in cultured fishes (Mohanty et al., 2008). *A. hydrophila* can be treated with one percent sodium hypochlorite solution and two percent calcium hypochlorite solution besides other disinfectants and antibiotics.

14.2.1.2. Edwardsiellosis

Edwardsiellosis is another septicaemic disease caused by *Edwardsiella tarda*, a Gram-negative bacterium, often due to poor waterquality and stress (Pressley et al., 2005; Mohanty and Sahoo, 2007). It causes small cutaneous lesions which develop into necrotic abscesses on the ventral body surface, gas filled abscesses in the muscle, distended abdomen and swollen anus due to accumulation of ascitic fluid. It has been reported to infect a widerange of fish like channel catfish, Japanese eel, Japanese flounder, tilapia, carp, largemouth bass, chinook salmon and rainbow trout. *E. tarda* have also been reported in Indian major carps (Sahoo and Mukherjee, 2002; Swain et al., 2002; Swain and Nayak, 2003; Mohanty and Sahoo, 2010). The control measure for this disease in general pond practices are treatment with idophor and improvement of water quality. However, few immunostimulants such as β-1, 3 glucan and levamisole found to be effective in rohu to prevent the mortality (Sahoo and Mukherjee, 2002).

14.2.1.3. Columnarisis disease

Columnarisis disease is one of such bacterial diseases that is currently affecting Indian aquaculture practices. *Flavobacterium columnare*, causal agent of columnarisis disease, is a facultative pathogen ubiquitous to fresh water environments. It may result in acute or chronic infections in both coldwater and warm water fishes. Epizootics of *F. columnare*...
disease frequently occur both as external or systemic infections that result in significant losses. Most species of fish are susceptible to *F. columnare* following environmental stress and when water temperatures rise. Fish with *F. columnare* usually have brown to yellowish-brown lesions (sores) on their gills, skin and/or fins. The bacteria attach to the gill surface, grow in spreading patches, and eventually cover individual gill filaments. Infected fish often show off-white to gray cotton-like patches on the head, fins, gills, body and particularly the mouth. Gill swelling may occur, gill filaments may stick together and excessive mucus may develop in the gill area. Columnaris occurs frequently in fish raised intensively and is mainly attributed to crowding stress. Once established, the infection can spread quickly and cause high mortality rates. While stressful conditions can contribute to *F. columnare* infections, the presence of *F. columnare* may also lead to secondary infections or other diseases. A sort bath in 500 ppm KMnO4 is found to be quite effective for curing this disease during general pond practices. However, there are few lytic bacteriophages like FCP1 phage which is found to quite effective in columnaris disease in catfish (Prasad et al., 2011).

Besides, there are many other bacterial pathogens affecting broodfish. *A. liquifaciens* infect the corneas of the eye causing vascularization leading to opacity and complete necrosis resulting in the death of the fish (Gopalkrishnan, 1961; Kumaraiah, 1971). *Streptococcus aureus* is also isolated in infected eye of fish (Shah and Tyagi, 1986; Mukherjee et al., 1992). *Pseudomonas fluorescens* can cause fin rot and tail rot.

14.2.2. Fungal diseases

14.2.2.1. EUS

Epizootic Ulcerative Syndrome (EUS) is a serious disease due to its epizootic spread and its clinical appearance. It affects a wide variety of fish species such as snakeheads, catfishes, minor carps, mullets and some Indian major carps. However, Chinese major carps, tilapia and milkfish are resistant to EUS. The etiological agents involved in the epizootics are complex and there is a controversy on primary etiological agent. It’s caused by a slow growing monoclonal species of fungus *Aphanomyces invadans*, which invade the tissues of susceptible fish (Chinabut and Roberts, 1999). There are some environmental factors which facilitate the entry of the fungus such as cooler or higher water temperature, low alkalinity and hardness, heavy rain, heavy influx of freshwater to estuaries reducing salinity below 2 ppt, parasites, bacteria and viruses. Even co-habitational challenge without skin damage was successful to produce EUS in *Channa* sp. (Mohan et al., 1999). The saprophytic fungi causing EUS lesions are *Aphanomyces* strains, *Achyla*, *Saprolegnia* and *Pythium* spp. Other than fungus many viruses, most frequently rhabdoviruses, couple of parasites were also identified in this disease. A number of bacteria involved in EUS outbreaks are *A. hydrophila*, *A. sorbiana* in freshwater and *Vibrio anguillarum* in brackishwater besides sporadic opportunists viz., *Micrococcus*, *Alcalagines*, *Pseudomonas*, *Bacillus* and *Nocardia* spp. The primary infection includes
darker body, anorexia, floating on surface, hyperactive and after this mortality may occur before developing lesions of acute dermatitis and ulcers. The acute symptoms include large red or gray shallow ulcers with brown, necrotic, fungus covered center at any parts of the body. A crude extract of *A. invadans* is found to elicit humoral immune response in snakehead fish (Thompson et al., 1997). Use of green water, ash, lime, and neem seeds or branches (*Azadirachta indica*) for prophylactic treatments of the EUS-infected fish in fish ponds are also effective sometimes (Inland Aquatic Animal Health Research Institute [AAHRI], Thailand, Internal Report, 2001). Immunostimulants such as Salar-bec (Miles et al., 2001) and CIFAX (developed in CIFA) are found to be better alternative towards prevention of this disease. However, lime or hydrated lime and/or salt are the widely accepted treatments.

### 14.2.2.2. Saprolegniasis

Saprolegniasis caused by *Saprolegnia parasitica* are characterized by a white to brown cotton like growth consisting of colonies of mycelium and filaments which appear as small to large patches on various parts of the body like fins, gills, mouth, eyes or muscle. This fungus is mostly seen in winter period of culture when water temperature is low and the immune system of fish is compromised along with any slight damage to skin architecture. It’s mostly a secondary pathogen of a surface lesion, but sometimes may be the direct cause of death. Frequent mass mortality due to saprolegniasis in the Indian major carps in the North Eastern hilly states of India was reported during winter season (Das et al., 2012). These infections can be recovered by treating the infected fish with 4g salt per litre of water for 2 min followed by dip treatment with 5ppm KMnO₄ for 10min, thrice every week for a period of 6 weeks. After recovery also the pond management practices such as removal of pond bottom soil, application of lime and replenishment with freshwater can prevent further infections.

Besides there are several other fungal diseases affecting fish such as *Aphanomyces pisci* where the scales became black and later fell off (Shrivastava, 1979). Branchiomyces can cause gill rot and invade the blood vessels of gills causing necrosis of the surrounding tissues (Jhingran and Pullin, 1988).

### 14.2.3. Virus infections

There are no reports of viral outbreaks in Indian freshwater fish farming. However, seropositivity of koi herpes virus (KHV) in ornamental fish has been reported from India without detectable virus in PCR.

### 1.2.4. Protozoan diseases

#### 14.2.4.1. Myxosporean infections

Myxosporean disease is caused by myxobolus cysts of varying sizes on the gills and kidney which can block the respiratory surface of the gill and excretory tubules of the
kidney. Besides scales and body surface are also infected by cysts of *Myxobolus bengalensis*, *M. catlae*, *M. hosadurgensis* and *Thelohanellus catlae*. Several species of this pathogen have been reported from various parts of the country (Chaudhuri and Chakravarty, 1970, Karamchandani, 1970; Senappa and Manohar, 1980; Senuullah and Ahmad, 1980; Ahmad, 1982; Dey et al., 1988). Since the spores are resistant to most of the chemicals, the prophylactic measures are the only remedy for this disease. However, disinfection of pond after dewatering with calcium oxide and drying for a month can be done.

Other than that ichthyophthiriasis or the white spot disease caused by *Ichthyophthirius multifiliis* leads to white nodular spots on the skin, fins and gills. Trichodiniiasis cause pale-color gill with a creamy coating due to excessive mucus secretion and mild hyperplasia of gills.

### 14.2.5. Helminthes infection

Dactylogyrosis and gyrodactylosis cause fading of gill and body colors and secretion of excessive mucus. The causative agents are *Dactylogyrus* sp. and *Gyrodactylus* sp. These monogeneans are very common when the environmental conditions deteriorate.

### 14.2.6. Crustacean infection

#### 14.2.6.1. Argulosis

Argulosis is one of the major diseases of freshwater carp aquaculture which leads to substantial production losses. This disease is caused by the branchiuranectoparasite, *Argulus* spp. which is a generalist parasite of tropical and temperate waters. A number of species such as *Argulus foliaceus*, *A. japonicas*, *A. bengalensis* and *A. siamensis* have been reported from India, *A. siamensis* being the most prevalent species of Indian subcontinent (Sahoo et al., 2011). *Argulus* infestation results in behavioral abnormalities in the fish like lethargy, irritation and loss of appetite. These ectoparasites feed on blood, besides feeding on mucus and epithelial cells by puncturing the host’s skin by the pre-oral stylet and injecting a cytolytic toxin (LaMarre and Cochran, 1992). These feeding sites become haemorrhagic and ulcerated, and provide a gateway to secondary infections by other parasites, fungi and bacteria, causing yet more economic losses (Saurabh et al., 2011). All the Indian major carp species are affected by this parasite and *Labeo rohita* is one the most susceptible species. To cope with the parasite, several control measures including treatment with parathion, hydrogen peroxide, dichlorvos andcypermethrin, trichlorofon and formalin have been in practice (Goven et al., 1980; Pike and Wadsworth, 1999; Toovey and Lynam, 2000; Klinger and Floyd, 2002). Alternative drug formulations includes medicinal plants such as methanolextracts of *Piper guineense* seeds (Ekanem et al., 2004), two bioactive compounds Osthol and Isopimpinellin (Wang et al., 2009) and azadirachtin (Kumar et al., 2012) reported as antiparasitic in few species. Besides, avermectin group of drugs and chitin inhibitors are also found to be highly effective, although drug resistance to many of the used agents are being marked by this parasite.
There are other parasitic diseases causing frequent loss. Ergasilosis caused by *Ergasilus* sp. infests gills, buccal cavity and operculum. Lernaeosis caused by *Lernaea chacoensis* and *L. bengalensis* results in sloughing off and ulceration.

### 14.3. Diseases in early stage growth

The early stage of fish are more prone to infections as their immune system is poorly developed and these are suddenly exposed to different shifts in environments (i.e. hatchery, nursery/seed rearing systems) with varied feed availability.

#### 14.3.1. Bacterial diseases

Fin rot and tail rot in young fish caused by mixed infection of *A. hydrophila* and *P. fluorescens* are very common. Edwardsiellosis can cause mass mortalities in spawn and anatomical deformities with grayish white discoloration of body in the young ones.

#### 14.3.2. Fungal diseases

The major infection in eggs, fry and fingerling stages is saprolegniasis caused by *S. parasitica* (Jhingran, 1991). It very often infects dead fertilized eggs and then spread to other live eggs resulting in their damage. The fry and fingerlings of major carps were quite susceptible to this disease (Gopalakrishnan, 1966). The fungus can be visually diagnose as a white or grey cotton wool-like growth smothering the eggs which shows a network of long, branched aseptate hyphae under a microscope.

#### 14.3.3. Viral diseases

There are few viral pathogens like rhabdoviruses which infect eggs before even hatching occurs and being transmitted by the gametes of their parents.

#### 14.3.4. Protozoan and helminthic diseases

Myxosporidia are one of the most important groups of pathogen capable of causing heavy loss in the early stages and juveniles due to emaciation and asphyxiation. Both dactylogyrosis and gyrodactylosis are major problems for fry and fingerlings. Ich, trichodiniasis, black grub diseases etc. are also being common in intensive seed rearing systems.

#### 14.3.5. Gas bubble disease

Though gas bubble disease is non-infectious, however, it’s very important to mention it here. Since fry are more susceptible to this disease which is caused due to excessive oxygen production by the ingested phytoplanktons as a result of photosynthesis inside the fry’s gut (Hoole et al., 2001). The affected fry showed symptoms such as gas-distended abdomens, impaired digestion and unusual swimming postures with ventral surface uppermost.
14.4. Management of diseases

14.4.1. Availability of diagnostics

Diagnostics of a disease is crucial before treatment or management strategy. It’s the combination of signs, symptoms, and test results that allows accurate therapy of the respective disease. The disease diagnostic methods can be microscopic, histological, microbiological, immunological or molecular. Most of the infected specimens can be directly put under a microscope to detect fungi, parasites and motile organisms. Tissue changes during infectious disease can be identified and described by preparation and staining of fish tissue sections to examine under a light microscope. Using immunohistochemical techniques certain infectious agents can also be detected in tissue sections. Bacterial diseases can be identified by various microbiology methods such as staining, motility test, culturing in selective medium and biochemical tests. Antibody-based (protein-based) immunodiagnostics can detect sub-clinical, latent or carrier state of infections and can also discriminate the antigenic differences. These involves relatively rapid, specific and sensitive techniques such as agar gel precipitation test, agglutination test, ELISA, dot ELISA, latex agglutination test and fluorescent antibody tests. Compared to all these techniques above molecular techniques such as polymerase chain reaction (PCR), restriction enzyme digestion, probe hybridization, in situ hybridization, and microarray are potentially faster and more sensitive to identify fish pathogens.

A large number of diagnostics are being developed for various fish diseases; however, few have been applied at farmers’ level. CIFA has developed both ELISA and PCR based diagnostics for some of the important bacterial diseases of finfish such as *A. hydrophila*, *E. tarda*, *Pseudomonas* sp. and PCR-based markers for species identification of *Argulus* present in Indian carp culture systems (Sahoo et al., 2011). Latex agglutination kits have also been developed in CIFE, Mumbai for various bacterial fish pathogens. However, commercialization and availability of these kits is still major drawback of the system.

14.4.2. Prevention and control measures

Prevention is always better than treatment. Therefore, the first and important criterion is water quality management in the pond ecosystem. The water quality parameters such as pH, dissolved oxygen, alkalinity, hardness, concentration of ammonia, nitrite and nitrate should be checked frequently and maintained within their normal range. The initial pond preparations and further maintenance play crucial role. Fishing appliances such as nets, buckets and hapas should be disinfected and dried before use. The broodstock may be treated with potassium permanganate before stocking or during sampling in the pond. Fish should not be kept at high stocked density. Since excessive pressure for space, food and oxygen may cause stress which promotes disease outbreaks. Few of the bacterial infections can be controlled by reducing these environmental stresses. Sometimes the uneaten feed sediments in the bottom and decomposes which attracts the growth of fungus and microorganisms. Use of salts may be effective for the
fiches showing high salt tolerance. There are few helminthes infections which could be controlled by just avoiding the secondary host such as bird and molluscs. Brood and juvenile fish should be stocked separately, since broodfish which are survivor of an infection may carry pathogens which may get transmitted to the young ones. Effective quarantine, biosecurity and better management practices are key to healthy seed production and brood raising. Exported or exotic species contains a high risk of pathogenic load which can enhance the biosecurity risk. Therefore, a proper health check is necessary for the export and import of fish with proper control strategies.

On the onset of disease, antibiotics or chemicals are the most popular way of disinfection in Indian freshwater aquaculture. However, the use of antibiotics has its own residual problems, leading to development of disease resistant pathogens, human carry-over and pollution. Chemotherapeutics, pesticides and avermectin group of drugs to tackle disease problems are also not a very good alternative due to deposition in water bodies and fish tissues. Vaccination is an effective means of disease prevention in many European and few Asian countries. However, there is no commercial vaccine available in India. Though a large numbers of laboratory scale formulations of vaccinessuch as formalin-killed whole cell bacterin, biofilm vaccine, outer membrane protein based vaccine, subunit vaccines, DNA vaccines for prevention of bacterial diseases have been described, however, commercialization is not successful for any of the pathogens in India. Probiotics are live beneficial microbes which can be supplemented with feed and influence the host’s intestinal microbial balance that in return compete with the infectious pathogens. However, the dose, mode and effects are major question for their availability and stability in commercial preparations. Chemical and herbal immunostimulants can stimulate the immune system of fish for disease control as reviewed in Sahoo et al., 2007; Sahoo and Sakai, 2010). The immunostimulants can raise the immune status of broods and the enhanced immunity could be passed to the off-spring for healthy seed production. The major immunostimulant products such as preparations of lactic acid bacteria, beta-glucans, vitamins C and E formulations, levamisole, herbal formulation etc. were being used in Indian carp aquaculture. CIFA has developed one immunostimulant, Immunoboost-C, for management of broodstock and healthy seed production. The strategy of “prevention” production of disease resistant stocks are more practicable in terms of a permanent or long term solution to diseases. By this method we can produce resistant variety seeds from already established resistant broodstocks. Approaches for resistant stocks have been made for many fish species such as common carp against dropsy (Kirpichnikov et al., 1993), rainbow trout against enteric redmouth disease, rainbow trout fry syndrome, viral haemorrhagic septicaemia (Henryonet al., 2002; 2005), Atlantic salmon against furunculosis, bacterial kidney disease and cold water vibriosis, infectious salmon anaemia (Standal and Gjerde, 1987, Gjedrem and Gjoen, 1995, Odegardet al., 2007), Atlantic cord against vibriosis (Kettunen and Fjalestad, 2006) and rohu against aeromoniasis (Sahoo et al., 2011). As this method of developing resistance stock is time consuming, attempts should be made only to
target commercially important stocks affected with economically important pathogens for which strong preventive or control measures are not available.

References


15. Off-season breeding in Indian major carps

Ashis Saha and Suresh Chandra Rath


15.1. Introduction

Indian major carp (IMC)- catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*)- are typical seasonal spawner under subtropical and tropical climates and normally do not spawn spontaneously in captivity and confined waters. They breed in flowing natural water bodies during monsoon months. In IMC the success of breeding depends on gametes recruitment and their growth as well as final maturation of gametes and spawning. These typical physiological processes are regulated by endocrine and neuro endocrine system and optimum environmental factors. These processes are naturally linked to the monsoon season confined to the period of June to August. Domestization of carp brood and scientific interventions of induced breeding system extended the spawning period of these carp towards pre-monsoon (March) and post-monsoon (September) month. During this period IMC could be induced bred for three to four times with a time gap of 45-60 day in between successive spawning. Despite, these technological progress the breeding season is confined between march to September while the lean period for IMC breeding is October to February, which is known as ‘off season’ for breeding in India.

The existence of off season in breeding is a major impediment in the seed production of the freshwater aquaculture. There are demands for the seed all across the year but the breeding is only confined to few months leading to a lean period for seed availability. Moreover, a large part of the India receives rain during June-July but the farmers have to wait even upto September to get the fingerlings for stocking in the ponds. In the seasonal water bodies, the loss of two to three months reduces the growing period as much as 30 to 40 %. Therefore, the breaking of seasonality is considered as research priorities in the freshwater aquaculture. It is in this context, the research on off season breeding has been carried out by Central Institute of Freshwater Aquaculture. The results achieved through a long period of work have been remarkable but a lot more need to be done for adoption in field conditions. The present paper deals with the principles involved in the development of off season breeding technology of IMC.
Ovarian development of the IMC during inter spawning period and induced breeding in the same season also been studied by some workers (Rath et al., 2002). The manipulation for gonadal maturation and spawning beyond the normal season i.e. during October to February is the focal point of off season development. Environmental manipulation in addition to other brood husbandry practices is a further step to promote gonadal maturity in off-season.

15.2. Review of work on seasonality of reproduction

15.2.1. Photoperiod

Seasonality of reproduction in fish is synchronized with the seasonal changes in climate, day length and food availability. For most animals and many fish species, the seasonally changing pattern of day length coordinates reproductive development. In order to use ‘the light’ as an important factor for the purpose, the perception of light and changing pattern of photoperiod must be measured. Such stimulus must be provided as a suitable “signal” for integration by the neuro-endocrine cascade, which ultimately modulates reproductive development (Chaudhuri, 1997; Baggerman, 1980). Thus, this pathway of regulation and methods has been used to operate off-season spawning to produce seed round the year (de Vlaming, 1975; Bromage et al., 1993; Thomas and Arnold 1993). Although the ‘light cue’ has long been recognized as a critical factor regulating time of reproductive development, recent information suggest that the annual cycle of reproduction is controlled by an endogenous rhythm or clock whose periodicity is ‘circannual’. And it is also envisaged that each fish species would respond in a different manner to photoperiodic entertainment, thus allowing gonadal development and spawning to occur at a particular time of the year and determines the species characteristic. For practicing this hypothesis, one would design the experimental alteration in photoperiod carefully considering the internal clock running under the ambient light cycle. As the concept of critical day lengths does not explain the results that have been reported for many fish species, the direction of change of photoperiod may be considered as an important factor in the entrainment of the internal clock and in determining the rate and timing of reproductive development (Bromage et al., 1993). Besides, the relationship of light and temperature to gonadal maturation in fish could be taken into care, as, it is clearly envisaged that temperature plays a crucial role in regulating reproductive cycle in many fish, particularly in carp (Davies et al., 1986)

15.2.2. Environment

The environmental impact on the regulation of reproduction is obviously a complex phenomenon (Fig.15.1). The combined effects of photoperiod and water temperature vary not only between species but also within same species during the gametogenesis (Billard & Breton 1981). The photoperiod manipulations have been applied in many farmed species to control out-of-season spawning (Bromage et al., 2001). In fact, the
manipulation of external factors to control any reproductive phase is generally limited by the ignorance of the precise mechanisms by which these factors act, and interact, on underlying physiological processes.

Temperature is also another critical factor and have synergetic effect for regulation of reproduction. It is thought that temperature exerts its effects on fish reproduction by direct action on gametogenesis, an action on pituitary gonadotropin secretion (Billard and Breton, 1978; Peter, 1981), an action on metabolic clearance of hormones (Peter, 1981); an action on the responsiveness of the liver to estrogen in the production of vitellogenins (Yaron et al., 1980) or and an action on the responsiveness of the gonad to hormonal stimulation (Jalabert et al., 1977). There is considerable evidence to suggest that the maturation of many carps and other cyprinids, catfish and other tropical species is affected by temperature (de Vlamming, 1975; Sundararaj and Vasal, 1976; Davies et al., 1986). Increasing temperature facilitated gonadal initiation, although a raised temperature regime alone could not initiate gonadal growth. Moreover, the specific requirement of temperature is not limited to the spawning period, which may lead to impaired egg quality, as shown in Arctic char (Gillet, 1991), rainbow trout (Davies and Bromage, 2002), Atlantic salmon (King et al., 2007), or halibut (Brown et al., 2006) and high temperatures have also been reported to inhibit ovulation and reduce fertility in certain species (Jobling et al., 1995). Thus, there is still continuing controversy as to whether the temperature effects are really direct ones and hence could be considered ‘permissive’ or whether temperature is acting as a proximate cue in its own right. A major problem with much of these data is the failure by many of the investigations to experimentally differentiate between photoperiodic effects from temperature effects.

The environmental control of the final maturation differs from the control of gonadal recrudescence (reviewed by Hontela & Stacey 1990). Although the critical event in both processes is an enhanced level of plasma GtH, the later stimulating steroid synthesis, initiating follicular rupture. Even though the gametogenesis progresses over the year, final gamete maturation will not occur as a consequence of the completion of gonadal growth. For example, in cyprinids there needs to be a rise in temperature in order to attain the ovulation state, whereas in salmonids, although vitellogenesis and spermatogenesis occur in summer, spawning is promoted by the decreasing temperatures and photoperiod of autumn (Billard et al., 1978, Breton et al., 1980, Worthington et al., 1982, Jafri 1989, Kestemont 1990). In the cyprinid Notemigonus crysoleucas, de Vlamming (1975) noticed that both temperature and photoperiod together have to be increased for the completion of gonadal development, whereas the increase in temperature alone may cause oocytes regression. Jafri (1989) observed in roach that exposure to a short photoperiod during the spawning season inhibited spawning and led to gonadal regression, whereas similar treatment during summer stimulated vitellogenesis in the liver. Exposure to warm water temperatures during the pre-spawning period reduced the gonadosomatic index in both sexes and caused atresia in the ovary.
15.3. Research in India

Research on manipulation of reproduction and spawning time in Indian fish though initiated a few decade back, but only restricted to different air-breathing fishes, such as, Heteropneustes fossilis (Sunderaraj and Sehgal, 1970 a, 1970 b; Sunderaraj and Vasal, 1976; Choube and Joy, 2002), Channa punctatus (Garg and Jain, 1985; Joy and Khan, 1991; Srivatsava and Singh, 1992, 1993) and Clarius batracus (Singh and Joy, 1998; Acharia et al., 2000), due to easy maintenance of them under laboratory conditions. Advanced and delayed maturation have been reported in a minor carp, Cirrhus reba through environmental manipulation (Vergheese 1967, 1970, 1975). Such manipulation of reproduction in IMC has been attempted recently through photoperiod alteration. However, the studies revealed that photoperiod per se plays an important role in the seasonal maturation of ovary in catla under ambient thermal condition (Dey et al., 2005). So far, little information is available on such manipulation of physiological rhythm of reproduction in Indian major carps. Neuro-endocrine rhythm through manipulation of external factors, such as photoperiod and temperature for off-season gonadal maturation spawning in IMC has been studied in CIFA. A schematic diagram of off season gonadal maturation of carps is shown in Fig. 15.1. For the first time CIFA reported off season breeding of Indian major carps during January-February (Sarkar et al., 2010).

Fig. 15.1. A schematic diagram of off season gonadal maturation of carps
15.4. Technological progress at CIFA

Advancement of sexual maturation and off-season breeding of Indian major carp (IMC) species, rohu (*Labeo rohita*), catla (*Catla catla*) and mrigal (*Cirrhus mrigala*) was obtained through photothermal manipulation during winter months. The spent fishes of preceding breeding season were subjected to long photoperiod (light: dark hours; 15.5 L : 8.5 D) in combination with above ambient temperature at 28.6°C ±1.2°C (LP–AAT, lights on at 0530 h), which led the fish for gonadal maturation. The mature fish were induced bred in eco-hatchery system with standard protocol (Rath et al 2007). The dawn and dusk periods were maintained with the help of low-watt fluorescence lamp fitted above each cistern. Artificial lights were provided in each tank with 23 W fluorescence lamps controlled with electronic timers for regulating the duration of illumination in different tanks. Tanks under long photoperiod regimes were provided with thermostat controlled heaters to maintain water temperature consistently during the experiment period. Both the sexes of the IMCs attained gonadal maturity for induced spawning between 100 and 124 days of rearing under LP–AAT condition during winter months. The spawning fecundity and spawn survival was similar with that of pre-monsoon breeding as reported by (Gupta and Rath 2005). The induced breeding response of individual carps ranged between 66 - 100%. The results clearly reveal that the photothermal manipulation is a potential tool to produce carp seed during winter months.

15.5. Future directions

The off-season maturation of carps under the influence of photoperiod reported believed to be a major step towards availability of carp seed round the year. For small fish species the influence of photoperiod is well known for spawning but in food fish like carp more research is needed to make it farmer friendly and economical in developing countries of SAARC region.

References


16. Identification of fish species using molecular tools

P. Das, L. Sahoo and P. Jayasankar


16.1. Introduction

Traditionally, identification of fish species is done by the examination of external morphological features, including body shape, pattern of colours, scale size and count, number and relative position of fins, number and type of fin rays and various measurements of body parts. In addition to this, gill rakers and otolith are also sometimes used to differentiate closely related species or for identification of fossils or stomach contents. In many cases, however, traditional identification using morphological features limits the differentiation, because they can show either considerable intraspecific variations or small differences between species. Furthermore, the identification of early life stages such as eggs and larvae is even more complicated than adult identification. Earlier methods involving protein analysis like iso-electric focusing, polyacrylamide gel electrophoresis (PAGE), starch gel isozyme pattern, high performance liquid chromatography (HPLC) and immunoassay systems such as Enzyme-Linked Immuno Sorbent Assay (ELISA) have been employed for species identification including fish. However, major disadvantages of protein analysis include the ease with which some proteins are denatured, thus preventing the comparison of cooked and raw products. In addition to this different proteins are expressed in different tissues of the same individual causing variation in protein profile for the same individual.

Advances in molecular biology technologies lead to the rapid development of identification methods owing the advantages of DNA molecule over the protein. DNA is more stable at high temperature, present in all tissue type and shows greater variation with genetic code. Secondly, it is resistant than proteins and can be amenable to PCR amplification even when partially degraded. Third, DNA can be retrieved from anything that contains few cells. Several DNA based technologies are employed for identification of aquatic organisms at species level. In the following sections, the main focus will be on the DNA markers/genes and methods used for species identification and potential applications (for a comprehensive review, refer to Teletchea 2009).
16.2. DNA markers as tools for species identification

DNA markers are important genetic tools that can be used to identify and evaluate important species, strains, stocks, populations and individuals. Identification of unique variations contributing to trait performance is the very first step in genetic improvement of cultivable species. The information gained from the above can be utilized for evolving proper breeding plans as well as for taking conservation measures for valuable populations or stocks. Suitable DNA marker for identification at the species level should be sufficiently variable between species (particularly the closest ones) and display either low or no intraspecific variations across the geographic area. Both nuclear and mitochondrial genes have been used as markers to identify fish species in the literature. Using nuclear markers to investigate species identities has the advantage that conclusions can be drawn by examining a number of independent loci.

A number of nuclear genes have been targeted for species and/or hybrid identification in fish (Table 16.1). However, slower evolution rate in nuclear genes has been an impediment for identifying closely related species. Certain characteristics of mitochondrial DNA make it suitable for species identification over nuclear DNA. Several copies of mitochondrial genome are present inside a cell amplifying preferentially a fragment within this genome than a fragment within the nuclear genome particularly when the DNA is degraded. In most of animal species mitochondrial DNA is maternally inherited, smaller in size, haploid in nature and does not undergo recombination making its use easier and straightforward. As far as rate of evolution is concerned, mtDNA

Table 16.1. Nuclear genes used for fish species/hybrid identification

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<th>Sl.</th>
<th>Gene</th>
<th>Selected references</th>
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<tbody>
<tr>
<td>1</td>
<td>5S rRNA</td>
<td>Chiara Tognoli et al., 2011; Aranishi 2005; Garcia et al., 2006</td>
</tr>
<tr>
<td>2</td>
<td>GNRH</td>
<td>Gross et al., 1996</td>
</tr>
<tr>
<td>3</td>
<td>ITS, ITS1</td>
<td>Goggin, C.L., 1994; Wyatt et al., 2006</td>
</tr>
<tr>
<td>4</td>
<td>SACTIN</td>
<td>Mc Dowell and Graves 2002</td>
</tr>
<tr>
<td>5</td>
<td>MN-32, MN-47, MN-81</td>
<td>Buonaccorsi et al., 1999</td>
</tr>
<tr>
<td>6</td>
<td>RAG2</td>
<td>Prado et al., 2012</td>
</tr>
<tr>
<td>7</td>
<td>Tropomyosin</td>
<td>Hashimoto et al., 2010, Hashimoto et al., 2011</td>
</tr>
<tr>
<td>8</td>
<td>IRBP (Interphotoreceptor Retinoid-Binding Protein- exon 1)</td>
<td>Oliveira et al., 2009</td>
</tr>
<tr>
<td>9</td>
<td>Parathyroid hormone-related protein gene</td>
<td>Kijewska et al., 2009</td>
</tr>
<tr>
<td>10</td>
<td>Rhodopsin</td>
<td>Sevilla et al., 2007</td>
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generally evolves much faster than nuclear DNA thus enabling identification of closely related species. Most frequently mtDNA gene used for identification of freshwater fish species is cyt b. Other mtDNA genes employed for identification of fish species are 16s RNA, 12s RNA, 5s RNA, COI, ND3/ND4, ATPase 8 and D-loop (Table 16.2). The most important feature of these genes is that they have both conserved regions allowing designing of universal primers amplifying a number of species, and highly variable regions allowing discrimination of even closely related species. However the efficiency of mtDNA is lost when employed for discrimination of hybrids. Because of its maternal inheritance, the hybrids are misidentified as the female species used for the production of hybrids. In contrast nuclear DNA is diploid in nature can be successfully used for identification of hybrids as one chromosome is received from each parent.

Table 16.2. Mitochondrial genes used for fish species identification

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<thead>
<tr>
<th>Sl. No.</th>
<th>Gene</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cytochrome b</td>
<td>Kyle &amp; Wilson 2007; Sevilla eat al., 2007; Baharum &amp; Nurdalilla, 2012</td>
</tr>
<tr>
<td>2</td>
<td>16S rRNA</td>
<td>Comesana et al., 2003; Chakrabortty et al., 2005; Cawthorn et al., 2012</td>
</tr>
<tr>
<td>3</td>
<td>12S rRNA</td>
<td>Kitano et al., 2007</td>
</tr>
<tr>
<td>4</td>
<td>D-LOOP</td>
<td>Quinteiro et al., 2001; Von Der Hyden et al., 2007</td>
</tr>
<tr>
<td>5</td>
<td>ATPase 6/8</td>
<td>McDowell and Graves 2002; Reid &amp; Willson 2006</td>
</tr>
<tr>
<td>6</td>
<td>COI</td>
<td>Dawnay et al., 2007; Ivanova et al., 2007</td>
</tr>
<tr>
<td>7</td>
<td>ND3/ND4</td>
<td>Gharret et al., 2001; Li et al., 2006</td>
</tr>
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</table>

We have used both mitochondrial and nuclear DNA fragments to identify Indian carp species. Species specific DNA signatures (DNA barcodes) of Indian and exotic carps viz. rohu, catla, mrigal, kalbasu and common carp have been established based on mitochondrial cytochrome c oxidase I (COI) gene. Amongother mitochondrial genes such as Cyt b, ATPase 6/8 and D-Loop (control region) have been studied for their usefulness in discriminating Indian major carps at species and population levels. MtDNA sequence alignment among species revealed comparative rates of divergence with considerably faster and more heterogeneous substitution rates for Control region as compared to Cytochrome b and ATPase 6/8. All the three genes have moderate to high variable regions allowing discrimination of these species.

16.3. Methods

The available literatures on species identification in fishes describe about ten methods (Table 16.2). Among them, the three methods such as PCR-RFLP, PCR-Sequencing and PCR-specific primers are more often used. The methods are described as follows.
16.3.1. PCR-RFLP

PCR-RFLP (Restriction Fragment Length Polymorphism) is a method in which a portion of the genome is amplified through PCR followed by restriction endonuclease digestion of the product resulting in distinguishable patterns DNA fragments between samples or species. A PCR is performed using two primers to get a specific product. After cleaning, the product is cut with a randomly selected set of restriction endonucleases, separately. Then the product is separated by agarose gel electrophoresis. This method is simple, robust, and much easier to perform. The main disadvantages are that incomplete digestion may occasionally occur and intraspecific variations could delete or create additional restriction sites. Besides, its dependence on restriction enzymes requires previous knowledge of the sample analyzed.

16.2. PCR-sequencing

This method is otherwise advocated under the name FINS: forensically informative nucleotide sequencing (Bartlett and Davidson 1992). The DNA sequence is obtained by sequencing the purified PCR fragment using the same primers. The sequences after editing are aligned between samples in order to detect species specific polymorphisms at the level of single nucleotide (SNP). The identified SNPs allow unambiguous discrimination between the species. By adopting pyrosequencing, a next generation sequencing technique it has been demonstrated that the detection of only 20 nucleotides following the sequencing primer is sufficient to identify the biological origin of samples by alignment with a reference sequence database (Balitzki-Korte et al, 2005).

16.3.3. PCR-specific primers

Sequence information of marker genes is available for many species. This facilitates alignment of sequences of phylogenetically different groups to detect single base polymorphisms and design species specific primers. Under suitable stringent reaction conditions, such primers generate a fragment which can be visualized by agarose gel electrophoresis only in the presence of DNA from a given species. This procedure is applicable only when some previous knowledge of the material analyzed is available. For example, universal primers are available for most of the mtDNA genes which can be used directly for PCR amplification of a gene across the species. One important consideration is that appropriate controls should be included to preclude the possibility of false positive or negative results.

16.3.4. PCR-SSCP

PCR-SSCP (polymerase chain reaction-single-strand conformation polymorphism) analysis is one of the simplest and perhaps one of the most sensitive methods for detection of mutations based on the relationship between the electrophoretic mobility of a single stranded DNA and its folded conformations. PCR products are heat denatured to make DNA single stranded and electrophoresed on a non-denaturing polyacrilamide
gel (PAGE) (Asensio 2001). The differential migration of the ssDNA occurs when there is a heterozygotic condition (alternative forms of gene i.e. alleles are different). The precision of this method to detect mutations is high. However, limitations in fragment size, necessity to run references samples side by side on the same gel and intra-species variation are some of the disadvantages of this technique.

16.3.5. PCR-DGGE

PCR-DGGE (Denaturing Gradient Gel Electrophoresis) method is based on the separation of PCR amplicons of the same size but with different sequences. Therefore, these fragments can be separated in a denaturing gradient gel based on their differential denaturation (melting) profiles. In DGGE gel, doublestrand DNA are subjected to an increasing denaturing environment with increasing concentrations of the denaturing agents (urea or formamide) and partially melt in discrete regions called “melting domains”. The melting temperature (Tm) of these domains is sequence-specific. Once the Tm of the lowest melting domain is reached, that part of the fragment becomes partially melted, creating branched “breaking” molecules. This behavior reduces the DNA mobility in the acrylamide gel. Therefore, DNA fragments of the same size but different base pair compositions will show a different response to the denaturing gradient. The different sequences of the DNA fragments will have melting domains with different Tm values that will run different distances in the DGGE.

16.3.6. PCR-RAPD

Random amplified polymorphic DNA (RAPD) is a molecular tool and refers to the random amplification of polymorphic DNA sequences from genomic DNA through PCR reaction using short primers. Short primers are 10 bases long and arbitrary in nature implying that no prior information on the target sequence is required. A range of short arbitrary primers are commercially available which can be used to generate a large number of PCR products at low stringency giving different fingerprint patterns. They are very useful in detecting polymorphisms at species and sub-species level. The method is very simple and straightforward having a couple of advantages such as no prior knowledge on DNA sequence is necessary, targets many loci (multilocus) at a time and speed in execution. Though RAPD analysis has become a valuable tool the technique has some drawbacks. It may not be practical to identify the species of origin in products containing mixtures of species. Second, it is not adequate for analysis of severely degraded material, because it is sensitive to amplification conditions and highly susceptible to slight changes in target-DNA quality and quantity.

16.3.7. PCR-AFLP

This technique is based on the detection of DNA restriction fragments by PCR amplification. Amplification of restriction fragments is achieved by the ligation of double standard adapter sequence to the ends of the restriction sites. These adapters contain
universal primer binding sequences that serve for primer annealing in PCR. In practice, the number of fragments simultaneously amplified is limited to 50-100 on denaturing polyacrylamide gels. The restriction fragments patterns generated by the AFLP fingerprints are a rich source for restriction fragment polymorphisms called AFLP. The first step in an AFLP analysis is restriction digestion of DNA with two different enzymes, generally a rare cutter and a frequent cutter. Next step is ligation of the restriction fragments with adapter. 3rd step is the amplification through PCR. The technique was originally adapted for constructing polymorphic marker maps. It has seen wide application both in plants and animals. AFLP combines the advantages of RAPD and RFLP, and thus displays higher reproducibility, resolution, and sensitivity at the whole genome level compared to these two techniques.

16.3.8. PCR-cloning and sequencing

This technique involves three main steps: i) the target fragment is PCR amplified ii) the PCR amplification products are cloned and iii) at last, several clones per amplification product are sequenced.

The major advantage of this technique is that it allows detecting whether several different target DNA sequences, potentially belonging to different species, are present within the studied sample. Yet, compared to the previous molecular methods, PCR-cloning and sequencing is more expensive and time-consuming, and due to which it has rarely been used for fish species identification.

Real time PCR and microarrays are the latest methods in the above series and have been in use recently. Quantitative PCR, also known as Real-time quantitative PCR, or qPCR method in which DNA amplification and quantification are evaluated simultaneously (Dalmasso et al., 2007). However, microarrays, also known as DNA chips are glass slides spotted with oligonucleotide probes that are complementary to the DNA target sequences to be analyzed. DNA microarrays enable the examination of complex mixtures of PCR products and, potentially, the identification of hundreds or even thousands of species simultaneously (Balitzki et al., 2005; Dooley et al., 2005).

16.4. Applications

The most common applications belong to the forensic, taxonomic and ecological fields. Commercially available edible products involving processed food fishes often face forensic examinations for commercial fraud with respect to false labeling, mixing of species and related things. Food poisoning is another important field of application where hazardous species are identified to eliminate from retail trade. Detection of GMO (genetically modified organism) is an important area of application where genetically altered food fishes can be identified through DNA tools (PCR) in few hours. Species discrimination and body parts identification are the important aspects that are considered under taxonomic applications. Other fields of potential application are the
identification of early life history stages, determining trophic relationships within an ecosystem, DNA bar-coding and analysis of ancient DNA by retrieving DNA sequences from museum specimens, fossil remains and other sources.

References


17. Carp hatchery construction and management

P.B. Bhakat


17.1. Introduction

In recent times, the development of aquaculture sector is gaining importance globally for its role in meeting the world’s protein food demand. Due to exponential growth for demand of fishes all around, the aquaculture is gradually transforming into aquaculture industry. However, the availability of quality seed is limiting factor for rapid expansion and growth of the sector. Rural fish seed production through small scale hatcheries is no longer in a position to supply seed to the entire sector. The epicenter of the entire sector lies in the availability of good seeds which demands for developments of the hatchery system. All efforts of hatchery design and construction for raising fish seed yields need optimization of natural riverine conditions by minimizing the limiting factors.

The suitability of site, soil and water quality, components of hatchery, construction of hatchery and its management are discussed in this chapter.

17.2. Site selection

The selection of suitable site with proper layout of hatchery components is the basic needs for proper management of the entire system. The site which fulfilled the following criteria, with minimum expense is adjudged as the best.

- The soil should be water retentive with minimum or no seepage loss and should have basic mineral nutrients.
- There should be adequate availability of water round the year.
- The all physical and chemical properties of the water are within acceptable limits.
- There should not be any source of pollution in the vicinity.
- There should be ease accessible road to the site.
- Adequate labour forces should be available nearby.
17.3. Soil and water quality

Soil at the pond’s bottom plays the most important role for water quality, retention of water for ponds and ultimately, the brood stock management.

The pH and mineral composition of soil have a direct effect on the acidity and presence of toxic metal in the water.

As most of the soils are mixture of sand, soil and clay particles, the water retention in pond is dependent on their percentage composition. The soil with more percentage of clay retains water for longer period with more than 90% water retentivity.

Silty clays, clay-loams, loams etc. generally make good quality soils for a fish pond. Sand, gravel and limestone areas must be avoided.

Water of desirable quality and quantity is another important requirement for hatchery operation. The usual sources of water for a carp hatchery are reservoirs, rivers, irrigation canals, open well, tube wells etc. Water from rivers, canals etc. carry a heavy load of silt along with organic impurities which is undesirable. It needs proper filtration before use. Water from underground sources often contain heavy metal impurities and also suffers from lacks of dissolved oxygen. Such water needs tertiary filtrations such as aeration, adsorption etc. Dissolved oxygen level in the water is increased by aeration.

17.4. Hatchery system

The hatchery system consists of various components which are enumerated below:

- The brood stock unit for rearing and management of carp broodstock with different size of ponds varying from 0.1 to 1.0ha.
- The nursery units for raising fry from stocked spawn. (4-5mm to 25-30mm)
- The rearing unit for raising fry to fingerlings (50mm and above)
- The packing and marketing unit.

The main hatchery unit (Eco-hatchery) for spawn production consists of the following essentials sub-units.

- Spawning chamber
- Egg incubation chamber
- Egg and spawn collection chamber
- Water supply system for the above units.

A detail design for various components for production of 30 lakhs/cycle has been explained here.
17.5. Spawning chamber/breeding pool

The following assumptions are made for designing the hatchery components

- 30 kg female and 30 kg male are required for 30 lakh egg production.
- 6 ppm D.O. in water is required for conducive spawning of fishes and 1 m$^3$ of water is required for 3.5 kg of brood stock.

Total brood stock for 30 lakh eggs production

$= 30 + 30 = 60$ kg

Amount of water required

$\frac{60}{3.5} = 17.14$ m$^3$

Assume height of tank = 1.20m + 0.3m (free board)

Now for diameter of the tank

Volume $= \frac{\pi}{4} d^2 h$

Where $d =$ diameter of the tank

$h =$ height/depth of the tank

$17.14 = \frac{\pi}{4} xd^2 x 1.2$

$d^2 = \frac{4 \times 17.14}{\pi \times 12} = 18.186$

$d = 4.26$ m

Say $d = 4.50$ m

Hence the diameter of the breeding tank is 4.50 m

Height of the tank = 1.2m + 0.3m = 1.5m

17.6. Incubation pool

Assume 1 m$^3$ of water is required for 7 lakh eggs production.

Quantity of water required for 30 lakh eggs.

$\frac{30}{7} = 4.3$ m$^3$

Assume the depth of chamber = 1.00m + 0.2m (free board)

A Central chamber is provided in the pool which is wrapped with 1/80nos net for
arresting the eggs from escaping and regulates circular flow for rolling of eggs and also allow the water to drainout during hatching through the central outlet. The ratio between the diameter of central and outer chamber should be 1:4.

Now D – diameter of outer chamber
d – diameter of central chamber

\[ \frac{d}{D} = \frac{1}{4} \Rightarrow D = 4d \]

For calculation of actual diameters for 30 lakh eggs production

\[ V = \frac{\pi}{4} D^2 x h - \frac{\pi}{4} d^2 x h \]

Where V – volume of water in incubation pool.
D – External diameter of incubation pool.
d-Internal diameter of the central chamber
h – Height of the pool

Now

\[ 4.3 = \frac{\pi}{4} x h x (D^2 - d^2) \]

\[ \Rightarrow 4.3 = \frac{\pi}{4} x 1.00 x ((4d)^2 - d^2) \]

\[ \Rightarrow 4.3 = \frac{\pi}{4} x 1.00 x (16d^2 - d^2) \]

\[ \Rightarrow 4.3 = \frac{\pi}{4} x 1.00 x (15d^2) \]

\[ d^2 = \frac{4.3 \times 4}{\pi \times 1 \times 15} = 0.365 \]

d=0.6042

Rounding to practicality
d=0.65 m and
D = 4d = 4 \times 0.65 = 2.6 m

Hence depth = 1 m + 0.2 m (free board) = 1.2 m
Diameter of the pool = 2.6 m
Diameter of the central chamber = 0.65 m
Considering production of 500 lakh spawn in a season, the proposed hatchery is planned to operate six cycles in one month to meet the requirement of spawn.

Infrastructure required:

(i) 01 no spawning pool : 4.50 mdia and 1.5 m.
(ii) 02 nos incubation pool : 2.60 m dia each and 1.20 m
(iii) Egg/spawn collection chamber : 1.5 m x 1.5 m x1.2 m (2 nos)
(iv) Overhead water tank : 30,000 ltrs (5 m X 3 m X 2 m)

Considering the requirement of standard velocity of flow in both the above breeding and hatching pool, the water requirement in breeding pool is 2 l/sec and in incubation pool is 0.5 l per second. Let us find out the minimum quantity of water required in each pool excluding initial filling. Breeding pool water requirement is 2 l./sec./pool i.e. 7.2 m³/hr. Hence, the overhead water tank is designed for 30 m³. The same water tank will take care of incubation pool for 6 - 8 hours and spawning pool for 3 -4 h separately. Therefore, the size of the tank may be 5m x 3m x 2m (h) with a minimum tagging height of 4.5m to regulate the flow in the spawning and incubation units.

17.7. Spawn and egg collection chamber

A brickmasonry rectangular cistern adjacent to spawning and breeding pool is required for collection of egg and spawn along with water flow from both the pool. The same chamber also can be divided in two parts for use of egg and spawn separately.

1.5 x 1.5 x 1.2m size egg collection chamber and 1.5 x 1.5 x 1.2m size spawn collection chamber may be constructed with complete drainage, overflow and screening arrangement.

17.8. Open well and tube well

A well or tubewell of 20,000 liters/hr. yielding capacity is required for the smooth operation of above hatchery complex. Accordingly a suitable pump may be installed in the pump house below overhead tank.

17.9. Ponds

10nos. of nursery ponds of size: 20 x 20 x 2m (d) each and 2nos of brood stock pond of size : 20 x 50 x 2.5m (d) each is required to be constructed as a minimum need of the project for spawn rearing and brood stock management.

17.10. Shed

A semi-open type shed of 10 x 6m size is required to be provided for housing the breeding and hatching pools etc.
17.11. Land

Minimum 1 ha. of low cost land is required for establishment of the above complex.

17.12. Constructional steps

- All the component/structures as mentioned above may be made with locally available materials with a view to brings down the cost.
- However, all the circular pools, chambers, channels etc., may be made with 1st class brick work (1:4) with cement concrete base. The open overhead water tank may be a RCC storage tank (15,000 ltrs) staged with RCC columns and brick wall. The space below the overhead tank may also be used as a room for pump house.
- PVC pipes and valves are preferred for water supply and drainage network of whole complex.
- 3" dia PVC pipes and valves may be used for egg and spawn collection and also for drain out.
- There may be two egg collection pipe from the centre of the breeding pool, while one leads to egg collection chamber, the other may connected to hatching pool for direct passing of eggs for hatching.
- Four inlets at 45 degree to breeding pool wall (one 2"dia and three 1" dia) may be provided at wall side sloped floor level in the pool with water control valve to create desired flow in the breeding pool.
- Six nos. of duck mouth inlets drawn from a 2"diaPVC pipe line with valve are required to be installed suitably at the floor level of hatching pool to create circular flow for movement of eggs for hatching. The 2" dia PVC pipe may be installed under the floor of the tank at the time construction while the valve chamber may be positioned just outside the wall.
- The water holding surface of all the pools and cisterns should be very smooth by providing proper neat cement finish and painting.
- Provision of showers may be provided to the breeding and hatching pools to use the facility as and when needed.

References (Suggested reading)


