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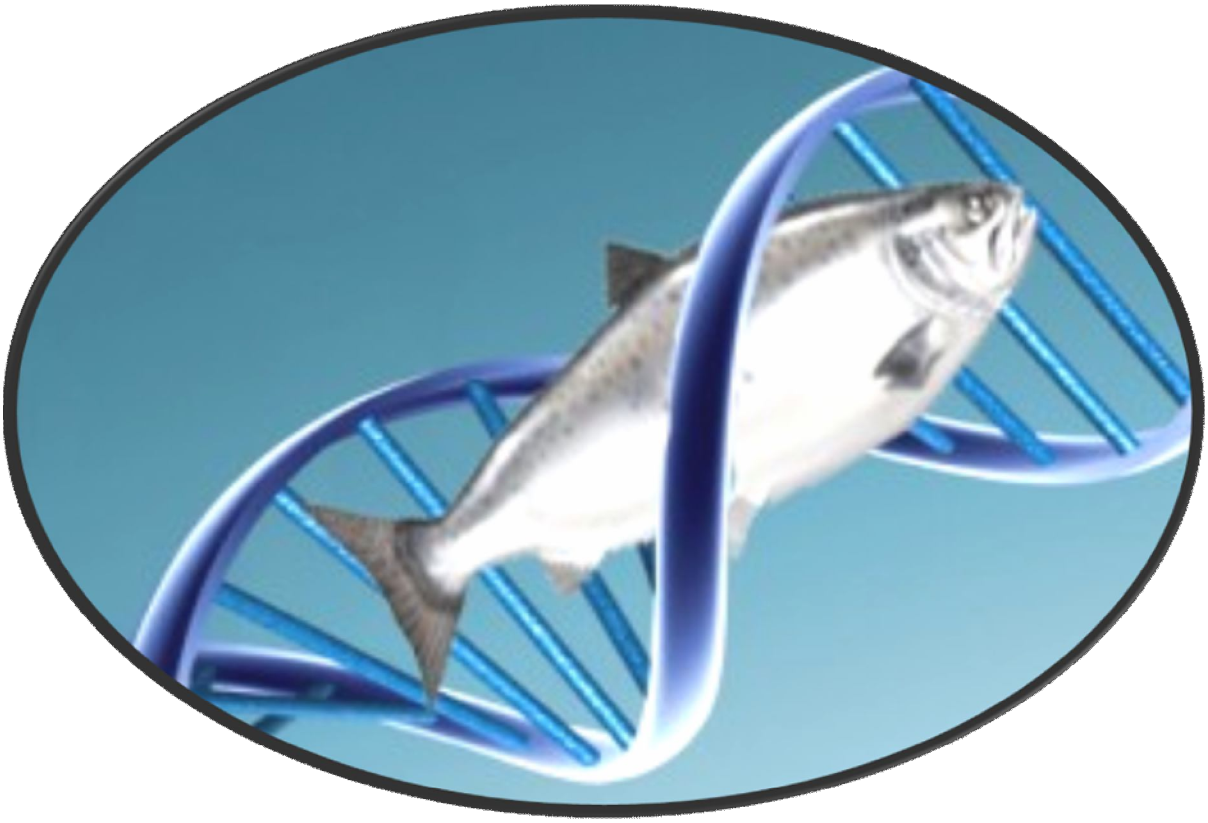
AQUACULTURE BIOTECHNOLOGY



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ENVIS Newsletter on Environmental Biotechnology is a half-yearly publication publishes articles related to the thematic area of the ENVIS Centre. Popular or easily intelligible expositions of new or recent developments are welcome.

Manuscripts should be typewritten (font should be Times New Roman and font size ought to be 12) on one side of the paper in double spacing with maximum of 6-8 typed pages.

Figures and typed table should be in separate pages and provided with title and serial numbers. The exact position for the placement of the figures and tables should be marked in the manuscript.

Articles should be sent to

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EDITORIAL

Rural population in India has been facing series of problems which affect their progress and quality of life. Most significant among these problems are lack of gainful employment leading to food insecurity, illiteracy and poor health. Biotechnologies have played an important role in the development of food products over centuries. Biotechnological research and development are growing at a very fast rate. The biotechnology has assumed greatest importance in recent years in the development of fisheries, agriculture and human health. The science of biotechnology has endowed us with new tools and tremendous power to create novel genes and genotypes of plants, animals and fish. The application of biotechnology in the fisheries sector is a relatively recent practice. It is a promising area to enhance fish production. The increased application of biotechnological tools can certainly revolutionise our fish farming besides its role in biodiversity conservation.

In this newsletter (Vol. no. 29), we have attempted to discuss the **Aquaculture Biotechnology** related issues.

(S. C. Santra)

IN THIS ISSUE:

AQUACULTURE BIOTECHNOLOGY

ABSTRACT

Aquaculture is the fastest growing food sector in the world with its increasing role for economy and safe food strategy. Due to the continuing depletion of the fish stocks, farming of aquatic organisms such as fish, crustaceans, molluscs and aquatic plants, is now a substantial global industry supplying a significant proportion of the aquatic products consumed. The demand for fish is rising rapidly. Aquaculture could help to meet increasing demand, and biotechnology can make a great contribution to improve aquaculture yields. Shortage in food supply and high prices are the possible important risks in the future. The aquatic products are the valuable sources of protein and essential nutrient components for global food security and eliminating malnutrition. Aquaculture also plays an important role in rural economies through creation of new employments. In these cases, aquaculture outputs will need to be enhanced several fold in order to meet the rising demands for fish and other aquatic products in coming years. Biotechnology has potential to affect aquaculture and can provide at least a partial solution to the problem of feeding the world's growing population because without dramatic increases in production this cannot be achieved. Biotechnology provides powerful tools for the sustainable development of aquaculture, fisheries, as well as the food industry. The applications of biotechnology options seem to be good potential for increasing aquacultural productivity, food security and environmental quality. Modern biotechnology has also opened up an opportunities to increase production and enhance the quality of fresh and processed farmed species. In addition, farmed species are now being developed to resist disease, and this will reduce losses and allows increased production on same area, and therefore bring possible benefits to rural areas. Finally biotechnology can contribute significantly to aquaculture industry, for example by helping to make more diversification in farmed species that will more attractive to consumers. This news letter discusses the biotechnological application in aquaculture.

1. State of Aquaculture in India.

The country has a long coastline of 8118 km and equally large areas under estuaries, backwaters, lagoons etc., conducive for developing capture as well as culture fisheries. The inland fishery resources include 1.96 lakh kms stretch of rivers and canals, 29.07 lakh hectare reservoirs, 24.40 lakh hectare ponds and tanks, 7.98 lakh hectare of beels, derelict water bodies and 12.40 lakh hectare brackish water areas (Handbook on Fisheries Statistics, 2014). Marine fish production was lower than inland fish production till 1995. In 2000 Marine and Inland fish production was almost equal. And then marine production crosses inland fish production from 2005 till date showing accelerated growth. Tough fish production is increasing in both Inland and marine resources (Table.1).

Table: 1 Fish production in India

Year	Inland (lakh tonnes)	Marine (lakh tonnes)
2014-15	65.77	34.91
2013-14	61.36	34.43
2012-13	57.20	33.20
2011-12	52.95	33.71
2010-11	49.81	32.50

Source: CIFRI & CMFRI

Fig: 1 Fish production in India

Aquaculture resources comprise a wide variety of animals and plants (and their genetic resources) such as fish; crustaceans, molluscs, seaweeds and other aquatic plants. Aquaculture contributed 43% of aquatic animal food for human consumption. Aquaculture remains important sources of food, nutrition, income and livelihoods for a large number of people around the world. Global fish production from capture has remained relatively stable over the past two decades while fish production through aquaculture has progressivly increased. It is a very important economic activity and a flourishing sector with varied resources and potentials. India is also an important country that produces fish through aquaculture in the world. India is home to more than 10% of the global fish diversity. Presently, the country ranks second in the world in total fish production with an annual fish production of about 9.06 million metric tonnes. It has been playing a significant role in the economy and in supporting the livelihood of an estimated 14 million people in the country (Fig.2). Its contribution includes

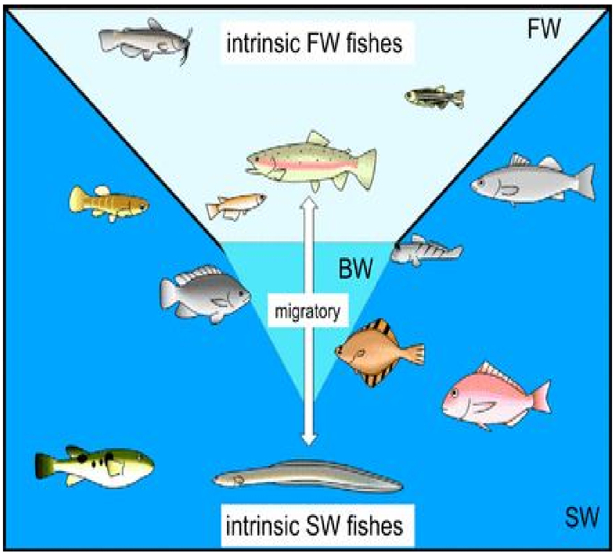


Fig: 2 The relative position of the fish to Fresh water (FW), brackish water (BK), or Sea water (SW) indicates the degree of "intrinsic" habitat to FW or SW fish.
Source:http://ajpregu.physiology.org/content/

✓ National income

Fisheries and aquaculture provides goods and services. It's share in the country's Gross domestic product (GDP). As per the estimates of the Central Statistical Organisation (CSO), of the Government of India, the value of GDP from fisheries sector during 2011–2012 was Rs 65 541 crores, which is 4.47% of the total GDP of agriculture and allied sectors.

✓ Fish production

The estimated fish production was 4.88 million tonnes consisting of 3.418 million tonnes from marine fisheries and 1.3 million tonnes from inland fisheries in the year 2014 (FAO, 2016). Considering the farmed aquaculture finfish production, the India was the third producer after China and Indonesia. In 2014 the India produces 4. 39 million tonnes of from Inland aquaculture and 0.090 million tonnes from marine/coastal aquaculture production.

✓ Eco-tourism

Several islands, beaches, backwaters and mangrove forests have become hot spots of eco-tourism. Which may be generating several millions of rupees as income.

✓ Foreign Exchange earnings

Foreign exchange earnings through export of aquaculture products is a top priority of the Union Government of India for sustaining economic growth of the country. Fisheries exports constitute about 18% of agricultural exports of the country. About 100 products of fish are exported to many countries. The export of marine products has steadily grown over the years. Marine products account for 1.1% of the total export from India.

✓ Livelihood support and employment

Fisheries remains a major source of livelihood for the rural poor, particularly the coastline areas of the country. The country has enormous inland and marine waters providing immense livelihood and employment opportunities to the fisherfolk. According to an estimate, about 14 million people derive livelihood and employment from fisheries (www.nfdb.org) (Fig 3).

Fig: 3 Rural livelihood development from Aquaculture

Aquaculture in India has evolved as a viable commercial farming practice from the level of traditionally backyard activity over last three decades with considerable diversification in terms of species and systems, and has been showing an impressive annual growth rate of 6-7 percent. While the carp-based freshwater aquaculture, mainly constituted by the Indian major carps, such as, catla, rohu and mrigal, has been contributing over 90% of the aquaculture production satisfying the domestic need, the shrimp-based coastal aquaculture contributes to only about 5% of the export earnings.

- ✓ **Development of Aquaculture in India**

Major improvements have been achieved through enhanced husbandry procedures, improved nutrition, enhanced disease diagnosis and therapies and the application of genetics to production traits. Although several aquaculture species have been greatly improved through the application of genetics, much greater improvements can be accomplished (Dunham et al., 2001). Genetics can greatly contribute to production efficiency, enhancing production and increasing sustainability. Resource utilization can be greatly improved and impediments to sustainability, such as slow growth of fish, inefficient feed conversion, heavy mortality from disease and the associated use of chemicals, loss of fish from low oxygen levels, inefficient harvest, poor reproduction, inefficient use of land space and processing loss, can all be diminished by utilizing genetically improved fish.

Several national and central institutions and state agricultural and other universities undertake fish genetic research. Some of these are the Central Institute of Freshwater Aquaculture (CIFA), Central Inland Fisheries Research Institute (CIFRI), National Bureau of Fish Genetic Resources (NBFGR), Central Institute of Fisheries Education (CIFE), University of Agricultural Sciences (UAS), Bangalore (at Fisheries Research Station, Hesaraghatta, and College of Fisheries, Mangalore), Centre for Cellular and Molecular Biology (CCMB), Madhurai Kamraj University (MKU) and Bose Institute etc.

2. Importance of Biotechnology in Aquaculture

Aquaculture faces many challenges over the next decade, notably, combating diseases and epizootics, broodstock improvement and domestication, development of appropriate feeds and feeding mechanisms, hatchery and grow-out technology, as well as water-quality management. These all present considerable scope for biotechnological and other technology interventions. Aquaculture biotechnology can be described as the scientific application of biological concepts that enhance the productivity and economic viability of its various industrial sectors (Liao and Chao, 1997) and biotechnology is one of the tools that hold much promise towards addressing these aquacultural problems. The relatively new tools of biotechnology offer significant opportunities to improve aquacultural productivity and environmental quality. In recent biotechnology has developed creative new methods to detect the gene liable for specific characteristics, such as disease resistance, nutrient composition, and insert them into another fish or aquatic organism (Fletcher *et al.*, 2011). Biotechnology offers tremendous potential for improving production and it provides opportunities to reduce the need for additives in feeds and the use of chemicals (*e.g.* hormones). Advanced biotechnology in feed are rapidly evolving and promise to improve the composition, digestibility and bio-availability of feed towards high growth rate (Brinker and Reiter, 2011). The application of different biotechnological tools in aquaculture contribute to increase the intensity and products at less economic and environmental cost, thereby helping to build global food security and more sustainable and environmentally friendly farming (Fig.4).

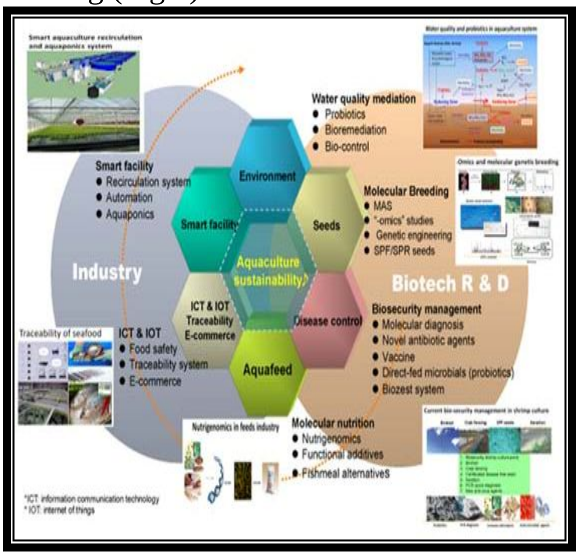


Fig: 4 Aquaculture biotechnology for research and industry
Source: http://www.eurekaselect.com/

It provides powerful tools for the sustainable development of aquaculture, fisheries, as well as the food industry. Increased public demand for seafood and decreasing natural marine habitats have encouraged scientists to study ways that biotechnology can increase the production of marine food products, and making aquaculture as a growing field of animal research. Modern biotechnology is already making important contributions and poses significant challenges to aquaculture and fisheries development. It has potential to affect aquaculture and can provide at least a partial solution to the problem of feeding the world's growing population. Modern biotechnology has also opened up opportunities to increase production and enhance the quality of fresh and processed farmed species. In addition, farmed species are now being developed to resist disease, and this will reduce losses and allows increased production on same area, and therefore bring possible benefits to rural areas. Finally biotechnology can contribute significantly to aquaculture industry, for example by helping to make more diversification in farmed species that will more attractive to consumers. Modern biotechnology will be a useful for the genetic improvement of aquacultured species and the protection and management of wild aquatic populations.

Biotechnology is offering valuable options such as protein expression, microsatellite, gene mapping and genomic, DNA vaccines, DNA chips, proteomics, transgenic technology and embryonic stem cell technology. This technology provides genetic manipulations, molecular cloning, embryo manipulation, genetically-engineered diagnostics, immunoprophylactic agents. All of these applications could help to improve the selective breeding, hybridization, productivity, health, growth, nutrition, cryopreservation and conservation of genetic resources in aquacultural stocks for the benefit of mankind. Products derived from marine organisms have been utilized to remediate the environment, increase food supply, develop new industrial processes and create new pharmaceuticals.

In India, biotechnology is making a significant contribution to the development of aquaculture:

- The improvement of fish feed quality
- Genetic engineering in order to improve the protein value of fishes
- The reproduction of recalcitrant species
- Fish disease diagnoses
- Hybridoma technology and genetic engineering that could be employed for the production of vaccines.
- The treatment of effluents and other pollutants generated by aquaculture

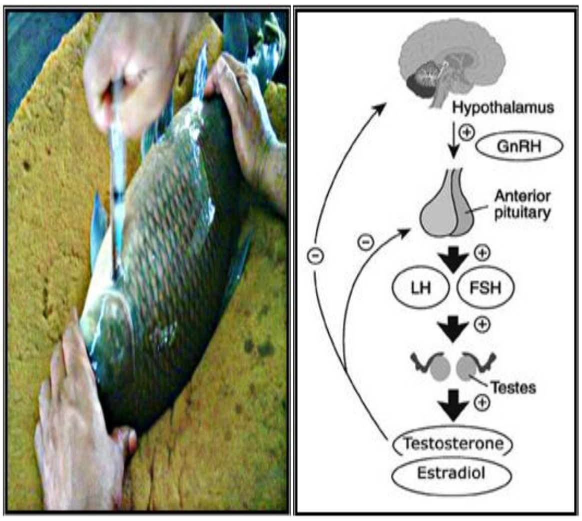
Many Funding agencies encourage institutions to do research on transgenic fish, cloning of the gene and introduction into the major carp induced multiple spawning.

a) Fish Productivity and Induced breeding

Carp culture forms the backbone to freshwater aquaculture practice in India. Carp culture in India was restricted to as homestead backyard pond activity in West Bengal and Odisha until late 1950s, with seed from riverine sources as the only input resulting in low level of production. Importance of fish culture as an economically promising enterprise was gradually implemented in India. By then, non-availability of quality fish seed and lack of scientific culture know-how constrained the growth and further development of carp culture.

Induced breeding of carps and catfishes, hatcheries for mass-scale spawning, seed rearing and carp polyculture are some of the epoch-making technologies actually guided by the freshwater aquaculture development(Fig 5). The sector has also shown considerable diversification in recent years with the adoption of other species such as catfishes and freshwater prawns, due to their higher market demand and economic values. While production of 4–5 tonnes under carp polyculture is quite common, farmers of several regions are able to produce 8–12 tonnes/ha/year. Integrated fish farming with livestock and horticulture has not only been able to utilize the by-products/wastes as principal inputs, but also made the farming practice highly remunerative and farmers' friendly. Development of improved rohu (Jayanti) through selective breeding with a record of 17% higher growth response per generation after seven generations is a major achievement. Availability of balanced supplementary feed for different life stages for diversified cultivable species and appropriate disease management measures are some of the important other developments.

The technological breakthrough in induced breeding of carps through hypophysation in 1957 revolutionized freshwater aquaculture of the country. With assured supply of quality seed, the techniques of seed rearing and grow-out culture of carps had undergone faster development and refinement through research and development made by the Pond Culture Division of the CIFRI and Government of India respectively, and further by their multi-locational trials by state governments. In fact, the development of freshwater aquaculture in the country only finally became recognized and established following the establishment of the Pond Culture Division at Cuttack in 1949 under CIFRI.



A **B**

Fig: 5 (A) Induced breeding of fish (B) Mechnisms of induced breeding
Source: http://mushahida.weebly.com/ and www.ads-uk.org

Numerous hormones have been used to induce reproduction. Gonadotropin releasing hormone (GnRH) is now the best available biotechnological tool for the induced breeding of fish. The induced breeding of fish is now successfully achieved by the development of GnRH technology (Lakra and Ayyappan, 2003). Mechnism of induced breeding.

b. Transgenic fish production

An organism that has a foreign or modified gene integrated in its genome using the *in vitro* genetic techniques of genetic engineering is called trsgenesis. Transgenic fish technology has great potential in the aquaculture industry. By introducing desirable genetic traits into fishes, mollusks, and crustaceans, superior transgenic strains can be produced for aquaculture. These traits include faster growth rates, improved food conversion efficiency, resistance to some known diseases, tolerance to low oxygen concentrations, and tolerance to extreme temperatures. This technology helps for modifying or improving the genetic traits of commercially important fishes, mollusks and crustaceans for aquaculture. Palmitter *et al*. (1982) first produced transgenic mouse by introducing metallothionein human growth hormone fusion gene (mT-hGH) into mouse egg. It show the dramatic increase in growth rate. So this technology triggered a series of attempts on gene transfer in economically important animals including fish (Fig.6)

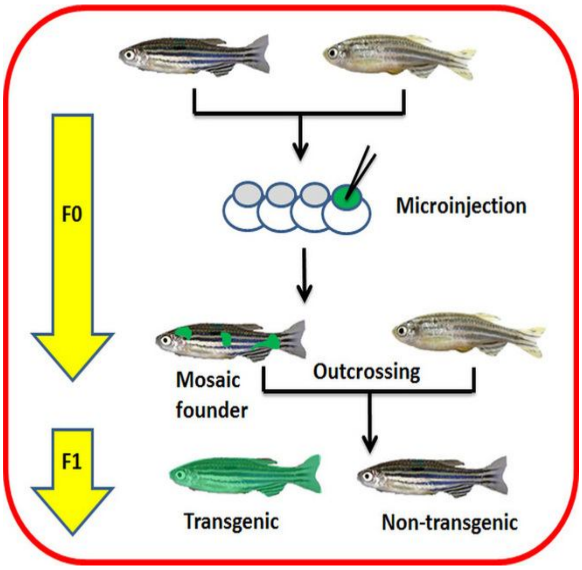


Fig: 6 Process of trasgenenic fish production
Source: http://zgenebio.weebly.com/transgenic-fish-services.html

Transgenic technique provides a rapid and efficient method for fish improvement across a wide range of species by direct gene manipulation. Gene transfer studies in fishes have been initiated for developing some superior strains useful in aquaculture. The first recorded instances of production of transgenics or genetically modified organisms (GMO) in aquatic species were in rainbow trout and in gold fish. Since then, many fish species have been used to produce GMOs in several parts of the world. The most popular gene used in aquatic species is the growth hormone (GH) with an aim to enhance the growth rate of cultivable species. Growth hormone genes of fish origin have been shown to be more effective than those of mammalian origin. Transgenic ornamental fish popularly called as “glow fish”, harbouring fluorescent genes isolated from jellyfish has recently opened new possibilities for producing new multi-coloured fluorescent fish. Fish gene transfer methods are

- Micro injection
- Electroporation
- Gene gun injection
- Use of Retroviral
- Vector Lipofection
- Use of embryonic stem cells
- Tissue injection
- Gene targeting method

Genetically modified fish are being developed for both academic and applied goals, allowing the production of useful model systems as well as new genetic strains with improved characteristics for aquaculture. A variety of genes have now been introduced into fish with the goal of influencing traits such as growth, maturation, freezing tolerance, flesh quality and disease resistance. The production of appropriate genetically modified organisms or GMOs offers considerable opportunities for wide range of aquacultural species. A number of transgenic fishes were developed in all over the world. Some examples are given in table-2.

- **Growth enhancement**

Growth rates of many fish species are slow. The efficiency of growth and feed-conversion can also be increased in finfish by creating transgenic fish that incorporate a gene construct encoding growth hormone. The generation of transgenic fish with the transfer of growth hormone (GH) genes has opened new possibilities for the manipulation of growth in economically important fish species. An important contribution of genetic engineering is the enhanced growth of farmed fish by transferring growth hormone (GH) genes (Fig. 5). The ability to manipulate growth rates through the introduction of additional growth hormone (GH) genes was demonstrated originally in mice (Palmiter et al., 1982), but has been applied successfully to a number of other animals, including fish e.g. (Du et al., 1992; Devlin et al., 1994). So it can provide advantages for aquaculture by shortening production times, enhancing feed conversion efficiency, and controlling product availability. Dramatic growth rate has been shown using this technique, especially in salmonids (Devlin et al., 1994). Secretion of GH typically occurs in bursts and varies seasonally in fish. However, the transgenic fish used in this study employ an antifreeze gene promoter from ocean pout to drive the expression of a GH transgene. The secretion of GH of transgenic fish is not under control of neuroendocrine factors, but occurs predominately in the liver and perhaps other tissues (Fletcher *et al*., 1985, 1990; Gong *et al*., 1992).

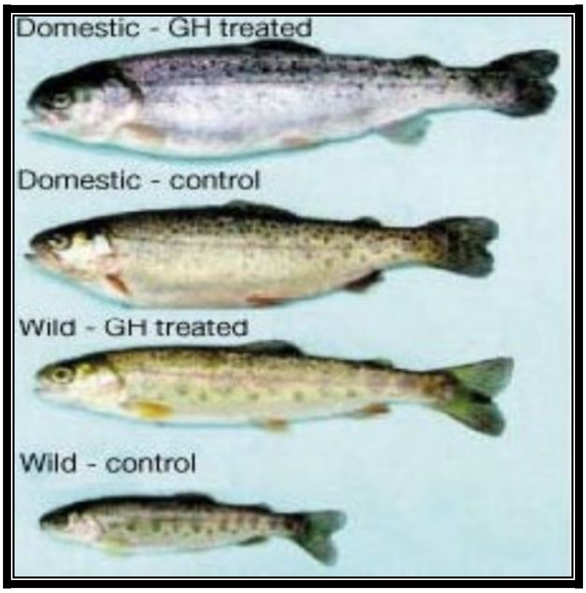


Fig: 7 Growth enhancement of transgenic fish http://www.slideshare.net/sumedhadahal/aquaculture-15133559

• Freeze and cold tolerance

An increased resistance of fish to cold temperatures has been another subject of research in fish transgenics for the past several years (Fletcher *et al*., 2001). Cold-water temperatures pose a considerable stressor to many fish, and few are able to survive water temperatures much below 1°C. However, some marine teleosts have high levels 10–25 mg/ml of serum antifreeze proteins AFP or glycoproteins AFGP which effectively reduce the freezing temperature by preventing ice-crystal growth. The introduction of AFPs to goldfish also increased their cold tolerance, to temperatures at which the control fish all died (12 h at 0 °C Fletcher *et al*., 2001 (Fig. 6).

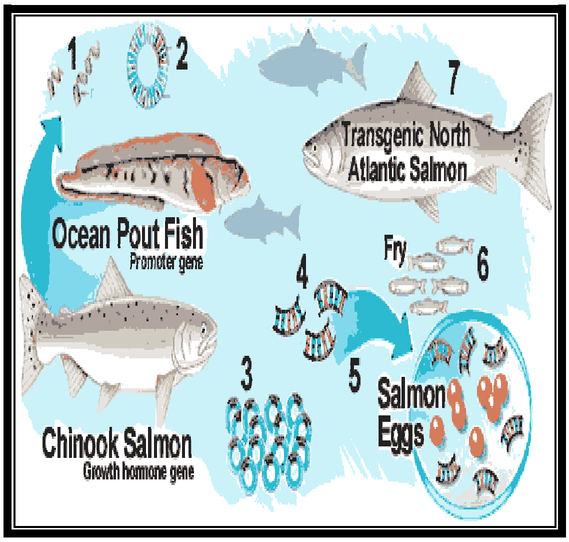


Fig: 8 Cold tolerance of transgenic fish Source:http://www.mindfully.org/

• Diseases resistance in transgenic fish

Both innate and adaptive immunity systems are required for fish and higher animals to overcome the pathogens. The adaptive immunity can protect the host from infection by specific microbial pathogens, and it requires a longer period of time for the host to develop antigen-specific antibodies and immunologic memory against the specific pathogen. Though fish are living under a lower temperature, it will require a longer time to develop adaptive immunity against infection of microbial pathogens (Boman 1995; Bonizzi and Karin 2004). It suggest that transgenic manipulation of antimicrobial peptide genes may lead to the production of fish strains with elevated resistance to bacterial, viral and protozoan pathogens.

Table: 2 Transgenic fishes of the world

Species	Gene(s) Introduced	Desired effect	Country
Atlantic Salmon	AFP-salmon GH	Cold tolerance, Increased	Canada

		growth and feed efficiency	
Coho Salmon	AFP-chinook salmon GH	Increased growth	Canada
Chinook Salmon	AFP - salmon GH	Increased growth and feed efficiency	New Zealand
Rainbow Trout	AFP - salmon GH	Increased growth and feed efficiency	Canada
Cutthroat Trout	AFP – chinook Salmon GH	Increased growth	Canada
Tilapia	AFP - salmon GH	Increased growth and feed efficiency	Canada, UK
Tilapia	CMV-tilapia GH	Increased growth	Cuba
Tilapia	Tilapia insulin gene	Production of human insulin	Canada
Salmon	Rainbow trout lysozyme, flounder pleurocidin	Disease resistance	United States,
Striped Bass	Insect cecropin	Disease resistance	United States
Mud Loach	Loach and mouse MT - Mud loach GH	Increased growth and feed efficiency	China, Korea
Channel Catfish	RSVLTR-GH	Increased growth	United States
Common Carp	Salmon and human GH	Increased growth, disease resistance, tolerance of low dissolved oxygen	China,United States
Indian Major Carps	Human GH	Increased growth	India
Goldfish	AFP-GH	Increased growth	China
Abalone	Various promoters coho salmon GH	Increased growth	United States
Oysters	Various promoters coho salmon GH	Increased growth	United States

Source:Transgenic fishes under development for use in aquaculture (FAO, 2000)

Application of transgenic technology in fish biology and aquaculture

- Improves economics of fish culture
- Increase growth rate
- Increase market size
- Improves feed conversion efficiency
- Utilize low cost diet
- Improve cold tolerance
- Improve freeze resistance
- Increase brood stock fecundity Control smolting, reproduction and sex Reduce aggression
- Improves disease resistance

c. Chromosome Engineering

Chromosome engineering is "the controlled generation of chromosomal deletions, inversions, or translocations with defined endpoints." Chromosome manipulations can be carried out easily in fishes due to their external fertilization and embryogenesis. A number of research works has been conducted worldwide on chromosome set manipulation. This techniques are important in the improvement of fish breeding as they provide a rapid approach for gonadal sterilization, sex control improvement (Fig. 8).

✓ Polyploidy

Polyploidy individuals possess one or more additional chromosome sets, bringing the total to three in triploids, four in tetraploids and so on. Induced triploidy is widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management. The induce triploids and other types of chromosome set manipulations in fishes and the applications of these biotechnologies to aquaculture and fisheries management are well described (Purdom, 1983; Chourrout, 1987 and Thorgaard, 1983).

✓ Gynogenesis

The gynogenesis process is the development of animal with excessive maternal inheritance. The application of gynogenesis in fisheries is of particular interest to breed high level of inbreeding in a single generation. The method of combining use of induced gynogenesis with hormonal sex inversion has been developed for several aquaculture species (Gomelsky *et al.*, 2000).

✓ Androgenesis

Androgenesis is the process by which used in aquaculture to generating homozygous lines of fish and in the recovery of lost genotypes from the cryopreserved sperms. Androgenetic individuals have been produced in some species of Cyprinids, Cichlids and Salmonids (Bongers *et al.*, 1994).

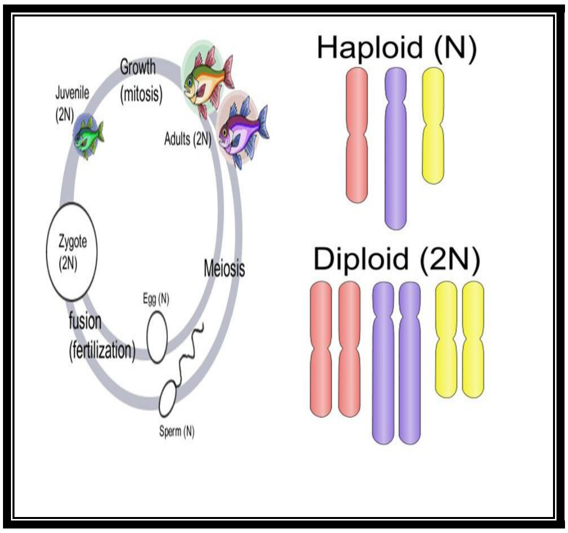


Fig: 8 chromosome engineering
Source:http://slideplayer.com/slide/5815921/

The researchers of University of Agricultural Sciences(UAS), Bangalore studies on the production of monosex population of *C. carpio* through hormone sex reversal and progeny testing as an alternative approach to overcome sexual maturation and unwanted reproduction. They also are carrying out research to evaluate performance of crossbreeds of Indian major carp in collaboration with the International Center for Living Aquatic Resources Management (ICLARM).

There are a number of breeding programmes that can be used to improve a population of fish genetically. Selective breeding and crossbreeding (also called "hybridization") are the two traditional approaches that have been used for years, and they have been used to improve all major crops and livestock grown by farmers. In early 1990s CIFA, Bhubaneswar in collaboration with the Institute of Aquaculture Research of Norway (AKVAFORSK), undertook genetic improvement of *L. rohita* through selective breeding. The fish *L. rohita* was chosen as the model fish, not only because of consumer preference, but also because of its poor growth in many polyculture systems and prone to parasitic infection (Fig.9).

Fig: 9 Selective breeding of fish
Source:http://www.fao.org/docrep/006/X3850E/X3850E03.htm

3. Fish health protection technology

Aquaculture faces many challenges such as diseases broodstock improvement, development of appropriate feeds and feeding mechanisms, hatchery and grow-out technology, as well as water-quality management. Disease problem is the major constraint for development of aquaculture. Aquaculture biotechnology can enhance the productivity (Liao and Chao, 1997) by solving the diseases. Biotechnological tools such as gene probes, polymerase chain reaction (PCR), use of vaccines and immunostimulants are showing great potential in this area and gaining popularity for improving the disease resistance in fish and shellfish in all over the world. Gene probes and PCR based diagnostic methods have developed for a number of pathogens affecting fish and shrimp (Karunasagar, 1999). In case of finfish aquaculture, numbers of vaccine against bacteria and viruses have been developed (Fig.10).

Fig: 10 Method for detection of pathogen in fish
Source: http://www.slideshare.net/

Genetic biotechnologies are being used to improve fish health through conventional selection for disease resistance and through the use of molecular investigation of pathogens for characterization and diagnosis. DNA-based technologies are being used now to characterize different species and strains of pathogens. Once the pathogen is characterized, DNA probes can be developed to screen for specific pathogens in tissue, whole animals and even in water and soil samples. These techniques are being used to detect viral diseases of marine shrimp throughout the world and for bacterial and fungal pathogens in fishes in many areas (Subasinghe 2009; Subasinghe and Bondad-Reantaso 2006).

4. Fish Feed biotechnology

Biotechnology offers opportunities for development of alternatives to fishmeal, especially plant based protein sources, by enhancing production and processing techniques. Other technologies also offer potential for enhancing the efficacy of feed delivery. As a result of these concerns with fish meal, researchers are using biotechnology to produce alternative plant-based protein source (Adelizi, 1998). Plant protein has the potential to address the problem of phosphorus pollution. For Prairie crops to be used as the main protein source for fish, they must be processed into a concentrate. Biotechnology is often used in this processing; plant protein also requires processing because plants contain what are called antinutritional compounds that serve as a defense mechanism. These compounds must be destroyed during processing or they could harm the fish or interfere with the fish’s ability to utilize the feed.

5. Bio-Remediation of pollution in Aquatic Environment

Farmed aquatic animals are much more sensitive to their immediate environment than land animals. The water, in which they depend for oxygen and a range of other important chemicals also takes up their waste products and may carry pollution from the nearby environment (Mandany *et al.*, 1996). The process of disease in aquaculture species is thus much more strongly connected to environmental factors. Biotechnology field that has developed in aquaculture, because of the nature of this relationship, is that of bio-remediation.

Currently, there are several microbial products in the market for aquaculture use. In addition to live bacterial cells, enzyme preparations, plant and yeast extracts are also used in aquaculture ponds. To mention few of them like Biostart, Liqualife, Pond pro VC, Nitro clear and Eutro clear and many probiotic formulations, which are marketed the elucidation that bacteria, which improve water quality, may be good for animal health. In aquaculture, heavily stocked ponds can become hypereutrophic. Using a combination of nitrifying bacteria with probiotic formula will address ammonia and reduce the organic sludge within the system.

6. Conservation of fish genetic resources

Cryopreservation of gametes is one of the important ex situ methods of conservation of germplasm and has wide ranging applications in aquaculture and fisheries management. The technology of cryopreservation of fish spermatozoa (milt) has been adopted for animal husbandry. The first success in preserving fish sperm at low temperature was reported by Blaxter (1953) who fertilizes Herring (*Clupea herengus*) eggs with frozen thawed semen. The spermatozoa of almost all cultivable fish species has now been cryopreserved (Lakra 1993). Aquatic gene bank however suffers from the fact that at present it is possible to cryopreserve only the male gametes of finfishes and there in no viable technique for finfish eggs and embryos (Fig. 11).

Short term preservation-

In which the sperm is stored for a few hours to a few weeks in an unfrozen state or at above the freezing temperature 0°C to - 6°C (Stoss, 1983; Martin *et al.*, 1994).

Long-term preservation-

Preservation of gametes along with suitable extender medium and cryoprotectants for long term in a frozen state at a very low temperature (-196 ± 1°C) (Scott and Baynes, 1980; Piironen, 1993). It involves long-term preservation and storage of biological material at a very low temperature usually at -196 ^{0}C, the temperature of liquid nitrogen. It is based on the principle that very low temperatures tranquilize or immobilize the physiological and biochemical activities of cell, thereby making it possible to keep them viable for very long period.

To date, successful cryopreservation of fish semen were reported in more than 200 freshwater species and 40 marine species worldwide (Gwo, 2000). Even though in general many successes have been achieved in fish semen cryopreservation, the technique remains as a method that is difficult to be standardized and use in all types of fishes.

The ICAR-NBFGR, India has built up a strong database on over 2800 indigenous fish species of the country; generated information on population genetic structure of over two dozen of prioritized endemic and commercially important finfish and shellfish species; development of molecular markers to document intra and inter-specific genetic divergence in fish species; development of functional genomic resources, DNA barcodes of over 500 aquatic species and about a dozen cell lines of important finfish species; developed sperm cryopreservation protocols of about 30 important finfish species; developed techniques for ex-situ gene banking of endangered species; impact assessment of exotic fish species and diagnostic capabilities for emerging pathogens of aquatic organisms; and contributed significantly in bringing out several policy documents for the country.

The NBFGR has a major role to play in ensuring the growth and sustainability of fisheries and aquaculture of the country by adopting timely and appropriate strategies to catalogue, providing scholarly information on genomic resources for effective utilization and managing the valuable aquatic genetic diversity for the future generations.

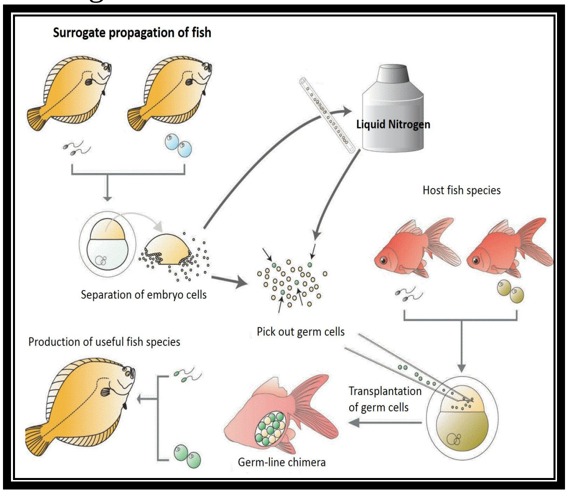


Fig: 11 Gamet preservation of fish
Source:http://www.fsc.hokudai.ac.jp

7. Technology Transferred (Lab to land Programme)

Technology transfer has been a key area in aquaculture which has improved substantially with the support from the government through involvement of the agencies. The sustainable development in aquaculture is always linked with policy support and proper strategies for technology transfer and adoption for effective resource utilization, market linkage, post-harvest product processing, etc. Aquaculture technologies developed and transferred in India. Some examples are given in table-3.

Table: 3 Technology developed and transferred in different organisations

Name of the technology & process techniques developed	Institute developed
Immunostimulant 'Immunoboost-C' developed for use in brood fish to raise healthy larvae.	CIFA, Bhubaneswar, Odisha
CIFACURE developed to protect bacterial and fungal infections of ornamental fish.	
Development of manual handy cryofreezer	
Diagnostic kits developed for diagnosis of bacterial diseases of freshwater fishes include: i) Dot-ELISA kit ii) Indirect ELISA kit, iii) Spot agglutination kit, iv) Antigen captured ELISA kit and v) Competitive ELISA kit for diagnosis of several pathogenic bacteria in freshwater fish species.	
Selective breeding of rohu with average of 17% higher genetic gain per generation after five generations	
35 microsatellite markers isolated and characterized in rohu.	
Species-specific RAPD profiles of M. rosenbergii, *M. malcolmsonii and M. gangeticum* generated.	
Process of unambiguous identification of intergeneric hybrid of rohu and catla (Family Cyprinidae) using molecular marker based kit.	
Immunostimulant for shrimp health management (aquastim)	College of Fisheries, Mangalore
Kit for rapid detection of Vibrio harveyi in hatcheries	
Combi kit for simultaneous detection of white spot syndrome virus (WSSV) and monodon baculovirus (MBV)	College of Fisheries, Mangalore
Development of recombinant protein vaccine against *Aeromonas hydrophila*	
Development of recombinant protein vaccine against *Edwardsiella tarda*	
Rapid detection of *edwardsiella tarda* by PCR	
Bio-control of luminous bacteria in shrimp hatcheries using bacteriophages	
Pearl production techniques through tissue culture in the Indian pearl oyster (*Pinctada fucata*) and the abalone, (*Haliotus varia* and other pearl producing mollusks	CMFRI, Chennai. India,
A product containing anti-inflammatory principles from green mussel *Perna viridis L.* and a process thereof	
A process to prepare antioxidant and anti-inflammatory concentrates from brown and red seaweeds and a product therof	
A product containing anti-inflammatory principles from brown seaweeds and a process therof	
Polyclonal antibody based immunodiagnostic kits for detection of different bacterial pathogens in finfish and shellfish	Central Institute of fisheries Education
Polyclonal antibody-based immunodiagnostic Assay for the detection of white spot syndrome virus	Anna University, Chennai and CAH Abdul Hakeem College, Malvishram
Bioreactors for prawn hatchery	Cochin University of Science and Technology

Source: www.nbfgr.res.in
www.cifa.in
http://www.cmfri.org.in/
http://www.cofmangalore.org/

8. Some Successful Case studies on Aquaculture Biotechnology in India

✓ PCR-based pathogen detection in shrimp aquaculture

Shrimp aquaculture sector has been continuously facing the challenge of new diseases, particularly viral pathogens. Advanced molecular research and biotechnology, DNA-based detection technologies such as PCR methodologies available for all the major shrimp viruses. A number of PCR, nested-PCR and hybridization tests have been developed for virus detection. The tests use a range of different PCR primers and hybridization probes targeted to different and poorly defined sites in the virus genome. Several RT-PCR tests are also available. The application of PCR detection of viruses of broodstock and postlarvae in both *Penaeus monodon* and *Penaeus vannameii* is now practised in all countries producing commercial shrimp at all levels (http://www.fao.org).

✓ Application of Probiotics as an Environmental Treatment and Feed Additive in the Production of Farmed Marine Shrimp in China.

In China, the first probiotic strain for aquaculture was reported by the Sichuan Agricultural University in 1995. By 2001, the Ministry of Agriculture had approved 13 probiotic strains for agriculture practices. Now, there are more than 400 companies producing *Bacillaceae*, yeast, *Rhodospirillaceae* and *Lactobacillus* probiotics for agriculture, with an annual production of 10000 tonnes.

✓ Antiviral property of marine actinomycetes against white spot syndrome virus in penaeid shrimps

Actinomycetes have provided many important bioactive compounds of high prophylactic and therapeutic value and are continually being screened for new compounds. In this communication, the results of a study made to determine the effectiveness of marine actinomycetes against the white spot disease in penaeid shrimps are presented. Twenty-five isolates of actinomycetes were tested for their ability to reduce infection due to WSSV among cultured shrimps. When these actinomycetes were made available as feed additives to the post-larvae of the black tiger shrimp *Penaeus monodon* for two weeks and challenged with WSSV, the post challenge survival showed variations from 11 to 83% (Kumar, 2010).

✓ Selective breeding of rohu (*Labeo rohita*) in India

Selective breeding was carried out to genetically improve one of the most preferred carp species (rohu). 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 (Das Mahapatra, 2012).

Reference

www.nbfgr.res.in

www.cifa.in

http://www.cmfri.org.in/

http://www.cofmangalore.org/

http://www.fao.org

http://ajpregu.physiology.org

http://www.eurekaselect.com

http://mushahida.weebly.com/ and www.ads-uk.org

http://zgenebio.weebly.com/transgenic-fish-services.html

http://www.mindfully.org

FORTHCOMING EVENTS		
Events	**Date**	**Place & Correspondence**
National Conference on Urban Environmental Management in India: Problem and Prospects	20 –21st January, 2017	Jaipur, Rajasthan, India http://mnit.ac.in/news/news.php?news_id=1692
124th International Conferences on Metallurgy Technology and Materials (ICMTM)	25- 26th January, 2017	Pune, Maharashtra, India http://theires.org/Conference2017/India/1/ICMTM/
International Conference on New and Renewable Energy Resources for Sustainable Future	2-4th February, 2017	Jaipur, Rajasthan, India http://www.iconrer2017.org/
7th International Conference on Environment Science and Engineering (ICESE 2017)	11-13th April, 2017	Seoul, South Korea http://www.icese.org/#
8th International Conference on Biotechnology and Food Science (ICBFS 2017)	11-13th April, 2017	Hanyang Institute of Technology (HIT), Hanyang University, Seoul, South Korea. http://www.icbfs.org/

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