**In this article we will discuss about:- 1. Meaning of Transgenic Fish 2. Development of Transgenic Fishes 3. Controlled Culture of Transgenic Fish and Feed 4. Gene Transfer Technology for Development 5. Applications 6. Environmental Concerns  7. Transgenic Fish could Threaten Wild Populations 8. Transgenic Fish Invasive Species.**

**Meaning of Transgenic Fish:**

A transgenic fish is one that contains genes from another species. A transgenic fish is an improved variety of fish provided with one or more desirable foreign gene for the purpose of enhancing fish quality, growth, resistance and productivity.

Typically, genes of one or more donor-species are isolated, and spliced into artificially constructed infectious agents, which act as vectors to carry the genes into the cells of recipient species. Once inside a cell, the vector carrying the genes will insert into the cell’s genome.

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A transgenic organism is regenerated from each transformed cell (or egg, in the case of animals), which has taken up the foreign genes. And from that organism, a transgenic variety can be bred. In this way, genes can be transferred between distant species, which would never interbreed in nature.

The application of genetic engineering to animals, like potatoes with built-in insecticide, could provide numerous benefits, including the possibility of a safer, cheaper food supply and the creation of new sources for inadequate pharmaceutical resources.

With the advancement in the field of genetic engineering, the application of its commercial use has also increased. Aquatic animals are being engineered to increase aquaculture production.

The use of genetic engineering and rDNA technology has done miracles in medical and industrial research. The transgenic fish are being promoted as the first marketable transgenic animals for human consumption.

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One of the most important aspects between fish and other terrestrial animals for cultivation and genetic improvement is that, usually, fishes have higher levels of genetic variation and hence more scopes for selection than most mammals or birds.

Using the gene transfer technology, scientists now have created a genetically engineered variety of Atlantic salmon that grow to market size in about 18 months, otherwise the fish takes about 24-30 months for becoming market size fish. It is also hoped that we can now modify a large number of fishes with fast growing characteristics and bring Blue Revolution.

**The following are the important points needed for genetic engineering (gene transfer) to produce transgenic fish:**

(1) A gene sequence is to isolate for the particular characteristics; for example, growth hormone gene.

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(2) These genes (gene sequence) are then inserted into a circular DNA known as plasmid Vector (enzymes endonucleases and ligases are used).

(3) Plasmids are harvested in the bacteria to produced billions of copies.

(4) Plasmids are introduced into linear DNA. The linear DNA is sometimes called a gene cassette because it contains several sets of genetic material in addition to new inserted gene; for example, growth hormone gene. The technology is available to integrate genes in germ line of developing individual (fish) and finally transmitted into further generations.

(5) Making the cassette a permanent part of fish’s genetic makeup.

**Development of Transgenic Fishes:**

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Development of transgenic fish has focused on a few species including salmon, trout, carp, tilapia and a few others. Salmon and trout are cash crops while the others primarily provide sources of protein. Currently, about 40 or 50 labs around the world are working on the development of transgenic fish.

About a dozen of them are in the U.S., another dozen in China, and the rest in Canada, Australia, New Zealand, Israel, Brazil, Cuba, Japan, Singapore, Malaysia, and several other countries. Some of these labs are associated with companies that expect to commercialize their fish in a few more years.

Many of the fish under development are being modified to grow faster than their wild or traditionally bred aquaculture siblings.

Faster growth is usually accomplished by transferring a fish growth hormone gene from one species of fish into another. The faster growing fish not only reach market size in a shorter time, they also feed more efficiently. Trout growth hormone (GH) was used to produce transgenic carp with improved dressing properties. Such transgenic carp are recommended for production in earthen ponds.

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**Transgenic Salmon:**

The Atlantic salmon is engineered with a pacific salmon, growth hormone driven by the arctic antifreeze promoter gene. The rapid growth of that transgenic salmon is achieved, not so much by the transgenic growth hormone as by the antifreeze gene promoter that functions in the cool water desirable for salmon flavour.

Devlin (1994) research scientists with Fisheries & Oceans, Canada, in West Vancouver, British Columbia has modified the growth hormone gene in Coho salmon by developing a gene construct in which all the genetic elements are derived from sockeye salmon.

The transgenic Coho grew on average 11 times faster than unmodified fish and the largest fish grew 37 times faster. The growth hormone levels in the transgenic fish are high year-round, rather than falling off in the winter as occurs in ordinary salmon. Devlin (2001). The modified salmon are large enough to be marketed after one year, in contrast to standard farmed salmon that do not reach market size for at least three years.

**Transgenic Tilapia:**

Tilapia fish, native to Africa, are cultured world-wide as **“poor man’s food”,** second only to carp as warm water food fish, and exceeding the production of Atlantic salmon (whose market value is twice that of tilapia). Tilapia has been exten­sively genetically modified and promoted as a transgenic fish exclusive for isolated or contained production.

Transgenic tilapia, which is modified with pig growth-hormone, has three times larger than their non-transgenic siblings. Tilapia genetically modified with human insulin grew faster than non-transgenic siblings, and could also serve as a source of islet cells for transplantation to human subjects.

**Transgenic Medaka Fish:**

Purdue animal scientist Muir and Howard (1999) used tiny Japanese fish, Oryzias latipes called medaka to examine what would happen if male med akas genetically modified with growth hormone from Atlantic salmon. Inserting a gene construct consisting of the human growth hormone driven by the salmon growth promoter into medaka produced the transgenic medaka.

The viability of groups of modified and conventional fish was measured at three days of age, and 30 percent fewer transgenic fish survived to that age. The researchers calculated that large males had a four-fold mating advantage, based on observations of wild-type medaka. In another experiment Silk moth genes were introduced into Medaka fish to create resistance to bacterial pathogens.

**Transgenic Zebra Fish:**

The tiny zebra fish (Bmchydanio rerio) that lives in aquariums, was genetically modified to produce a fluorescent red pigment, and is being promoted for sale as a household aquarium pet, the **“goldfish”.**

The goldfish caused a stir in the United States because regulation of such transgenic pets is murky and none of the major regulatory agencies: Food and Drug Administration (FDA), United States Department of Agriculture (USDA) or Environment Protection Agency (EPA), has been willing to take the lead in regulating the goldfish (even though USDA does deal with pet animals).

The goldfish is available for sale from January 5, 2004 without regulatory approval in United States (Fig. 43.1).

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Gong (2003) developed novel varieties of the Zebra fish. Three**“living colour”** fluorescent proteins, green fluorescent protein (GFP), Yellow fluorescent protein (YFP), and red fluorescent protein (RFP or dsRed), were expressed under a strong muscle-specific mylz2 promoter in stable lines of transgenic zebra fish.

These transgenic zebra fish with vivid fluorescent colours (green, yellow, red or orange) fluorescent proteins can be seen with naked eyes under both daylight and ultraviolet light in dark. The green fluorescent protein (GFP) is originally isolated from the jellyfish (Aequorea tictoria).

**Transgenic common Carp:**

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Thomas T. Chen, director of the Biotech­nology Centre at the University of Connecticut, Storrs, transferred into common carp the growth hormone DNA from rainbow trout fused to a sequence from an avian sarcoma virus.

The genetic material was injected into fertile carp eggs with microinjection. The offspring of the first generation of transgenic fish grew 20 to 40% faster than their unmodified siblings. Chen is also developing transgenic catfish, tilapia, striped bass, trout, and flounder.

Research associate Amy J. Nichols and Professor Rex Dunham (1999) in the department of fisheries and allied aquaculture at Auburn University, Auburn, Ala., have developed transgenic carp and catfish that grow 20 to 60% faster than standard farmed varieties.

They use microinjection and electroporation to inject another copy of a fish growth hormone gene into fertile fish eggs. The growth of the resulting modified carp and catfish is stimulated by extra fish growth hormone.

In India, research in transgenic fish was initiated in Madurai Kamaraj University (MKU), Centre for Cellular and Molecular Biology (CCMB), Hyderabad and National Matha College, Kollam with borrowed constructs from foreign scientists.

The first Indian transgenic fish was generated in MKU in 1991 using borrowed constructs. Scientist in India has developed experimental transgenic of rohu fish, zebra fish, catfish and singhi fish.

Genes, promoters and vectors of indigenous origin are now available for only two species, namely rohu and singhi for engineering growth. Transgenic rohu recently produced from indigenous construct at Madurai Kamaraj University has proved to be eight times larger than the control siblings. This transgenic rohu attains 46 to 49 grams body weight within 36 weeks of its birth.

**Auto-Transgenesis:**

Indian scientists are concentrating on developing transgenic fish through auto-transgenesis which involves just increasing the copies of growth hormone genes present in a fish as opposed to allotransgenesis which amounts to transfer of genes from different species.

The increase in growth homone genes leads to an increase in flesh content. Indian scientists feel that auto-transgenesis is safer and less controversial. According to T.J. Pandian of the school of biological sciences in the Madurai Kamaraj University, the generation time of most fish species is shorter and breeding frequency is relatively higher.

A single female can produce several hundred or thousand eggs and thus provide a larger number of genetically identical eggs. Besides, the most important advantage is that the fertilization is external and can be readily controlled by experimental manipulation.

According to Pandian, “the limited availability of transgenes of piscine origin had been the major hurdle in production of transgenic fish. However, with advancements in molecular biology, more than. 8500 genes and cDNA sequences of piscine origin have been isolated, characterized and cloned in the world.”

**Controlled Culture of Transgenic Fish and Feed:**

Pond commercial culture is effective for carp and tilapia, but more difficult with salmon and trout. Currently, pond culture is suitable for carp and tilapia because the fish are vegetarians, carnivorous salmon and trout depend on a diet of fish and fishmeal but the worldwide stock of feed fish has diminished and suitable vegetable meat substitutes must be found.

Atlantic salmon (as typical cold water carnivores) cannot thrive on a diet of rapeseed oils but the fish can achieve maturity if finished with fish oils at least 20 weeks near the end of their maturity cycle.

GM oil rapeseed with enhanced production of long chain fatty acids are proposed to serve as feed for pond cultured fish. And glyphosate- tolerant GM canola meal has been pronounced substantially equivalent to non-GM canola as feed for rainbow trout.

**Gene Transfer Technology for Development of Transgenic Fishes:**

The most commonly used methods in fish biotechnology are chromosome manipulation and hormone treatments, which can be produced triploid, tetraploid, haploid, gynogenetic and androgenetic fish.

Other popular methods of gene transfer in fish are microinjection, electroporation of sperms, electroporation of eggs and incubation of sperms. Following are the main steps in gene transfer for development of transgenic fish.

**A. Preparation of DNA Construct:**

Desired transgene should be a recombinant gene or DNA construct, which is constructed in plasmid that contains an appropriate promoter-enhancer element and a structural DNA sequence.

The foreign genes are typically introduced with strong genetic signals, promoters and/or enhancers, which enable the foreign genes to be expressed at very high levels continuously (or constitutively), effectively placing those genes outside the normal metabolic regulation of the cell, and of the transgenic organism resulting from the trans­formed cell.

**There are three main types of transgenes:**

**(1) Gain-of-Function:**

These transgenes are able to increase particular function in transgenic individual after their expression. For example growth hormone genes from mammal and fish linked to appropriate promoter-enhancer element and a structural DNA sequence to produce GH transgene.

This GH transgene when express in transgenic individuals increases production of growth hormone leading to enhanced growth of transgenic animal.

**(2) Reporter Function:**

These transgenes are able to identify and measure the strength of promoter-enhancer element.

**(3) Loss of Function:**

This transgene is not yet used for modification of transgenic fish. Such transgenes are used for interfering with the expression of host genes. The promoter-enhancer elements of transgenes are linked to a growth hormone gene of fish.

Hence transgenic fish contain extra DNA sequences that are originally derived from same species. Gene construct is then introduced into fertilized egg or embryo, so that transgene be linked to genome of each cell of egg or embryo.

**B. Gene Transfer by Microinjection:**

Microinjection is most successfully and widely used technique for gene transfer in fish. One method of microinjection technique involves the use of fine injection needle for introducing DNA into cut site in the cell. While doing so it destroys those cells that are in direct contact with the injected DNA.

To ensure the integration of the DNA it should be injected to intact cells close to the cut site. The injection apparatus consists of a dissecting stereomicroscope and two micromanipulators, one with a glass micro-needle for delivering transgene and other with a micropipette for holding fish embryo in place (Fig. 43.2).

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The success of microinjection technique depends on the nature of egg chorion. The soft chorion facilitates the microinjection while the thick chorion limits the ability to visualize the target for injection of DNA. In many fishes (Atlantic salmon and rainbow trout) the egg chorion gets tough and hard just after the fertilization or to contact with the water and provides a difficulty in injecting the DNA.

**But using the following methods can solve this problem:**

(1) By using the micropyle (an opening on the egg surface for sperm entry during the fertilization) for inserting the injecting needle.

(2) By using microsurgery for making an opening on the chorion.

(3) By digesting the chorion with enzymes.

(4) By using 1mM glutathione for initiating fertilization and reducing hardness of chorion.

(5) By direct injection to the unfertilized eggs.

Another technique of gene transfer is intra-nuclear microinjection, which involves direct physical approach using a fine needle to deliver DNA into cell or even nuclei.

To facilitate rate of microinjection protoplast with partially reformed cell wall may be attached to a solid support with artificially bound substrate -without damaging the cells. Solid support may be of either glass cover slips or slides.

**Steps of Microinjection Technique:**

(1) Desired eggs and sperms are stored separately at the optimum conditions.

(2) Add water and sperms and initiate the fertilization.

(3) Ten minutes after the fertilization, eggs are dechorionated by trypsinization.

(4) Fertilized eggs are microinjected with desired DNA just within a few hours of fertilization. DNA is released into the centre of the germinal disc to the first cleavage in dechorionated eggs. The time available for microinjection is first 25 minutes and that too between fertilization and first cleavage.

(5) After microinjection the embryos are incubated in water until hatching takes place.

Survival rates of microinjected fish embryos is seem to be about 30-80% depending the fish species.

**Advantages of Microinjection Technique:**

**This technique has the following merits:**

(1) Optimum quantity of DNA can be delivered per cell, increasing chances for integrative transformation.

(2) The delivery of DNA is precise, even into nuclei of target cell again improving chances for integrative transformation.

(3) The small structure can be injected.

(4) It is a direct physical approach, hence it is a host range independent.

**Disadvantages of Microinjection Technique:**

(1) A single cell can be injected at a time, hence it is time consuming process.

(2) It requires sophisticated instruments and specialized skills.

(3) Limited embryonic time restricts injection to more eggs and a low transformation rate.

**C. Gene Transfer by Electroporation:**

It is a simple, fast, efficient and convenient method for transferring gene. This method involves an electrical pulse to deliver DNA into cells (Fig. 43.3). The cells are exposed to a short electrical shock, which make the cell membrane temporarily permeable to DNA.

The desired DNA fragment is placed in direct contact of protoplast membrane, which enters into the cell upon electric shock. Hole may be created as a result and stabilized by a favourable
dipole interaction with electric field.

Electroporation involves a chain of electrical pulses for permeation of cell membrane, thereby allowing the entry of DNA into fertilized eggs. The rate of DNA integration in electroporated embryo is more than 25% is the surviving rate, which is slightly higher in comparison of microinjected ones.

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**Advantages of Electroporation Technique:**

(1) It allows simultaneous entry of DNA constructs.

(2) It is more suitable method for those species, which has very small eggs for microinjection.

(3) This method does not require specialized skill.

**D. Antifreeze Protein Gene Transfer:**

Many teleost inhabiting icy marine water in the Polar Regions produce antifreeze glycoproteins (AFGPs) or antifreeze proteins (AFPs) in their sera to protect them from freezing. This protein lowers the freezing temperature of solution without altering its melting temperature.

Thermal hysteresis, the difference between the freezing and melting temperature, is a unique property of these proteins. AFPs and AFGP have been demonstrated to bind to ice crystals and inhibit ice crystal growth.

Despite their similar antifreeze properties, these proteins are quite different in their protein structures. There are one type of AFGP and three types of AFP. Recently fourth type of AFP has also been reported in longhorn sculpin.

The Atlantic salmon Salmo salar, lacks any of these AGFPs or AFPs gene(s) and are unable to survive in sub-zero sea water temperature. An inability to tolerate temperature below – 0.6 °C to – 0.80 °C is one of the major problems of sea cage farming in Northern Atlantic coast. Hew and his co-workers developed antifreeze-resistant Atlantic salmon containing the AFP or AFGP genes using gene transfer technology.

They used genomic clone (2A-7) encoding the major liver-type AFP (wflAFP-6, previously known as (HPLC-6) from the winter flounder (Pleuronectus amaricanus) was used as a candidate for gene transfer.

Flounder AFPs belonged to the type I AFPs that are small polypeptides and high in alanine and helical content . Flounder AFPs is multi-gene family of 80-100 copies encoding two different isoforms, namely the liver type and skin type AFPs.

The liver type AFPs such as wflAFP-6 or wflAFP-8 (HPLC-8), are synthesized exclusively in the liver as prepro AFPs. In contrast the skin-type AFPs, including wfsAFP-2 and wfsAFP-3, are expressed widely in many peripheral tissues as intracellular mature AFPs.

**E. Growth Hormone Gene Transfer:**

Recently scientists have developed an **“all fish”** growth hormone model. They have cloned and sequenced the grass carp and common carp carbonic anhydrase (CA) gene and growth hormone gene Hew et al., (1992). The grass CA gene (beta-actin) promoter has been linked to a grass carp growth hormone cDNA to form a high efficiency expression vector called pCAZ.

Using the CAT gene as receptor gene, a pCA grass carp growth hormone was microinjected into fertilized, non-activated common carp via the micropyle, generating “all fish” transgenic carp. The presence of transgene was detected by reverse transcriptase PCR and Northern blotting. These transgenic fish showed about 137% high growth rate of the control.

**F. Disease-resistance Gene Transfer:**

In China scientists piloted a gene contributing resistance to the grass carp haemorrhagic virus (GCHV). Eleven different gene fragments encoding protein was cloned and isolated from translation in vitro using GCHV genomic single gene fragments.

Based on the information of capsid protein SP6 and SP7 gene cDNA, 3 oligonucleotides were synthesized and fused with SV40 MT promoter and transferred into grass carp cytokine-induced killer (CIK) cells via a constructed expression vector and transfected with GCHV. The result indicated that the mortalities were reduced by one order after challenge with the virus.

**Applications of Transgenic Fish:**

**Transgenic Fish may be better used for the following purposes:**

(1) For increasing fish production to meet the growing due to demand of food due to increase in world population.

(2) For production of pharmaceutical and other industrial products from piscine origin.

(3) For development of transgenic native glow fish varieties for aquarium.

(4) As fish biosensors for monitoring aquatic pollution.

(5) For isolation of genes, promoters and synthesis of effective gene constructs.

(6) For researches in embryonic stem cells and in-vitro embryo production.

(7) For production of anti-freeze protein.

**Environmental Concerns about Transgenic Fish:**

The primary environmental concerns about releases of transgenic fish, for example, include competition with wild populations, movement of the transgene into the wild gene pool, and ecological disruptions due to changes in prey and other niche requirements in the transgenic variety versus the wild populations.

**Transgenic Fish could Threaten Wild Populations:**

West Lafayette, Ind. — Purdue University researchers have found that releasing a transgenic fish to the wild could damage native populations even to the point of extinction. Transgenic fish could present a significant threat to native wildlife.

“Transgenic fish are typically larger than the native stock, and that can confer an advantage in attracting mates”, Muir says. **“If, as in our experiments, the genetic change also reduces the offspring’s ability to survive, a transgenic animal could bring a wild population to extinction in 40 generations”.**

Although at Canadian research facilities, elaborate precautions are being taken to prevent the release of transgenic fish into the environment. The fish are often raised in ponds covered with nets to keep birds out; enclosed by electric fences to keep muskrats, raccoons, and humans out; and the outlets are fitted with screened drains to prevent the loss of small fishes or eggs.

**Gene Flow:**

One of the larger environmental concerns raised by transgenic fish is the possibility that a transgenic species raised in open water pens will escape and spread novel traits into the ecosystem by breeding with wild relatives, a biological process known as **“gene flow.”**

Gene flow between transgenic or conventionally bred fish and wild populations is an environmental concern, because it may present a threat to natural biodiversity.

Some researchers believe that the genetic differences introduced to a transgenic fish may impact its net fitness, a scientific term meaning an organism’s ability to survive and pass its genes to future generations.

The concept, which factors in characteristics such as the juvenile and adult viability of a fish, the number of eggs produced by a female, and the age at which a fish reaches sexual maturity, provides a useful barometer for discussing some gene flow scenarios.

**According to one scientific model, if a transgenic fish escapes and mates with a wild fish, gene flow could follow one of three scenarios:**

**Purge Scenario:**

When the net fitness of a transgenic fish is lower than that of its wild relatives, natural selection will quickly purge from the wild population any novel gene(s) introduced by the transgenic fish. In theory, evidence of the novel trait will disappear from subsequent generations.

**Spread Scenario:**

When the net fitness of a transgenic fish is equal to or higher than the net fitness of a wild mate, gene flow is likely to occur and the genes of the transgenic fish will spread through the wild population. This means evidence of the transgenic genome would persist in subsequent generations.

**Trojan Gene Scenario:**

When the net fitness of a transgenic fish is altered such that the fish has enhanced mating success but reduced adult viability (i.e., chances of surviving long enough to mate), introduction of that fish into the wild population could result in a rapid decline of the wild population.

Essentially mating success would ensure the spread of the novel gene throughout the population, but the inability to survive would reduce the population size of subsequent generations and potentially lead to extinction.

A declining fish population would also have secondary impacts on other aquatic species that feed on, or otherwise depend on it. Populations unable to’ successfully switch over to another food source, or those whose survival or reproduction depends directly on the declining population, would also suffer.

**Transgenic Fish Invasive Species:**

Even if they do not breed with wild relatives, transgenic fishes that escape into natural ecosystems could be an environmental nuisance by becoming an invasive species.

This danger mainly arises for those transgenic fishes endowed with new genes that improve such fitness traits as breeding capabilities and the ability to withstand harsh conditions. The establishment of a thriving transgenic fish population in an ecosystem where it has never existed could crowd out native fish populations.

**Risk Mitigation:**

It is important to note that developers of transgenic fish are attempting to reduce or eliminate both gene flow and invasive species risks by sterilizing transgenic fish. Sterilization is relatively easy and inexpensive but success rates are highly variable.

In addition, sterilization does not necessarily neutralize environmental risks. Academic scientists note that an escaped, sterile fish might still engage in courtship and spawning behaviour, disrupting breeding in wild populations. Waves of escaped sterile fish could also create ecological disruptions as each group is replaced by another equally strong group of transgenic sterile fish.

**Food Safety Issues:**

One important food safety issue involves the extent to which fish absorbs and stores environmental toxins, such as mercury, high levels of which could pose a danger to humans who eat the contaminated fish.

Some scientists worry that discrete biological changes induced by the genetic engineering process might enable transgenic fish to absorb a toxin that conventional fish cannot absorb or to better tolerate higher levels of a toxin already known to cause concern.

Some scientists have expressed concern that the genetic engineering process could increase the allergic potential of fish, particularly through the introduction of novel proteins that never before existed in the food chain.

However, it is equally possible that genetic engineering will form their diet. Genetically engineered plant crop had faced protest in various countries regarding safety for food and environment. There is a need for the regulating transgenic animals for debate.

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