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TRANSGENIC FISH TECHNOLOGY AND ITS APPLICATION IN AQUACULTURE



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* Transgenic Fishes : Introduction

- Organisms into which heterologous DNA (transgene) has been artificially introduced and integrated in their genomes are called transgenics.
- Genetically modified fish (GM fish) are organisms from the taxonomic clade which includes the classes Agnatha (jawless fish), Chondrichthyes (cartilaginous fish) and Osteichthyes (bony fish) whose genetic material (DNA) has been altered using genetic engineering techniques. In most cases, the aim is to introduce a new trait to the fish which does not occur naturally in the species, i.e. transgenesis.
- GM fish are used in scientific research and kept as pets. They are being developed as environmental pollutant sentinels and for use in aquaculture food production. In 2015, the Aqua-Advantage salmon was approved by the US Food and Drug Administration (FDA) for commercial production, sale and consumption, making it the first genetically modified animal to be approved for human consumption. Some GM fish that have been created have promoters driving an over-production of "all fish" growth hormone. This results in dramatic growth enhancement in several species, including salmonids, (Shao *et al.*,1992) carps (Robert *et al.*,2001) and Tilapias(Rahman *et al.*,2005; Hackett and Alvarez,2000).



TRANSGENIC FISHES: INTRODUCTION

- The first transgenic fish were produced in China in 1985(Dunham & Winn,2014). As of 2013, approximately 50 species of fish have been subject to genetic modification. This has resulted in more than 400 fish/trait combinations.
- Most of the modifications have been conducted on food species, such as Atlantic salmon (*Salmo solar*), *tilapia* (genus) and common carp (*Cyprinus carpio*).[Menozzi *et al.*,2013]

What can transgenic technology offer?

- Success in aquaculture depends on six factors:
 - (1) complete control of the reproductive cycle of the fish species in culture
 - (2) excellent genetic background of the brood-stock
 - (3) efficient prevention and detection of disease infection
 - (4) thorough understanding of the optimal physiological, environmental, and nutritional conditions for growth and development
 - (5) sufficient supply of excellent quality water
 - (6) application of innovative management techniques.

By improving these factors, the aquaculture industry has developed to a remarkable extent during the last decade. To sustain this growth, the applications of various newly developed technologies in molecular biology including transgenesis have proven their worth in modern aquaculture industry. These technologies can be employed to enhance growth rates, control reproductive cycles, improve feed compositions, produce new vaccines, and develop disease resistant and hardier genetic stocks.

* METHODS OF TRANSGENIC FISH PRODUCTION

A Transgene Constructs

- A transgene used in producing transgenic fish for basic research or application should be a recombinant gene construct that produces a gene product at an appropriate level in the desired tissue(s) at the desired time(s). Therefore, the prototype of a transgene is usually constructed in a plasmid to contain an appropriate promoter-enhancer element and a structural gene sequence.
- Depending on the purpose of gene transfer studies, transgenes are grouped into three main types: (1) gain-of-function, (2) reporter function, and (3) loss-of-function.
- The gain-of-function transgenes are designed to add new function to the transgenic individuals or to facilitate the identification of the transgenic individuals if the genes are expressed properly in the transgenic individuals. Trans genes containing the structural genes of fish and mammalian growth hormone (GH or their cDNA) are fused to the promoter, which led to the greater production of growth hormones. Chicken and fish β actin gene promoters are the example of the gain of function transgenes.
- The reporter function trans-genes are commonly used to identify the success of gene transfer effort. Bacterial chloramphenicol acetyl transferase (CAT), β -galactosidase or luciferase genes fused to functional promoters are example of reporter function trans genes. Following gene transfer the expression of the reporter gene activity is used to determine the transcriptional regulatory sequence of a gene or strength of the promoter.
- The loss of function trans genes are constructed for interfering with the host genes. These genes might encode a catalytic RNA (Ribozyme) that can cleave specific mRNA and thereby cancel the production of normal gene products.

* METHODS OF TRANSGENIC FISH PRODUCTION: CONTD...

B. Selection of Fish species: Gene transfer techniques have been studied on channel catfish,

common carp, gold fish, Japanese medaka, loaches, northern pike, salmon, rainbow trout, tilapia and zebra fish etc.

- The Japanese medaka (*Oryzias latipes*) and zebra fish (*Brachydanio rerio*) have short life cycle (three months from hatching to mature adult), produce hundreds of eggs on regular basis without exhibiting a seasonal breeding cycle, and can be maintained in the laboratory for two or three years.
- Eggs from these two fishes are relatively large(diameter 0.7-1.5mm) and possess very thin, semi-transparent chorion suitable for easy micro injection of DNA into the eggs. Besides availability of inbred lines and various morphological mutants of both these fish species have made them most suitable organism for conducting gene transfer experiment.

* METHODS OF TRANSGENIC FISH PRODUCTION: CONTD...

C. Methods of gene transfer

- Various techniques such as –calcium phosphate precipitation, direct microinjection, lipofection, retroviral infection, electroporation and particle gun bombardment have been used to introduce foreign DNA into animal cells, plant cells, germ line of mammals and other vertebrates.

1. Micro injection of eggs and embryo

- Micro injection of foreign DNA into newly fertilized eggs have been adopted for introducing trans genes into Atlantic salmon, common carp, cat fish, gold fish, medaka, rainbow trout, tilapia and zebra fish..
- The gene constructs that were used in these studies include human or rat growth hormone(GH) gene, rainbow trout and salmon GH cDNA, chicken δ crystalline gene protein, winter flounder antifreeze protein gene, Escherichia coli β galactosidase genes and E. coli hygromycine resistance gene.

Steps of microinjection:

- eggs and sperms are collected in separate dry containers and fertilization is initiated by adding water and sperm to the egg with gentle stirring.
- The injection apparatus consists of a dissecting stereo microscopes and two micromanipulators , one with glass micro needle for delivering trans genes and other with a micropipette holding the embryo in place.
- Fertilized eggs are then micro-injected within first two hours of fertilization. Routinely, about 10^6 - 10^8 molecules of a linearized trans genes with or without plasmid DNA in about 20nL is injected into the egg cytoplasm.
- Depending upon the fish species the survival rate of embryo ranges from 35 to 80% while the rate of DNA integration ranges from 10 to 70% in the survivors.
- The tough chorion of fertilized egg of some fish species like Rainbow trout and Atlantic salmon can frequently make insertion of these glass needle difficult. These difficulties can be over come by any one of the following methods- 1. inserting the injection needle through the micropyle, 2. making an opening in the egg chorion by micro surgery, 3. removing the chorion by mechanical or enzymatical means, 4. reducing chorion hardening by initiating fertilization in a solution containing 1 mM Glutathione or 5. injecting the unfertilized egg directly.

* **METHODS OF TRANSGENIC FISH PRODUCTION: CONTD...**

2. Electroporation:

- Electroporation is a successful methods for transferring foreign DNA into bacteria, yeast, plants and animal cells in culture.
- This technique has become popular for transferring transgenes into fish embryo in last three years.
- Electroporation uses a short electrical pulses to permeate cell membrane thereby permitting the entry of Na molecules into embryo.
- The pattern of electrical impulses can be emitted in a single impulse of exponential decay form or high frequency multiple picks of square waves.
- Although the overall rate of DNA integration in transgenic fish produced by electroporation may be equal to that of micro injection or slightly higher than that of microinjection technique, but the actual amount of time required for handling a large number of embryos by electroporation in order of magnitude is much less than the time required for micro injection.
- Hence electroporation is considered as an efficient and versatile massive gene transfer technology.

* METHODS OF TRANSGENIC FISH PRODUCTION: CONTD...

3. Transfer of trans genes by infection with pantropic retroviral vectors

- Although the trans genes can be reproducibly introduced into various fish species by micro-injection and electroporation techniques, but the resulting P₁ transgenic individuals possess mosaic germ lines as a result of delayed trans genes integration.
- Furthermore these two gene transfer methods are not effective or successful in producing transgenes in marine fish and invertebrates.
- Recently a new gene transfer vector, a defective pantropic retro viral vector has been developed (Burns *et al.*,1993).
- The vector contains the long terminal repeats(LTR) sequence of Moloney murine leucemia virus (MoMLV) and trans genes packed in a viral envelop with the gene protein of the vesicular stomatitis virus (VSV).
- Because the entry of the VSV into cell is mediated by the interaction of the VSV G proteins with a phospholipid component of the cell, this pseudo-typed retroviral vector has a broad host range and is able to transfer trans genes into many different cell types using the pantropic pseudo typed defective retrovirus as a gene transfer vector trans gene containing neo^R or β galactosidase has been successfully introduced into zebra fish.

APPLICATIONS OF TRANSGENIC FISH TECHNOLOGY IN AQUACULTURE

- In recent years growth hormones(GH) cDNA and genomic DNA have been isolated and characterized for several fish species . Expression of rainbow trout or striped bass GH cDNA in E. coli cells results in production of large quantity of recombinant GH polypeptide. Because the GH polypeptide is highly hydrophobic and contains six cystein residue, the newly synthesized recombinant GH polypeptide forms insoluble inclusion bodies in E. coli cells rendering the hormone inactive. Cheng *et al.*,1995 has developed the re-naturation techniques for restoring the impact of GH in E . Coli.
- Although exogenous application of biosynthetic GH results in a significant growth enhancement in fish, it may not be cost effective because of the following reasons--1. high cost of producing large scale purified biosynthetic GH, 2. treating individual fish with the hormones is labour intensive, 3. The optimal hormonal dosage for each fish species is difficult to determine and, 4. GH uptake into fish from an exogenous source is insufficient.
- Hence three aspects of the fish growth characteristics that could be improved for aquaculture by active intervention of transgenic fish technology-1. Initial growth rate so that they reach maturation earlier, 2. enhanced somatic growth rate as adult to provide larger body size for market, 3. fish with improved feed conversion efficiencies. Among these three, enhanced somatic growth rate by manipulation of GH or insulin like growth factor gene show considerable promises.
- Most dramatic growth enhancement in transgenic fish has been obtained by introducing Chinook salmon GH cDNA drive by the promoter of the ocean pout antifreeze protein into Atlantic salmon embryos. Some of these transgenic animals grow faster than their controls. Lu *et al.*,(1994) have shown that both P¹ and F¹ medaka carrying a chicken β actin genes promoter or human GH constructor tilapia carrying carp β actin promoter –n GH₁cDNA exhibited a significant growth enhancement when compared with the non transgenic siblings. The IGF-1 transgenic medaka and tilapia not only developed faster during embryonic development they also exhibited a significant degree of growth enhancement.

APPLICATIONS OF TRANSGENIC FISH TECHNOLOGY IN AQUACULTURE: CONTD...

Applications of Transgenic Fish technology in Research

- Transgenic fish are used in research covering five broad areas:
 1. Enhancing the traits of commercially available fish
 2. Their use as bioreactors for the development of bio-medically important proteins
 3. Their use as indicators of aquatic pollutants
 4. Developing new non-mammalian animal models
 5. Functional genomics studies
- Most GM fish are used in basic research in genetics and development. Two species of fish, zebrafish and medaka, are most commonly modified, because they have optically clear chorions (shells), develop rapidly, the 1-cell embryo is easy to see and micro-inject with transgenic DNA.
- Zebrafish have the capability of regenerating their organ tissues. They are also used in drug discovery. GM zebrafish are being explored for benefits of unlocking human organ tissue diseases and failure . mysteries. For instance, zebrafish are used to understand heart tissue repair and regeneration in efforts to study and discover cures for cardiovascular diseases.
- Transgenic rainbow trout (*Oncorhynchus mykiss*) have been developed to study muscle development. The introduced transgene causes green fluorescence to appear in fast twitch muscle fibres early in development which persist throughout life. It has been suggested the fish might be used as indicators of aquatic pollutants or other factors which influence development.
- In intensive fish farming, the fish are kept at high stocking densities. This means they suffer from frequent transmission of contagious diseases, a problem which is being addressed by GM research.
- Grass carp (*Ctenopharyngodon idella*) have been modified with a transgene coding for human lactoferrin, which doubles their survival rate relative to control fish after exposure to *Aeromonas* bacteria and Grass carp hemorrhage virus.
- Cecropin has been used in channel catfish to enhance their protection against several pathogenic bacteria by 2–4 times.

APPLICATIONS OF TRANSGENIC FISH TECHNOLOGY IN AQUACULTURE: CONTD...

Application in Recreation and game fishing

Some of the transgenic fishes have been designed to treat them as pet.

- GloFish is a patented technology which allows GM fish (tetra, barb, zebrafish) to express jellyfish and sea coral proteins giving the fish bright red, green or orange fluorescent colors when viewed in ultraviolet light.
- Although the fish were originally created and patented for scientific research at the National University of Singapore, a Texas company, Yorktown Technologies, obtained rights to market the fish as pets. They became the first genetically modified animal to become publicly available as a pet when introduced for sale in 2003.
- Other transgenic lines of pet fish include Medaka which remain transparent throughout their lives and pink body colour transgenic angelfish (*Pterophyllum scalare*) and lion head fish expressing the Acropora coral (*Acroporo millepora*) red fluorescent protein.
- The ocean pout type III antifreeze protein transgene has been successfully micro-injected and expressed in goldfish. The transgenic goldfish showed higher cold tolerance compared with controls.

APPLICATIONS OF TRANSGENIC FISH TECHNOLOGY IN AQUACULTURE: CONTD...

Application in Food and feeding industry

- One area of intensive research with GM fish has aimed to increase food production by modifying the expression of growth hormone (GH). The relative increases in growth differ between species. They range from a doubling in weight, to some fish that are almost 100 times heavier than the wild-type at a comparable age.
- This research area has resulted in dramatic growth enhancement in several species, including salmon, trout and tilapia. Other sources indicate an 11-fold and 30-fold increase in growth of salmon and mud loach, respectively, compared to wild-type fish.
- Transgenic fish development has reached the stage where several species are ready to be marketed in different countries, for example, GM tilapia in Cuba, GM carp in the People's Republic of China, and GM salmon in the US and Canada. In 2014, it was reported that applications for the approval of transgenic fish as food had been made in Canada, China, Cuba and the United States.
- Over-production of GH from the pituitary gland increases growth rate mainly by an increase in food consumption by the fish, but also by a 10 to 15% increase in feed conversion efficiency.
- Another approach to increasing meat production in GM fish is "double muscling". This results in a phenotype similar to that of Belgian Blue cattle in rainbow trout. It is achieved by using transgenes expressing follistatin, which inhibits myostatin, and the development of two muscle layers.

APPLICATIONS OF TRANSGENIC FISH TECHNOLOGY IN AQUACULTURE: CONTD...

Application in Food and feeding industry(Contd.): AquAdvantage salmon

- In November 2015, the FDA of the USA approved the AquAdvantage salmon created by AquaBounty for commercial production, sale and consumption. It is the first genetically modified animal to be approved for human consumption.
- The fish is essentially an Atlantic salmon with a single gene complex inserted: a growth hormone regulating gene from a Chinook salmon with a promoter sequence from an ocean pout. This permits the GM salmon to produce GH year round rather than pausing for part of the year as do wild-type Atlantic salmon. The wild-type salmon takes 24 to 30 months to reach market size (4–6 kg) whereas the GM salmon require 18 months for the GM fish to achieve this.
- AquaBounty argue that their GM salmon can be grown nearer to end-markets with greater efficiency (they require 25% less feed to achieve market weight) than the Atlantic salmon which are currently reared in remote coastal fish farms, thereby making it better for the environment, with recycled waste and lower transport costs.
- To prevent the genetically modified fish inadvertently breeding with wild salmon, all the fish raised for food are females, triploid, and 99% are reproductively sterile. The fish are raised in a facility in Panama with physical barriers and geographical containment such as river and ocean temperatures too high to AquAdvantage would not have a significant effect on the environment in the United States. A fish farm is also being readied in Indiana where the FDA has approved importation of salmon eggs.

APPLICATIONS OF TRANSGENIC FISH TECHNOLOGY IN AQUACULTURE

Application in detection of aquatic pollution

- Several research groups have been developing GM zebrafish to detect aquatic pollution. The laboratory that developed the GloFish originally intended them to change colour in the presence of pollutants, as environmental sentinels.] Teams at the University of Cincinnati and Tulane University have been developing GM fish for the same purpose.
- Several transgenic methods have been used to introduce target DNA into zebrafish for environmental monitoring, including micro-injection, electroporation, particle gun bombardment, liposome-mediated gene transfer, and sperm-mediated gene transfer. Micro-injection is the most commonly used method to produce transgenic zebrafish as this produces the highest survival rate.

ISSUES RELATED TO TRANSGENIC FISH TECHNOLOGY IN AQUACULTURE

Regulations and issues related to transgenic fish technology

Regulation of the release of genetic modified organisms

- The regulation of genetic engineering concerns the approaches taken by governments to assess and manage the risks associated with the development and release of genetically modified crops. There are differences in the regulation of GMOs between countries, with some of the most marked differences occurring between the US and Europe. Regulation varies in a given country depending on the intended use of the products of the genetic engineering. For example, a fish not intended for food use is generally not reviewed by authorities responsible for food safety.
- The US FDA guidelines for evaluating transgenic animals define transgenic constructs as "drugs" regulated under the animal drug provisions of the Federal Food and Cosmetic Act. This classification is important for several reasons, including that it places all GM food animal permits under the jurisdiction of the FDA's Center for Veterinary Medicine (CVM) and imposes limits on what information the FDA can release to the public, and furthermore, it avoids a more open food safety review process.
- The US states of Washington and Maine have imposed permanent bans on the production of transgenic fish.

Genetically modified food controversies

- Critics have objected to use of genetic engineering per se on several grounds, including ethical concerns, ecological concerns (especially about gene flow), and economic concerns raised by the fact GM techniques and GM organisms are subject to intellectual property law.
- GMOs also are involved in controversies over GM food with respect to whether using GM fish as safe is safe, whether it would exacerbate or cause fish allergies, whether it should be labelled, and whether GM fish and crops are needed to address the world's food needs. These controversies have led to litigation, international trade disputes, and protests, and to restrictive regulation of commercial products in most countries.
- There is much doubt among the public about genetically modified animals in general. It is believed that the acceptance of GM fish by the general public is the lowest of all GM animals used

ISSUES RELATED TO TRANSGENIC FISH TECHNOLOGY IN AQUACULTURE

Ethical Issues Concerned with Transgenic Fish Technology

- In transgenic fast-growing fish genetically modified for growth hormone, the mosaic founder fish vary greatly in their growth rate, reflecting the highly variable proportion and distribution of transgenic cells in their bodies. Fish with these high growth rates (and their progeny) sometimes develop a morphological abnormality similar to acromegaly in humans, exhibiting an enlarged head relative to the body and a bulging operculum. This becomes progressively worse as the fish ages. It can interfere with feeding and may ultimately cause death. According to a study commissioned by Compassion in World Farming, the abnormalities are probably a direct consequence of growth hormone over-expression and have been reported in GM coho salmon, rainbow trout, common carp, channel catfish and loach, but to a lesser extent in Nile tilapia?
- In GM coho salmon (*Oncorhynchus kisutch*) there are morphological changes and changed allometry that lead to reduced swimming abilities. They also exhibit abnormal behaviour such as increased levels of activity with respect to feed-intake and swimming. Several other transgenic fish show decreased swimming ability, likely due to body shape and muscle structure.
- Genetically modified triploid fish are more susceptible to temperature stress, have a higher incidence of deformities (e.g. abnormalities in the eye and lower jaw, and are less aggressive than diploids).
- Other welfare concerns of GM fish include increased stress under oxygen-deprived conditions caused by increased need for oxygen. It has been shown that deaths due to low levels of oxygen (hypoxia) in coho salmon are most pronounced in transgenics. It has been suggested the increased sensitivity to hypoxia is caused by the insertion of the extra set of chromosomes requiring a larger nucleus which thereby causes a larger cell overall and a reduction in the surface area to volume ratio of the cell.

ISSUES RELATED TO TRANSGENIC FISH TECHNOLOGY IN AQUACULTURE

Ecological Issues Concerned with Transgenic Fish Technology

- Transgenic fish are usually developed in strains of near-wild origin. These have an excellent capacity for interbreeding with themselves or wild relatives and therefore possess a significant possibility for establishing themselves in nature should they escape biotic or abiotic containment measures.
- A wide range of concerns about the consequences of genetically modified fish escaping have been expressed. For polyploids, these include the degree of sterility, interference with spawning, competing with resources without contributing to subsequent generations. For transgenics, the concerns include characteristics of the genotype, the function of the gene, the type of the gene, potential for causing pleiotropic effects, potential for interacting with the remainder of the genome, stability of the construct, ability of the DNA construct to transpose within or between genomes.
- One study, using relevant life history data from the Japanese medaka (*Oryzias latipes*) predicts that a transgene introduced into a natural population by a small number of transgenic fish will spread as a result of enhanced mating advantage, but the reduced viability of offspring will cause eventual local extinction of both populations.
- GM coho salmon show greater risk-taking behaviour and better use of limited food than wild-type fish. Transgenic coho salmon have enhanced feeding capacity and growth, which can result in a considerably larger body size (>7-fold) compared to non-transgenic salmon. When transgenic and non-transgenic salmon in the same enclosure compete for different levels of food, transgenic individuals consistently outgrow non-transgenic individuals. When food abundance is low, dominant individuals emerge, invariably transgenic, that show strong agonistic and cannibalistic behaviour to cohorts and dominate the acquisition of limited food resources. When food availability is low, all groups containing transgenic salmon experience population crashes or complete extinctions, whereas groups containing only non-transgenic salmon have good (72%) survival rates. This has led to the suggestion that these GM fish will survive better than the wild-type when conditions are very poor. Successful artificial transgenic hybridization between two species of loach (genus *Misgurnus*) has been reported, yet these species are not known to hybridize naturally.
- GloFish were not considered as an environmental threat because they were less fit than normal zebrafish which are unable to establish themselves in the wild in the US.

ISSUES RELATED TO TRANSGENIC FISH TECHNOLOGY IN AQUACULTURE

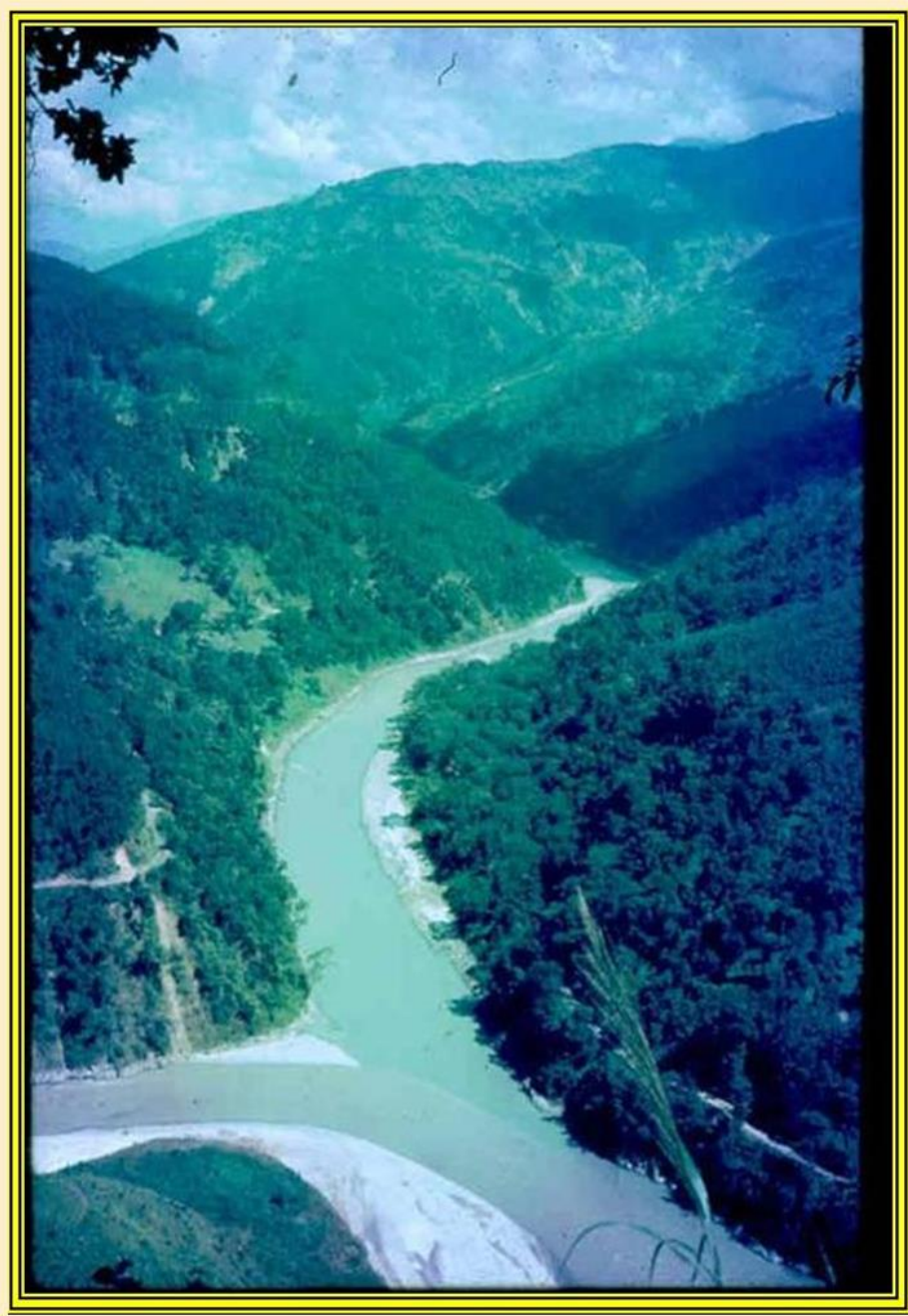
Issues related to aquAdvantage salmon

- The FDA has said the AquAdvantage Salmon can be safely contained in land-based tanks with little risk of escape into the wild, however, Joe Perry, former chair of the GM panel of the European Food Safety Authority, has been quoted as saying "There remain legitimate ecological concerns over the possible consequences if these GM salmon escape to the wild and reproduce, despite FDA assurances over containment and sterility, neither of which can be guaranteed."
- AquaBounty indicates their GM salmon can not interbreed with wild fish because they are triploid which makes them sterile. The possibility of fertile triploids is one of the major short-falls of triploidy being used as a means of bio-containment for transgenic fish. However, it is estimated that 1.1% of eggs remain diploid, and therefore capable of breeding, despite the triploidy process. Others have claimed the sterility process has a failure rate of 5%.. Large scale trials using normal pressure, high pressure, or high pressure plus aged eggs for transgenic coho salmon, give triploidy frequencies of only 99.8%, 97.6%, and 97.0%, respectively. AquaBounty also emphasizes that their GM salmon would not survive wild conditions due to the geographical locations where their research is conducted, as well as the locations of their farms.
- The GH transgene can be transmitted via hybridization of GM AquAdvantage Salmon and the closely related wild brown trout (*Salmo trutta*). Transgenic hybrids are viable and grow more rapidly than transgenic salmon and other wild-type crosses in conditions emulating a hatchery. In stream mesocosms designed to simulate natural conditions, transgenic hybrids express competitive dominance and suppress the growth of transgenic and non-transgenic salmon by 82% and 54%, respectively. Natural levels of hybridization between these two species can be as high as 41%. Researchers examining this possibility concluded "Ultimately, we suggest that hybridization of transgenic fishes with closely related species represents potential ecological risks for wild populations and a possible route for introgression of a transgene, however low the likelihood, into a new species in nature."
- An article in Slate Magazine in December 2012 by Jon Entine, Director of the Genetic Literacy Project, criticized the Obama Administration for preventing the publication of the environmental assessment (EA) of the AquAdvantage Salmon, which was completed in April 2012 and which concluded that "the salmon is safe to eat and poses no serious environmental hazards." The Slate article said that the publication of the report was stopped "after meetings with the White House, which was debating the political implications of approving the GM salmon, a move likely to infuriate a portion of its base". Within days of the article's publication and less than two months after the election, the FDA released the draft EA and opened the comment period.



TRANSGENIC FISH : CONCLUSION

- Transgenic fish technology has a great potential in revolutionizing the aquaculture industry by introducing desirable genetic traits into fin fish or shell fish , superior transgenic fish strains can be produced for aquaculture.
- These traits may include elevated growth enhancement, improved food conversion efficiencies, resistance to some known diseases , tolerance to low oxygen concentration and sub zero temperature.
- The transfer, expression and inheritance of fish growth hormone and IGF-I transgenes have been successfully achieved in several fin fishes and the resulting animals grow substantially faster than their control siblings.
- But if we want to achieve the full fledged goal and potentials of the transgenesis and other biotechnological applications in aquaculture several important scientific breakthrough are required like-
 1. Identifying genes of desirable traits for aquaculture and other applications,
 2. developing targeted gene transfer technology, such as embryonic stem cell gene transfer method or ribozyme gene inactivation method.
 3. Identifying suitable promoters to direct the expression of trans genes at optimal levels during the desired developmental stages,
 4. determining physiological, nutritional, immunological and environmental factors that will maximize the performance of the transgenic individuals and,
 5. assessing safety and environmental effects of transgenic fish.
- Hence all the issues raised need to be scientifically resolved to attain the full potentials of transgenic fish technology in aquaculture.



THANK YOU