E-ISSN: 2618-0618
P-ISSN: 2618-060X

www.agronomyjournals.com
2024; SP-7(3): 45-52
Received: 27-01-2024
Accepted: 05-03-2024

Pradnya Garud
M.Sc. Aquaculture and Aquatic Resources Management, Asian Institute of Technology, Khlong Luang, Pathum, Thani, Thailand

Sagar Vitthal Shinde
ICAR-Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai, Maharashtra, India

Prakash Patekar
ICAR-Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai, Maharashtra, India

Swapnil Narsale
ICAR-Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai, Maharashtra, India

Shamika Sawant
ICAR-Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai, Maharashtra, India

Samad Sheikh
ICAR-Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai, Maharashtra, India

Corresponding Author:
Pradnya Garud
M.Sc. Aquaculture and Aquatic Resources Management, Asian Institute of Technology, Khlong Luang, Pathum, Thani, Thailand

Revolutionizing aquaculture: Exploring the diverse applications of biotechnology for sustainable growth and enhanced productivity

Pradnya Garud, Sagar Vitthal Shinde, Prakash Patekar, Swapnil Narsale, Shamika Sawant and Samad Sheikh

DOI: https://doi.org/10.33545/2618060X.2024.v7.i3Sa.385

Abstract
As aquaculture plays a pivotal role in global food production, addressing challenges related to efficiency and sustainability is imperative. Genetic improvement strategies have emerged as potent tools, revolutionizing fish farming by enhancing key traits such as production yields, disease resistance, and ecological compatibility. This thorough review explores three major facets of genetic improvement in aquaculture: Polyploidy induction, Selective Breeding, and Trans-genesis. Each segment delves into the fundamental concepts, methodologies, benefits, limitations, and practical applications of various genetic modification methods. The study also highlights the ecological and social concerns associated with the deployment of genetically modified organisms in aquaculture, emphasizing the necessity for responsible and ethical use of genetic improvement technologies. By comprehensively examining these genetic enhancement approaches, this review aims to contribute to the ongoing dialogue surrounding the sustainable and responsible future of aquaculture.

Keywords: Polyploidy induction, selective breeding, methodologies, benefits, limitations

1. Introduction

1.1 Polyploidy induction in Aquaculture

Polyploidy can be defined as the genetic state of an organism have in one or more additional set of chromosomes, has been harnessed as a tool in aquaculture for achieving sterility in fish populations. In aquaculture most of the fishes are grown for consumption purposes. However, most of the nutrition of a juvenile fish contribute towards the reproduction and hence increasing the time of production of table size fishes leading to higher cost of production. (Rizzo and Spagnolo, 1996) [18] and hence sterility plays an important role in growth management diverting all the energy towards growth rather than reproduction. Higher occurance of disease is another common problems associated with sexual maturation. Hence, sterility becomes a measure to encounter such problem by producing all sterile population. Although sterility is normally seen in natural population, with technological advancement of technology sterility can be achieved artificially by induction of polyploidy, particularly triploidy (Piferrer, 2009) [16].

Polyploids can be triploid, having three sets of chromosomes or a tetraploid, having four sets of chromosomes. Polyploidy is occurs naturally in wild and also in farmed fishes. Two types of natural polyploidy are observed in vertebrates. (Stöck *et al*., 2002) [20]. Auto polyploidy, polyploidy caused due to changes in meiotic or mitotic division in the animal within the species and Allopolyploidy, Caused by reproductive contact among different species (Piferrer, 2009) [16].

1.2 Induction of polyploidy

Various techniques, including temperature hocks, hydrostatic pressure, anesthetics, and chemical shocks, are employed to prevent the extrusion of polar bodies during fertilization. Additionally, interploidy crossing, involving fertilization of normal haploid eggs by diploid spermatozoa, offers an alternative method for achieving triploidy.

In shell fish mature eggs are arrested at meiosis I, metaphase state while in fin fish the eggs are

at metaphase stage of meiosis II and hence the further process of cell division resumes on entry of sperm into the oocyte leading to extrusion of both (first and second) polar body in shellfish whereas only second polar body extrusion in finfish. So here at this stage (After fertilization) prevention of extrusion of first (Shellfish) or second (Shell fish and finfish) polar body is achieved by various techniques like temperature shocks (hot or cold), hydrostatic pressure, anesthetics, or chemical shocks (Thorgaard *et al*., 1981; Wolters *et al*., 1981a; Chourrout and Itskovich, 1983; Benfey and Sutterlin, 1984a; Chourrout, 1984; Cassani and Caton, 1986a; Curtis *et al*., 1987; Johnstone *et al*., 1989) [21, 24, 4-5, 2, 6, 12]. Another method of achieving triploidy by interploidy crossing, in which normal haploid eggs are fertilized by diploid spermatozoa from a tetraploid male (Wang *et al*., 2002; Nam and Kim, 2004; Francescon *et al*., 2004) [22, 15, 9].

1.3 Advantages of polyploidy

Production of sterile fishes by triploidy helps to achieve higher growth and feed conversion like transgenic fishes in to the wild thereby protect the genetic diversity of wild population. In addition to this, unauthorized breeding of farmed shrimp can be prevented with successful production of sterile triploid shrimp, additionally showing higher growth than diploid fish. (Sellars and others 2006) [19] Production of sterile triploid can be beneficial to check the profiling breeding of certain species like tilapia and hence protecting the other native populations. (Dunham 2004) [7].

1.4 Constraints and limitations

Although triploidy helps to achieve sterility but complete success is not guaranteed and some triploids have reported to revert their cells to diploid state. (Wang and others, 2003; Dunham, 2004) [23, 7]. Induction polyploidy can also show some adverse effect on some the traits including lesser survival (Rasmussen, 2007) [17]. Sterile triploids are also happens to show sexual behavior (Although sexually impotent), participates in mating behavior and hence affect the natural spawning process of sexually active population (Dunham, 2004) [7]. At last the polyploidy induction technique may not be economically cost effective when produced in a higher scale.

Although polyploidy has greater potential in achieving significant production in aquaculture, a few aspects needs further research to achieve complete 100% sterility to avoid risk. Ecological impact of the triploids in natural ecosystem must assessed fully to understand any potential threat caused by them to natural ecosystem. Although, interploidy crossing (crossing between diploid and tetraploid) is an effective method to produce triploids, production of tetraploid is difficult with requirement of special containment. Hence more research must carried out on achieving tetraploid population must be given emphasis. Overall, Polyploidy holds a greater scope and potential future prospective in aquaculture and more detailed study must be carried out to carry on further investigation.

2. Selective Breeding

Selective breeding is a beneficial approach in aquaculture to produce efficient domesticated stock needed to enhance production. Selective breeding can be defined as a breeding programme engaged to improve the breeding value of the population by selectively mating the individuals showing better desirable traits (growth, disease resistance, meat high FCR &FCE, Meat quality, cold tolerance, fecundity, color enhancement etc.), and which can subsequently be transferred to their progeny (Tave, 1995; FAO) [59].

Higher genetic gain is normally observed in aquatic species which can be attributed to their high fertility and broad genetic variation of traits of economic interest (Gjedrem, 2014) [60].

2.1 Selection Methods

The first report of selective breeding can be traced back to 1920s, selection of brook trout against reduced mortality due to furunculosis. (Embody and Hyford, 1925) [61] Subsequently, selection approach for common carp was taken in same 1920s with the development of two productive strains (Kirpichnikov, V.S. (1987) [62]. But after this intial investigation, very little work has been done up to 1970. But several research carried out in the period from 1970 to present contributing significantly to advancement in selective breeding approaches. (Dunham, 1996; Dunham *et al.*, 2001) [64, 63]. Various selection approaches have been implemented in selective breeding to yield higher genetic gain in different species including, Atlantic salmon, Coho salmon Rainbow trout, tilapia, carp, Channel catfish, Sea bream, oyster, scallops etc.

2.1.1 Individual selection

It is also called as character or mass selection. Here the selection is based on the performance of each individual. The selection method is quite easy to perform and has been the most common method for many years. Yet, selection of individuals by this method is only possible for measurable traits or traits that can be recorded like body weight and length. There are some serious drawbacks for this selection method like inbreeding depression which has led to failure of many experiments (Hulata, Wohlfarth, and Halevy, 1986; Huang, and Liao, 1990) [66, 67]. Another disadvantage is that efficiency of this selection relies to a large extent on environmental conditions. Hence if the animals are held in different ponds, tanks or cages having different environmental conditions, it will reduce the success chance of selection. Hence maintaining the same parameters in all tanks or ponds is most important for this type of selection.

2.1.2 Family selection

Family selection is mostly carried out where individual selection is inefficient due to low heritability of the phenotype is low. Low heritability means low heritable component of phenotypic variance that implies the measurable differences among the individual are largely due to non-heritable source of variance (Environment variables). Hence by selection according to family backgrounds, most of the environment variance can be nullified and selection of fishes can be done on genetic variance. (Tave, 1995, FAO Paper) [59]. Family selection is done by multiple crossing between selected parents and selecting the progeny (Evaluating their performances) from best families are taken for further rearing.

There are two types of family selection, between family and within family selection.

In between family selection, mean values of each family are determined and ranked and the families having highest mean values are selected. Where as in within family selection, the selection occurs within the family where each family is assumed as a sub-population and the fishes of each family is ranked based on their performances and the best fishes from each family are saved for further rearing.

2.1.3 Combined selection

To resolve the problems faced in both between-family and within-family selection combined selection is practiced.

Here both types of selection (between-family and within-family)

are integrated into two step breeding programme where between selection is done to select the best family and then within-family selection is done to select best performing individuals within the selected families. (Tave, 1995, FAO Paper) [59]

Some of the examples of the combined selection performed successfully includes:

1. The GIFT project (Philippines), reported to obtain 12% to 17% Genetic gain per generation in Nile tilapia (Eknath *et al.*, 1998) [69].
2. The Jayanti Rohu selective breeding project (India), reported to acquired 17% genetic gain per generation. (Reddy *et al.*, 1999; Mahapatra, 2004) [70, 71].
3. Malawian indigenous Tilapia (*Oreochromis shiranus*) selection project, reported to have acquired 13% genetic gain over two generation (Maluwa, 2005) [72].

2.1.4 Selection within cohorts and exchange of breeders

Poor broodstock management is the major cause of genetic detoriation encountered in the hatcheries of India. (Eknath, 1991) [73]. In order to rectify such problem, he suggested that the broodstock could be randomly divided into groups (cohorts). And on the rotational basis, mating could be performed to avoid the inbreeding depression.

2.1.5 Progeny testing

Progeny represents a random collection of respective parent gene allele for the concerned trait (50% of each of the parents) and hence the performance of the progeny of a certain male or female gives a good expression of its breeding value for the concerned trait. Progeny testing can be advantageous for the traits that cannot be measured on live breeding fish like disease resistance and product quality. In spite of that progeny testing imposes a lot of disadvantages, most important of them is the extension of generation interval. As in carps, where it takes one to two years for breeding, would slow down the selection work by 20-30% as compared to others which take less time. Also, it becomes a prime concern for the species that spawns only once or have high mortality rate after spawning, where progeny testing is of no relevance. Therefore, for this reason progeny testing is rarely used in aquaculture and hence rarely used.

2.2 Success of selective breeding in aquaculture

Selective breeding programmes on a number of species has been done in aquaculture with more promising results. Some of the successful breeding programme are mentioned here

2.2.1 Atlantic salmon

The first family based selective breeding programme was started by AKVAFORSK in 1975 for Atlantic salmon (Gjedrem 2000) [74]. The selection study initiated with the aim of growth enhancement, subsequently breeding goal extended to disease resistance, age at sexual maturity and a number of traits related to product quality. A genetic gain of 14% per generation was achieved for six generations (Gjerde and Korsvoll 1999) [75].

2.2.2 Rainbow trout

Selection of rainbow trout (*Oncorhynchus mykiss*) for growth rate has been successful with attained genetic gain of 10% to 13% per generation. Selective breeding programmes for rainbow trout has been carried out for different traits like growth rate and early sexual maturation (Kause *et al*. 2005) [76], early spawn date (Siitonen and Gall 1989) [77], resistance to IPN virus (Okamoto *et al*. 1993) [78] and muscle lipid content (Quillet *et al*. 2005) [79] and significant results were achieved.

2.2.3 Coho Salmon

Selection programme for coho salmon was carried out at University of Washington in Seattle for improvement of traits of importance for saltwater net-pen industry with increased growth rate of 15% per generation is achieved (Hershberger *et al*. 1990) [80].

2.2.4 Tilapia

The GIFT (Genetic Improvement of Farmed Tilapias) Tilapia project was initiated in 1988 on selective breeding of Nile Tilapia in Philippines by collaboration of ICLARM (Currently World Fish Centre) and AKVAFORSK and several other National research institutions of the country. The family based selection process carried out by taking eight strains; four wild strains from Africa and four farmed strains as base population. An average response of 17% genetic gain per generation was achieved in first 5 generations.

2.2.5 Carps

A family-based selection process was carried out for Indian Rohu (*Labeo rohita*) Central Institute Freshwater Aquaculture (CIFA), Bhubaneswar, India in collaboration with AKVAFORSK, with enhanced growth rate as major focus area. The base population for the selection were taken from 5 north Indian rivers and one farmed stock. A genetic gain of 18.3% achieved after eight generations of selective breeding (A rasal *et al*., 2017) [81].

2.2.6 Channel Catfish (*Ictalurus punctatus*)

Selective breeding of Channel Catfish (*Ictalurus punctatus*) was carried out for improved growth rate with an average growth rate of 13% was achieved per generation for six generations (Dunham (2006) [82].

2.2.7 Sea Bream (*Sparus aurata*)

A selective breeding programme for Sea Bream **(***Sparus aurata***)** for increased growth rate, reduced incidence of deformities, and improved external pigmentation carried out in by Kego S.A (Greece) in cooperation with AFGC. The genetic gain of 12% growth rate per generation was reported.

2.2.8 Shrimp

A breeding programme for *L. vannamei* was carried out by Ceniacua (Colombia) in cooperation with AFGC for increased growth rate, overall survivability, and survival against WSSV resistance. The average response was 4.2% growth rate, 5.7% for survival (under absence of specific pathogens) and 1.7% for survival against WSSV (Gitterle *et al*. 2006) [83].

Another selection study for *L. vannamei* was carried out at the Oceanic Institute in Hawaii for growth rate and resistance against Taura syndrome virus (TSV) and a response of 4.4% growth rate and 12.4% higher survival against TSV was reported.

2.2.8 Oyster

Different selective breeding programme were carried out for Pacific oyster (*Crassostrea gigas*) for increased live weight yield. The average response was an average genetic of 9.5% per generation for seven generations. A higher growth rate of 17% and 20% was obtained from selection studies done in European oyster (*Ostrea edulis*) Newkirk and Haley (1983) [84] and Barber *et al*. (1998) [85] respectively. Nell and Hand, 2003 [86]; reported reduced mortality 22% after two generations of selection of Sydney rock oyster populations (*Saccostrea glomerata*) in New

South Wales, Australia against a parasite (*Marteilia sydneyi*).
Continued research is recommended to address challenges such as poor broodstock management and extended generation intervals. Innovations in family selection and the exchange of breeders are suggested to enhance the success of selective breeding programs.

3. Application of Transgenesis in Aquaculture

3.1 Introduction

Transgenesis is the technique of developing genetically modified organisms by making heritable changes to the host genome by integration of exogenous DNA (transgene) into the host genomic DNA (Cebeci, 2020) [3]. Transgenesis is a novel method of incorporating desirable genetic traits and improvement of character of economic interest in fish for more profitable and sustainable aquaculture. Transgenesis finds its application through various genetic improvements in terms of growth enhancement, cold tolerance, disease resistant, production of ornamental fishes and use of transgenics in environment monitoring has been achieved contributing significantly to aquaculture (Cebeci, 2020) [3]. Hence considering the rising global population, transgenesis can be considered as a boon to the aquaculture to address the malnutrition and nutritional gap.

The first transgenic fish in aquaculture was done in rainbow trout followed by in gold fish (Zhu *et al*., 1984) [14]. Till now over 35 species of fishes are studied for transgenesis, of which many having significant importance in aquaculture (Zbikowska, 2003) [25].

3.2 The basic procedure of transgenesis

The basic procedure for transgenesis includes.

1. Preparation of gene construct.
2. Method of transfer of transgene into host.
3. Screening of fishes for transgenesis.
4. Study of inheritance of transgene in F_1 generation (Levy *et al*., 2000) [11].

3.2.1 Preparation of transgene

A transgene construct consist of 3 components, a promoter, a structural gene coding for gene of interest and a termination sequence. Selection of a suitable promoter has been a challenging factor the development of transgene. The common promoters used in 1980s were mammalian or viral promoters and mammalian growth hormone genes, for example, mouse metallothionein-1 (mMt-1), Rous Sarcoma Virus and SV40. But with the development of biotechnology, promoter and gene of piscine origin like anti-freeze protein (AFP) and carp β-actin were used with more efficient expression of target gene (Levy *et al*., 2000) [11].

3.2.2 Methods of gene transfer

There are several gene transfer technologies developed for transferring the transgene into host genome. Those includes microinjection via cytoplasm or germinal vesicle, electroporation, lipofection, retrovirus infection, particle-gun bombardment and sperm mediated transfer. Among these microinjection and electroporation proved to be more efficient in transferring the transgene into host genome (Chen 2001, Dunham and Winn 2014) [8].

3.3 Journey of fish transgenesis over time

The journey of transgenesis can be traced back to 1973 with development of first transgenic organism *Escherichia coli* (Cohen *et al*., 1973) [26]. This was followed by development of first transgenic animal in 1974 with the development of a transgenic mouse carrying SV40 viral DNA (Jaenisch and Mintz, 1974) [27]. Transgenesis in fish began its journey with the report of microinjection of cloned DNA into Rainbow trout (Mclean and Talwar, 1984) [13] with 5% success in resultant fish. This achievement was followed by successful integration of metallothionein promoter fused with human growth hormone gene in gold fish (Zhu *et al*., 1985) [14]. Further investigations on integration of human growth hormone gene through micro-injection into fertilized eggs of channel catfish (*Ictalurus punctatus*) and Nile tilapia (*Oreochromis niloticus*) was achieved (Dunham, *et al*., 1987) [28], (Brem *et al*., 1988) [29]. In 1987, successful transfer of transgene in to the offspring was reported in common carp and rainbow trout (Mclean *et al*., 1987) [30]. Further development on creating all fish constructs consisting promoter and gene sequence from fish species as in GH-transgenic carp by incorporating rainbow trout GH gene into fertilized carp eggs (Zhang *et al*., 1990) [31]. This led to the development of cold resistant transgenic salmon by using antifreeze gene sequence from winter flounder (*Pseudopleuronectes americanus*) (Shears *et al*., 1991) [32].

A significant development of transgenesis achieved with development of transgenic salmon with higher growth rates by injecting a gene construct containing anti-freeze protein (AFP) promoter from ocean pout (*Zoarces americanus*) and Chinook salmon Growth hormone cDNA (Du *et al*., 1992) [32]. Later this fish was commercially produced for consumption as AquAdvantage Salmon by Aquabounty Company. Recently in 2015 Food and Drug Association (FDA) of USA declared Aqu Advantage salmon is safe for human consumption. Further research carried out to prepare construct from the same fish species as in transgenic mud loach produced by injecting gene construct containing mud loach loach's growth hormone gene and β-lactin promoter (Nam *et al*., 2001) [33]. 2003 marked the production of first ornamental transgenic zebra fish commercialized as GloFish™ (Gong *et al*. 2003; Tonelli *et al*., 2017) [34, 35].

3.4 Application of transgenesis in aquaculture

3.4.1 Growth enhancement

Several studies on growth enhancement of fishes through transgenesis has been done with significant achievement by achieving up to 35 fold increase in size compared with the control non-transgenic fishes. (Nam *et al*. 2001) [33]. Higher growth was achieved by integrating growth hormone transgene for a few species including Nile tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*), mud loach (*Misgurnus mizolepis*), coho salmon (*Oncorhynchus kisutch*), and rohu carp (*Labeo rohita*) (Barman *et al*., 2015; Devlin *et al*., 1994; Nam *et al*., 2001; Rahman *et al*., 1998; Zhang *et al*., 1990) [36, 33, 31, 37, 38].

Aqu. Advantage Salmon

The greatest achievement in the field of transgenesis is the development of AquAdvantage salmon by the scientist of memorial university of Newfoundland, Canada. The early phase of research was focused on developing cold resistant salmon by inserting antifreeze gene from winter flounder into fertilized Atlantic salmon eggs to improve the temperature tolerance of salmon below freezing point of salmon blood(-0.7 ^{0}C). Failure of this experiment lead turn the attention of scientist toward developing fast growing salmon by inserting growth hormone gene (GH cDNA (opAFP-GHc2) from Chinook salmon (*Oncorhynchus tshawytscha*) combined with anti-freeze protein (AFP) gene promoter from ocean pout (*Macrozoarces*

americanus) into fertilized Atlantic salmon eggs (du *et al*., 1992) [32]. AquAdvantage salmon acquires an enhanced growth of 4-5 kg from eyed egg stage in 16-20 months compared to 28-32 months for non-transgenic farmed salmon (aquabounty 2016). As compared to other salmon, AquAdvantage salmon requires 25%less feed with efficient protein utilization (Aquabounty 2019). It took a long roller coaster journey for Aquabounty Company to get FDA approval for human consumption. The company applied for FDA approval in 1995 and after a series of investigation and assessment FDA finally declared AquAdvantage salmon safe for human consumption in 2015 (FDA, 2015) [40].

3.4.2 Cold temperature resistant

Cold resistance is observed in many teleost fishes like winter flounder and ocean pout whose body fluid does not freeze at the freezing point of sea water (-1.7 °C to -2 °C) (Rasmussen, 2006). The fishes produce anti-freeze proteins to protect them from freezing. Hence research started for producing cold tolerant transgenic fish producing AFP proteins with the initial objective of culturing salmon at cold temperature region of east coast of Canada. However, the AFP levels achieved in salmon were not adequate to achieve the desired cold tolerance (Gomez, 2018). However significant results were achieved by integrating cold tolerance genes in goldfish (Wang *et al*., 1995) [41] and tilapia (Wu *et al*., 1998), which protected the fishes from cold temperatures in winter.

3.4.3 Disease resistance

Disease resistance can be addressed by transgenic technology by incorporating antimicrobial peptide genes into fish. Cecropin is an anti-microbial protein having antimicrobial activity against number of bacterial species. Enhanced disease resistance and higher survival was recorded against *Edwardsiella ictaluri* and *Flavobacterium columnare* by introducing Cercopin gene into the genome of channel catfish (*Ictalurus punctatus*) (Dunham and others 2002b). Correspondingly on Japanese medaka (*Oryzias latipes*) higher disease reisistance was observed against *Pseudomonas fluorescens* and *Vibrio anguillarum* by injecting *insect cercopin* or pig *cercopin transgene* linked to a CMV promoter. Sarmasik and others 2002). Another distinct approach to use fish lysozyme as an antibacterial agent was done in zebra fish. The used hen egg white lysozyme gene and Japanese flounder (*Paralichthys olivaceus*) keratin promoter in zebra fish and recorded 60% survival compared to 100% death in control.

3.4.4 Environmental monitoring

Transgenic fishes can be used as biosensor to monitor the presence of toxic chemicals detectable to very minute concentrations (Cebeci, 2020) [3]. Transgenic lines of fishes used in the field of ecotoxicology are developed by integrating a reporter gene with a DNA response element that can be stimulated by the presence of certain toxic chemicals or pollutant. The toxic chemicals accumulated in the fish tissue are responded by genome response elements which activates the reporter gene. The expression of reporter gene activity is assayed by gene expression studies to calculate the amount of toxic chemicals present in the fish. (Zbikowska, 2003 [25]). *Transgenic zebra* fish developed to detect cadmium toxicity using HSP70 gene promoter to enhanced Green Fluorescent protein as reporter gene sensed concentration as low as 22.5 µg/L. (Blechinger *et al*. 2002) [44]. Other studied carried out for environmental toxicity and stress factor include aryl hydrocarbon mediated toxicity, oxidative stress through induction of an electrophile-responsive element, estrogenecity via vitellogenin / choriogenin / estrogen receptor-responsive elements stimulating luciferase or GFP(Green Fluorescent Gene) as reporter genes (Mattingly *et al*., 2001, Petersen *et al*., 2013; Zeng *et al*., 2005) [45, 46].

3.4.5 Ornamental fishes

Development of elegantly colored transgenic fishes contributed significantly to the ornamental fish sector. GFP construct tailored with zebrafish muscle specific promoter of the myosin light polypeptide 2 (mylz2) gene is injected to zebra fish showed consistent expression (ju *et al*. 2003) [48]. Using the same zebra fish mylz2 promoter fluorescent medaka, and farmed rohu and white skirt tetra, (*Gymnocorymbus ternetzi*), were successfully produced later. (Mohanta *et al*., 2014; Pan *et al*., 2008; Zeng *et al*., 2005) [49, 50, 47]. Glo fish, which is a milestone in development of transgenic ornamental fishes is commercially marketed in six different fluorescent color varieties comprises Starfire red, galactic purple, sun burst orange, electric green, cosmic blue and moonrise pink (Cebeci, 2020) [3].

3.4.6 Ecological and social issues

Even if the transgenic animals possess enormous potential to contribute significantly to aquaculture production and to augment nutritional demand there are a few concerns arises of detrimental effect of this transgenic animals on ecosystem. Hence proper risk assessment must be done to know fully about undesirable effect of accidental entry of these fishes in to wild. (Kapuscinski 2005) [51]. Accidental escape of these transgenic animal is a major concern for environmental safety. Mating of these transgenic fishes with the wild population may cause alteration of gene pool (McGinnity and others 2003, Roberge *et al*., 2006) [52, 53]. Although production of sterile animals by chromosome manipulation may prove to be an alternative to prevent pollution of gene pool in wild stock, no method that are available at present can produce 100% sterile offspring and hence can't eliminate the risk completely (Dunham, 2004) [7] Superior traits like enhanced growth and disease resistance of transgenic animal may attribute to enhanced predation and competition with indigenous wild population leading to their extinction and will subsequently lead to disruption in natural biodiversity. (Devlin *et al*., 1999) [54]. In addition, transgenic animal may carry certain pathogens from farm to wild causing disease outbreak in wild populations (Naylor *et al*., 2005) [55].

3.4.7 Health concerns

Insertion of a transgene may lead to production of allergens or toxins which were inactive previously in the fish body (Galli, 2002; Kelly 2005) [56, 57]. Increased disease resistance in transgenic fishes may enable them as a suitable host to carry disease causing new pathogen which can be passed to human causing zoonotic diseases (FAO, 2000; Rasmussen). Hence all these health concerns welcome public criticism and resistance that possess a problem for its commercialization.

4. Conclusion

Transgenesis has opened a new horizon in higher production of fish protein contributing to sustainable aquaculture development and augmenting nutritional deficiency. Recent innovation in transgenesis like gene transfer technologies has made it possible to the development of genetically modified fishes with more desirable traits like higher growth, cold resistance, disease resistance and elegant coloration. The major breakthrough in transgenesis can be conferred to AquAdvantage salmon, which

is the first food fish to approved as safe for human consumption. However, inspite of this phenomenal success several concerns like escape of transgenic fishes to the wild possess risk for environmental and health issues. Escape of fishes to wild may lead to breeding with wild stocks subsequently polluting the gene pool. Although creation sterile population by polyploidy can address such problems, however 100% success in such chromosomal manipulation techniques have not been achieved. In addition to environmental and health issues, consumer acceptance has been a major bottleneck for the transgenic fish production. For which detailed promotion and explanation on creation of genetically modified fish is required to gain the trust of consumers. Hence, we can conclude that transgenic fishes can be proven to be a major tool to address the nutrition deficiency in near future.

5. Future directions

Integrating polyploidy induction, selective breeding, and transgenesis is the next frontier in genetic improvement. Fish populations with improved characteristics, such as fast growth, disease resistance, and ecological adaptation, may arise from investigating ways to strategically combine these techniques. To optimize the synergistic impacts of various genetic enhancement techniques, this integrated strategy should be driven by a thorough understanding of their interconnections. By refining existing techniques, addressing emerging challenges, and fostering responsible deployment, the aquaculture industry can unlock the full potential of genetic technologies for sustainable, efficient, and environmentally conscious fish farming.

6. Sustainable Implementation and Global Collaboration

As genetic improvement techniques advance, it is crucial to focus on sustainable implementation practices. This involves not only refining genetic technologies but also addressing economic considerations, environmental sustainability, and social acceptance. Global collaboration and knowledge-sharing platforms should be established to facilitate the exchange of information, experiences, and best practices in genetic improvement, ensuring that advancements benefit diverse aquaculture contexts worldwide.

7. References

1. Benfey TJ, Sutterlin AM. Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). Aquaculture. 1984a;36:359–367.
2. Cassani JR, Caton WE. Efficient production of triploid grass carp (*Ctenopharyngodon idella*) utilizing hydrostatic pressure. Aquaculture. 1986a;55:43-50.
3. Cebeci A, Aydin I, Goddard A. Bigger, stronger, better: Fish transgenesis applications and methods. Biotech Studies. 2020;29(2):85-97.
4. Chourrout D, Itskovich J. Three manipulations permitted by artificial insemination in tilapia: induced diploid gynogenesis, production of all-triploid populations and intergeneric hybridization. In: Fishelson L, Yaron Z, compilers. International Symposium on Tilapia in Aquaculture. Tel Aviv University, Tel Aviv, Israel; c1983. p. 246.
5. Chourrout D. Pressure induced retention of second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids and heterozygous and homozygous diploid gynogenetics. Aquaculture. 1984;36:111–126.
6. Curtis TA, Sessions FW, Bury D, Rezk M, Dunham RA. Induction of polyploidy in striped bass, white bass and their hybrids with hydrostatic pressure. Proceedings Annual Conference Southeastern Association of Fish Wildlife Agencies. 1987;41:63–69.
7. Dunham RA. Aquaculture and fisheries biotechnology: genetic approaches. Cambridge, Mass.: CABI Publishing; c2004. p. 372.
8. Dunham RA, Winn RN. Production of Transgenic Fish. In: Transgenic Animal Technology; c2014. p. 305–334.
9. Francescon A, Libertini A, Bertotto D, Barbaro A. Shock timing in mito gynogenesis and tetraploidization of the European sea bass, *Dicentrarchus labrax*. Aquaculture. 2004;236:201–209.
10. Gómez A. Genetically modified fish in aquaculture. Frontiers in Sciences and Engineering. 2018;8(1):61-68.
11. Levy JA, Marins LF, Sanchez A. Gene transfer technology in aquaculture. Hydrobiologia. 2000;420:91–94.
12. Johnstone R, Knott RM, MacDonald AG, Walsingham MV. Triploidy induction in recently fertilized Atlantic salmon ova using anaesthetics. Aquaculture. 1989;78:229–236.
13. Mclean N, Talwar S. Injection of cloned genes into rainbow trout eggs. J Embryol. Exp. Morphol. 1984;82:187.
14. Zhu Z, He L, Chen S. Novel gene transfer into the fertilized eggs of gold fish (*Carassius auratus* L. 1758). Journal of Applied Ichthyology. 1985;1:31-34.
15. Nam YK, Kim DS. Ploidy status of progeny from the crosses between tetraploid males and diploid females in mud loach (*Misgurnus mizolepis*). Aquaculture. 2004;236:575–582.
16. Piferrer F, Beaumont A, Falguiere JC, Flajshans M, Haffray P, Colombo L. Polyploid fish and shellfish; Production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture. 2009;293:125–156.
17. Rasmussen RS, Morrissey MT. Biotechnology in aquaculture: Transgenics and polyploidy. Comp. Rev. Food Sci. F. 2007;6:2–16.
18. Rizzo G, Spagnolo M. A model for the optimal management of sea bass *Dicentrarchus labrax* aquaculture. Mar. Resour. Econom. 1996;11:267–286.
19. Sellars M, Degnan BM, Preston NP. Recent advances in *Marsupenaeus japonicus* polyploid induction: Mitotic tetraploidy and polar body I triploidy. Meeting Abstract #55. AQUA, Florence, Italy. May 10–13, 2006.
20. Stöck M, Lamatsch DK, Steinlein C, Eppeln JT, Grosse W-R, Hock R, *et al*. A bisexually reproducing all-triploid vertebrate. Nat. Genet. 2002;30:325–328.
21. Thorgaard GH, Jazwin ME, Stier AR. Polyploidy induced by heat shock in rainbow trout. Transactions of the American Fisheries Society. 1981;110:546–550.
22. Wang Z, Guo X, Allen SK, Wang R. Heterozygosity and body size in triploid Pacific oysters, Crassostrea gigas Thunberg, produced from meiosis II inhibition and tetraploids. Aquaculture. 2002;204:337–348.
23. Wang Z, Li Y, Yu R, Gao Q, Tian C, Zheng X, Wang R. Growth comparison between triploid and diploid Pacific oyster during the reproductive season. Am Fish Soc Symp. 2003;38:285–289.
24. Wolters WR, Chrisman CL, Libey GS. Induction of triploidy in channel catfish; c1981a.
25. Zbikowska HM. Fish can be first--advances in fish transgenesis for commercial applications. Transgenic research. 1996;12:379-89.
26. Cohen SN, Chang ACY, Hsu L. Nonchromosomal

antibiotic resistance in bacteria: Genetic transformation of Escherichia coli by R-factor DNA. Proc. Natl. Acad. Sci. USA. 1972;69:2110–2114.
27. Jaenisch R, Mintz B. Simian virus 40 DNA sequences in DNA of healthy adult mice derived from pre-implantation blastocysts injected with viral DNA. Proc. Natl. Acad. Sci. USA. 1974;71:1250-4.
28. Dunham RA, Eash J, Askins J, Townes TM. Transfer of the metallothionein-human growth hormone fusion gene into channel catfish. Trans Am Fish Soc. 1987;116:87-91.
29. Brem G, Brenig B, Hörstgen-Schwark G, Winnacker EL. Gene transfer in tilapia (*Oreochromis niloticus*). Aquaculture. 1988;68:209-219.
30. Mclean N, Penman D, Zhu Z. Introduction of novel gene in fish. Nat Biotechnol. 1987;5:257-261.
31. Zhang PJ, Hayat M, Joyce C, Gonzalez-Villaseñor LI, Lin CM, Dunham RA, *et al*. Gene transfer, expression and inheritance of pRSV-rainbow trout-GH cDNA in the common carp, *Cyprinus carpio* (Linnaeus). Mol. Reprod. Dev. 1990;25:3-13.
32. Du SJ, Gong ZY, Fletcher GL, Shears MA, King MJ, Idler DR, *et al*. Growth enhancement in transgenic Atlantic salmon by the use of an "all fish" chimeric growth hormone gene construct. Biotechnology. 1992;10:176-181.
33. Nam YK, Noh JK, Cho YS, Cho HJ, Cho KN, Kim CG, *et al*. Dramatically accelerated growth and extraordinary gigantism of transgenic mud loach *Misgurnus mizolepis*. Transgenic Res. 2001;10:353-362.
34. Gong Z, Wan H, Tay TL, Wang H, Chen M, Yan T. Development of transgenic fish for ornamental and bioreactor by strong expression of fluorescent proteins in the skeletal muscle. Biochem. Biophys. Res. Commun. 2003;308:58-63.
35. Tonelli FMP, Lacerda SMSN, Procópio MS, Lemos BLS, de França LR, Resende RR. Gene delivery to Nile tilapia cells aiming transgenesis and the role of PI3K-c2α in angiogenesis. Sci Rep. 2017;7:44317.
36. Barman HK, Mohanta R, Patra SK, Chakrapani V, Panda RP, Nayak S, *et al*. The β-actin gene promoter of rohu carp (Labeo rohita) drives reporter gene expressions in transgenic rohu and various cell lines, including spermatogonial stem cells. Cell Mol. Biol. Lett. 2015;20(2):237-247.
37. Devlin RH, Yesaki TY, Biagi CA, Donaldson EM, Swanson P, Chan WK. Extraordinary salmon growth. Nature. 1994;371(6494):209-210.
38. Rahman MA, Mak R, Ayad H, Smith A, Maclean N. Expression of a novel piscine growth hormone gene results in growth enhancement in transgenic tilapia (*Oreochromis niloticus*). Transgenic Res. 1998;7(5):357-370.
39. Zhang PJ, Hayat M, Joyce C, Gonzalez-Villasenor LI, Lin CM, Dunham RA, *et al*. Gene transfer, expression and inheritance of pRSV-rainbow trout-GH cDNA in the common carp, *Cyprinus carpio* (Linnaeus). Mol. Reprod. Dev. 1990;25(1):3–13.
40. FDA. U. S. Food & Drug Administration. FDA Consumer Health Information. FDA has determined that the Aqu. Advantage salmon is as safe to eat as non-GE salmon; c2015 Nov.
41. Wang R, Zhang P, Gong Z, Hew CL. Expression of the antifreeze protein gene in transgenic goldfish (*Carassius auratus*) and its implication in cold adaptation. Mol Mar Biol. Biotechnol. 1995;4(1):20–26.
42. Wu S, Hwang P, Hew C, Wu J. Effect of Antifreeze Protein on Cold Tolerance in Juvenile Tilapia (*Oreochromis mossambicus* Peters) and Milkfish (Chanos chanos Forsskal). Zoological Studies. 1998;37(1):39-44.
43. Yazawa R, Hirono I, Aoki T. Transgenic zebrafish expressing chicken lysozyme show resistance against bacterial diseases. Transgenic Res. 2006;15(3):385-391.
44. Blechinger SR, Warren JT, Kuwada JY, Krone PH. Developmental toxicology of cadmium in living embryos of a stable transgenic zebrafish line. Environ Health Perspect. 2002;110(10):1041–1046.
45. Mattingly C, McLachlan J, Toscano W. Green fluorescent protein (GFP) as a marker of aryl hydrocarbon receptor (AhR) function in developing zebrafish (Danio rerio). Environ Health Perspect. 2001;109(8):845-849.
46. Petersen K, Fetter E, Kah O, Brion F, Scholz S, Tollefsen KE. Transgenic (cyp19a1b-GFP) zebrafish embryos as a tool for assessing combined effects of oestrogenic chemicals. Aquat Toxicol. 2013;138:88-97.
47. Zeng Z, Liu X, Seebah S, Gong Z. Faithful expression of living color reporter genes in transgenic medaka under two tissue-specific zebrafish promoters. Dev. Dyn. 2005;234(2):387-392.
48. Ju B, Chong S, He J, *et al*. Recapitulation of fast skeletal muscle development in zebrafish by transgenic expression of GFP under the mylz2 promoter. Dev Dyn. 2003;227(1):14-26.
49. Mohanta R, Jayasankar P, Das Mahapatra K, Saha JN, Barman HK. Molecular cloning, characterization and functional assessment of the myosin light polypeptide chain 2 (mylz2) promoter of farmed carp, Labeo rohita. Transgenic Res. 2014;23:601-607.
50. Pan X, Zhan H, Gong Z. Ornamental expression of red fluorescent protein in transgenic founders of white skirt tetra (*Gymnocorymbus ternetzi*). Mar Biotechnol (NY). 2008;10:497-501.
51. Kapuscinski AR. Current scientific understanding of the environmental biosafety of transgenic fish and shellfish. Rev Sci Tech. 2005;24:309-322.
52. McGinnity P, Prodohl P, Ferguson A, *et al*. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proc Biol Sci. 2003;270:2443–50.
53. Roberge C, Einum S, Guderley H, Bernatchez L. Rapid parallel evolutionary changes of gene transcription profiles in farmed Atlantic salmon. Mol Ecol. 2006;15(1):9–20.
54. Devlin RH, Johnsson JI, Smailus DE, Biagi CA, Jonsson E, Bjornsson BT. Increased ability to compete for food by growth hormone-transgenic coho salmon Oncorhynchus kisutch (Walbaum). Aqua Res. 1999;30:479–82.
55. Naylor R, Hindar K, Fleming IA, *et al*. Fugitive salmon: assessing the risks of escaped fish from net-pen aquaculture. BioScience. 2005;55(5):427–37.
56. Galli L. Genetic modification in aquaculture: A review of potential benefits and risks. Bureau of Rural Sciences, Canada.
Available from: http://www.affa.gov.au/corporate_docs/publications/pdf/ruralscience/landuse/GM in Aquaculture.pdf
57. Kelly L. The safety assessment of foods from transgenic and cloned animals using the comparative approach. Rev Sci. Tech. 2005;24(1):61-74.
58. FAO. The state of the world fisheries and aquaculture (SOFIA). FAO, Rome. Available from: http://www.fao.org/sof/sofia/index en.htm.

59. Tave D. Selective breeding programmes for medium-sized fish farms. FAO Fisheries Technical Paper. No. 352. Rome, FAO; c1995. p. 122.
60. Gjedrem T, Robinson N. Advances by Selective Breeding for Aquatic Species: A Review. Agric. Sci. 2014;5:1152-1158.
61. Embody GC, Hyford CD. The Advantage of Rearing Brook Trout Fingerlings from Selected Breeders. Trans Am Fish Soc. 1925;55:135-138.
62. Kirpichnikov VS. Selection and New Breeds of Pond Fishes in the USSR. In: Tiewes K, ed. Selection, Hybridization and Genetic Engineering in Aquaculture. 1987;2:461-473.
63. Dunham RA. Contribution of genetically improved aquatic organisms to global food security. In: International Conference on Sustainable Contribution of Fisheries to Food Security. Government of Japan and FAO, Rome; c1996.
64. Dunham RA, Majumdar K, Hallerman E, *et al*. Review of the status of aquaculture genetics. In: Subasinghe RP, Bueno P, Phillips MJ, Hough C, McGladdery SE, Arthur JR, eds. Technical Proceedings of the Conference on Aquaculture in the Third Millennium. Bangkok, Thailand, 20–25 February 2000. NACA, Bangkok and FAO, Rome; c2001. p. 129–157.
65. Gjedrem T, Baranski M. Selective Breeding in Aquaculture: An Introduction. Springer, 2009, 221.
66. Hulata G, Wohlfarth GW, Halevy A. Mass Selection for Growth Rate in the Nile Tilapia (*Oreochromis niloticus*). Aquaculture. 1986;57:177-184.
67. Huang CM, Liao IC. Response to Mass Selection for Growth Rate in *Oreochromis niloticus*. Aquaculture. 1990;85:199-205.
68. Zuma A. Term paper on Practical Genetics and Selective Breeding in Aquaculture; c2021.
69. Eknath AE, Dey MM, Rye M, *et al*. Selective breeding of Nile tilapia for Asia. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production. 1998;27:89–96.
70. Reddy PVGK, Jana RK, Mahapatra KD, *et al*. Selective breeding of rohu, May 1992 – March 1996 and Genetic improvement of rohu for growth through selective breeding, April 1997 – June 2003. Final report on The Indo-Norwegian Collaboration Project; c2003. p. 56.
71. Mahapatra KD, Gjerde B, Saha JN, *et al*. Realized genetic gain for growth in rohu (Labeo rohita). In manuscript; c2004.
72. Maluwa AOH. PhD Thesis. Norwegian University of Life Sciences, Norway; c2005.
73. Eknath AE, Bentsen HB, Gjerde B, *et al*. Approaches to national fish breeding programs: Pointers from a tilapia study. NAGA the Quarterly, April; c1991. p. 10-12.
74. Gjedrem T. Genetic improvement of cold-water fish species. Aquac. Res. 2000;31:25–33.
75. Gjerde B, Korsvoll A. Realized selection differentials for growth rate and early sexual maturity in Atlantic salmon. Abstracts, Aquaculture Europe 99, Trondheim, Norway; c1999 Aug. p. 73-74. 7-10
76. Kause A, Ritola O, Paananen T, Wahloos H, Mäntysaari E. Genetic trends in growth, sexual maturity and skeletal deformations, and rate of inbreeding in a breeding programme for rainbow trout (*Onchorhynchus mykiss*). Aquaculture. 2005;247:177–187.
77. Siitonen L, Gall GAE. Response to selection for early spawn date in rainbow trout, *Salmo gairdneri*. Aquaculture. 1989;78:153–161.
78. Okamoto N, Tayaman T, Kawanobe M, *et al*. Resistance of a rainbow trout strain to infectious necrosis. Aquaculture. 1993;117:71–76.
79. Quillet E, Guillou SL, Aubin J, Fauconneau B. Two-way selection for muscle lipid content in pan-size rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2005;245:49–61.
80. Hershberger WK, Myers JM, Iwamoto RN, Mcauley WC, Saxton AM. Genetic changes in the growth of coho salmon (*Onchorhynchus kisutch*) in marine net-pens, produced by ten years of selection. Aquaculture. 1990;85:187–197.
81. Rasal M, Patnaik K, Murmu P, *et al*. Genetically Improved Jayanti Rohu: A Boon to Freshwater Aquaculture in India. World aquaculture; c2017 Dec. p. 23-24.
82. Dunham RA. Comparison of six generations of selection, interspecific hybridization, intraspecific crossbreeding and gene transfer for growth improvement in Ictalurus catfish. IAGA, 26–30 June, Montpellier, Abstract; c2006. p. 22.
83. Gitterle T, Johansen H, Erazo C, *et al*. Response to multi-trait selection for harvest weight, overall survival, and resistance to white spot syndrome virus (WSSV) in Penaeus (*Litopenaeus*) vannamei. IAGA, 26–30 June 2006, Montpelier, Abstract. 2006, 35.
84. Newkirk GF, Haley LE. Selection for growth rate in the European oyster, Ostrea edulis: Response of second generation group. Aquaculture. 1983;33:149–155.
85. Barber B, Davis C, Hawes R. Genetic improvement of oysters for the marine aquaculture industry. Abstract of the first Annual Northwest Aquaculture Conference and Exposition; c1998. p. 35.
86. Nell JA, Hand RE. Evaluation of the progeny of second-generation Sydney rock oyster *Saccostrea glomerata* (Gold, 1850) breeding lines for resistance to QX disease *Marteilia sydneyi*. Aquaculture. 2003;228:27–35.
87. Ibarra AM, Ramirez JL, Ruiz CA, *et al*. Realized heritabilities and genetic correlation after dual selection for total weight and shell width in catarina scallop (*Argopecten ventricosus*). Aquaculture. 1999;175:227–241.