

## APPLICATION OF GENETIC IMPROVEMENT TECHNIQUES IN AQUACULTURE INDUSTRY

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### ABSTRACT

*The purpose of this review is to emphasize the implemented success of genetic improvement techniques in aquaculture industry. The review illustrates the application of genetic improvement technology, that involves classical and modern genetic technology, in diverse aquatic species. Such as, the application of selection breeding technology, integration breeding technology (intra-specific crossbreeding and inter-specific cross breeding), chromosome set manipulation (artificial androgenesis, artificial gynogenesis, and polyploid breeding), and modification breeding technology (represented by transgenic breeding). Some cultured species received concentrated breeding effort, whereas other major cultured species received limited attention, and a few species have not been genetically improved. Genetic improvement of aquaculture species offers a substantial opportunity for increasing production efficiency, health, product quality, profitability in aquaculture enterprises. Therefore, Genetic improvement technology has a major role to ensure the continued expansion and intensification of aquaculture to meet the growing demand.*

**KEYWORDS:** *Genetic Improvement, Technology, Selective Breeding, Chromosome Set Manipulation, Crossbreeding, Modification Breeding*

### INTRODUCTION

Throughout the last decades, aquaculture has continued to represent the fastest growing animal food producing sector of the world. Increased aquaculture production is clearly needed to meet the rising demand for high-quality protein in the future, because the world fisheries are showing a rapid declining due to overfishing, pollution, and habitat destruction.

Increased demand for aquaculture production involves the need to developed fish strains, that can improve cultured stock. To address this, researchers have used a variety of genetic improvement techniques to produce new strains that have desirable traits such as rapid growth rate, high flesh quality, and disease resistance (Xu et al. 2015). The various approaches to genetic improvement can be divided into these categories: selective breeding, intra-specific cross breeding and inter-specific crossbreeding, chromosome set manipulation, and modification breeding. Selective breeding involves selection and breeding of individuals in population that have desirable traits (Hussain et al. 2002).The classical approach to traditional selective breeding is to choose and breed only individuals that exhibit desirable characteristics for one or multiple traits such as growth rate, meat quality, and disease resistance (Xu et al. 2015).Intra-specific crossbreeding and inter-specific crossbreeding involves the integration of two or more groups to obtain a combine of desirable traits from the donors(Bakos & Gorda 1995). Chromosome set manipulation involves the production of mono-sex and sterile species by using temperature, hydrostatic pressure, or chemical shock

(Donaldson 1996). Modification breeding involves the creation of transgenic fish by the transfer of genetic material from a donor to a recipient via micromanipulation (El-Zaeem 2004). The purpose of integration breeding, chromosome set manipulation, and modification breeding is to alter the genotype and phenotype of offspring. Selection breeding can be used subsequent to integration or modification breeding to ensure the persistence and emphasis of desirable inherited characteristics (Xu et al. 2015).

Genetic improvement techniques have opened a new window for development of genetic resources in aquaculture. Genetic improvement technology can be utilized in aquaculture for a variety of reasons, not just to improve production but also marketability, culture ability and the conservation of natural resources (Omole 2017).

### **Selective Breeding Techniques**

Selective breeding program is the classical method of genetic breeding. The main objective of selective breeding is to select for desirable genetic traits in individual or groups. Selective breeding has been successfully applied to enhance desirable traits in various species. Including rainbow trout *Oncorhynchus mykiss* (Kause et al. 2005), Silver carp *Hypophthalmichthys molitrix* (Gheyas et al. 2009), channel catfish *Ictalurus punctatus* (Rezk et al. 2003), and so on. The first published selection experiments in aquaculture started in the USA as early as the 1920s. These experiments were carried out to improve disease resistance against the furunculosis in brook trout *Salvelinus fontinalis*. The surviving brook trout was selected from a population with endemic furunculosis, to increase the survival rate from 2 % in the initial population to 69 % after three generations of selection (Embry & Hyford 1925). The pioneering success of these experiments was followed by the production of "Donaldson" strain of rainbow trout *Oncorhynchus mykiss*. Donaldson started selective breeding program with rainbow trout in 1932 and reported a significant increase in fecundity and growth after 35 years of individual selection. This strain was widely distributed and appreciated by trout culturists (Donaldson & Olson 1955). In spite of these successes, few selective breeding researches were carried out in aquacultured species before 1970. In the mid 1960s, the mass selection in common carp *Cyprinus carpio* Linnaeus showed no response to improve growth rate in this species (Moav & Wohlfarth 1976). This negative result to selection was very strong among aquaculturists when, the mass selection program for disease resistance and for high growth rate was established to improve growth rate and resistance against the dropsy in common carp (Kirpichnikov et al. 1993). The breeding program consisted of selection within the local and Siberian wild carps from the river Amur, the selection was successful and resistance to dropsy was improved. Due to this successful selection, three stocks of common carp were developed, and crossbreds among them were used for commercial production in the region of Krasnodar (Kirpichnikov et al. 1993). Another mass selection program with carp species was started to improve the growth rate of silver barb *Barbonymus gonionotus* in Bangladesh and Thailand, and common carp *Cyprinus carpio* in Vietnam. The results of these selection programs showed an acceptable response to the mass selection in early generations up to fourth or fifth, declining sharply thereafter (Hussain et al. 2002). Moreover, breeding work with Indian rohu carp was carried out at the Central Research Institute of fresh water aquaculture (CIFA) in India in collaboration with AKVAFORSK. This breeding work was to improve the growth rate in Indian rohu carp *Labeo rohita*. The base population for the program was sampled from five Indian river systems and one farmed stock. Four generations of selection were performed with rohu reared in monoculture, while for two generations rohu were reared in poly culture together with the carp species mrigal and catla (Gjedrem & Baranski 2009). The overall response was 29.6% per generation, which was particularly high and proved considerable genetic variation for growth rate in rohu carp. The response was slightly higher in monoculture compared with poly culture. This result showed possibility

to double the growth rate in rohu carp less than four generations by selecting for only growth rate (Gjedrem & Baranski 2009). Selective breeding program for growth and survival rate was successful in common carp (Nielsen et al. 2010).

Several large scale selection experiments and breeding programs, trying to increase growth rate were conducted during 1970s to 1980s, resulting in significant genetic gain per generation. The selection for improved growth rate in channel catfish *Ictalurus punctatus* has been performed with significant response. In the USA, the selection experiment was carried out for growth rate in channel catfish during 1970s. Body weight changes, measured as deviations from control line, were around 20% in different tests carried out in cages and tanks (Bondari 1983). Another significant response to selection for body weight in channel catfish was obtained, when response and heritability for body weight were determined for three strains of channel catfish. The strains had different histories of domestication 10 years, 20 years, and 60 years. Significant response about 18 %, 17 % and high heritability were obtained in strains with shorter periods of domestication (Dunham & Smitherman 1983). Sea bream was also undergone selective breeding program. Seabream body weight had a moderate heritability and showed response to selection under controlled laboratory conditions. An industrial selection program was established to increase body weight in seabream using mass selection. The response of this selection ranged from 5 to 10 % per generation (Knibb 2000).

The three first reports of selective breeding in tilapia *Oreochromis niloticus* produced discouraging results (Teichert-Coddington on 1983; Hulata et al. 1986; Huang & Liao 1990). All applied individual selection for growth rate with negligible response to selection. However, in 1988 a major selection program with Nile tilapia was initiated in the Philippines by ICLARM. The project was named GIFT (Genetically Improved Farmed Tilapia). Four wild strains from Africa and four farmed strains from Philippines were used to improve the growth rate. Initial strain comparison illustrated that, the fastest growth rate was obtained in African strains. The strain comparison work was followed by family based selection scheme to improve growth rate, which showed a significant response during five cycles of selection about 17 % per generation (Eknath et al. 1998). Other successful breeding experiments with Nile tilapia reported similar impressive responses to selection for growth rate. (Bolivar & Newkirk 2002). Although the GIFT Nile tilapia and, the Norwegian trout and salmon breeding programs have to be comprehensively and critically described in full details, the products from these breeding programs are commercially used by the industry in the respective countries. Several of these breeding programs, that carried out as trail projects, have been expanded to include the industry such as, Atlantic salmon or continued as a private companies such as, rainbow trout, Atlantic salmon, Nile tilapia, channel catfish (Hulata 2001).

Despite the perceived potential for increased shrimp production, the sustainability of shrimp farming is currently threatened by low production efficiency and susceptibility of farmed stocks to diseases. In particular, viral diseases have had disastrous effects in all the main shrimp production countries. One approach to overcome this threat is to develop domesticated breeds selected for traits such as improved growth rate and diseases resistance.

Domestication and selective breeding of Kuruma shrimp *Penaeus Japonicus* in Australia commenced in 1993 (conduct research trails in collaboration with a commercial farm). Following the success of these trails (Hetzl et al. 2000), reported significant response to selection for growth rate in Kuruma shrimp, that enhanced the use of selectively bred stocks. Selective breeding for disease resistance began in the mid 1990s in response to outbreaks of Taura Syndrome, that caused by Taura Syndrome Virus (Moss et al. 2012). The Oceanic Institute operated a selective breeding program for Pacific white shrimp *Litopenaeus vannamei*, based on a selection for improved growth rate and resistance to Taura Syndrome Virus (TSV). Significant improvements in growth rate and TSV resistance were achieved in Pacific white

shrimp through selective breeding program, after one generation (Argue et al. 2002). The response to selection for white spots syndrome virus (WSSV) resistance was too low to support an economically viable industry in heavily WSSV affected areas (Cock et al. 2008). However, significant resistance of *L. vannamei* against WSSV under controlled condition was achieved (Cuéllar-Anjel et al. 2012).

Oyster has the highest production of all aquaculture species worldwide, and has a long history of farming in Asia, Europe and America. Most common of all species farmed is the Pacific oyster *Crassostrea gigas*. Selection program will lead to significant gains in the traits of concern to the oyster industry, and the industry will propagate and maintain the most valuable lines (Ward et al. 2000). Selection was undertaken in the USA for increased live weight yield, which is a combination of individual growth rate and survival. Response to selection ranged from 0.4 to 25.6% improvement in yields of families from selected brood stock compared with families from wild brood stock. The average response to selection over seven trials resulted in a genetic gain per generation on average 9.5% (Langdon et al. 2003). The products from these breeding programs have significantly increased the use of genetically improved stocks in commercial aquaculture.

### **Integration Breeding Technologies**

#### **Intra-Specific Cross Breeding**

Intra-specific crossbreeding (crossing of different strains) may improve profitable traits of aquacultured species, but heterosis (differences between progeny and parents) may not be achieved in every case. A few commercially aquacultured species have been improved by crossbreeding. Variable percentage of crossbreds exhibiting heterosis for growth rate trait was achieved in common carp, channel catfish, rainbow trout and Pacific oyster. Heterosis was also found in survival, disease resistance and reproductive traits. Common carp crossbreds that exhibited positive heterosis for growth rate were the basis for carp aquaculture in Vietnam, China, Israel and Hungary (Wohlfarth, 1993; Bakos & Gorda 1995). A good example of the relative success of crossbreeding was the crossing of common carp lines in Szarvas. Since 1965, more than 100 crosses were tested under different production conditions. Three crosses were chosen for culture purposes, based on about 20 percent improvement in growth rate and other qualitative traits, compared to parent and control lines. Approximately 80 percent of common carp production comes from Szarvas crossbreds (Bakos & Gorda 1995). Hungarian crossbreeding program involved also in the production of gynogenetic female lines and gynogenetic sex-reversed inbred male lines from common carp with the best combining ability. A higher heterosis was observed from crossing inbred lines. Whereas, the growth rate of first generation crossbreds was about 10 percent higher than control lines (Bakos & Gorda 1995). In Indonesia, strain development using artificial gynogenesis and sex-reversal resulted in 10 common carp inbred lines, which were used for crossbreeding (Sumantadinata 1995). Significant heterosis was achieved in first generation of crossbreds among eight local Vietnamese varieties of common carp and foreign strains introduced from Ukraine, Czech Republic, Indonesia and Hungary. Double crosses among Vietnamese, Indonesian and Hungarian strains had subsequently been used as the starting material for carp selective breeding program in Vietnam (Thien & Trong 1995). In China, the common carp intra-specific crossbreds (Feng carp, Heyuan carp, Yue carp, Tri-crossed carp) were produced with good characters such as, higher growth rate, lower feed conversion rate. As a result of these characters, they were the main varieties of cultured common carp *C. carpio* throughout China (Penman et al. 2005).

Twelve experiments on intra-specific crossbreeding of channel catfish *Ictalurus punctatus* for improvement of body weight were evaluated and reviewed. There was negligible heterosis for growth rate in crossbred fingerlings, that resulted from mating of unrelated first generation crossbred populations (Dunham & Smitherman 1983).

However, crossbreds resulting from pure strain exhibited an average increase of 10.3 percent in growth above the fastest growing parent strain (Dunham & Smitherman 1983). In addition, Crossbreeding among rapidly growing domestic strains, Marion x Kansas, Auburn x Kansas and Auburn x Uvalde resulted in fastest growing crossbreds to harvest size evaluated between 8 and 13 percent increase in growth rate (Dunham & Smitherman 1983). All crossbreds of channel catfish made with Auburn females exhibited heterosis. Channel catfish crossbreds produced from Marion x Kansas strain showed superior growth and reproductive performance in previous studies, and had better *E. ictaluri* resistance compared to the other crossed strains (Wolters & Johnson 1995). Crossbred channel catfish were widely cultured in the southeastern states of the USA. The superiority of crossbred catfish over pure strains were highly appreciated by farmers (Hulata 2001). Significant heterosis to improve yield was also observed in crossing strains of rainbow trout (Gall, 1975; Ayles & Baker 1983), and Pacific oyster (Hedgecock et al. 1995). However, crossbreeding among seabream and among Atlantic salmon strains yielded little heterosis (Gjerde & Refstie 1984; Knibb et al. 2000).

### **Inter-Specific Crossbreeding**

Inter specific crossbreeding or hybridization was successfully used in numerous species of fish and shellfish to increase growth rate, manipulate sex ratios, improve flesh quality, produce sterile animals, increase disease resistance, improve environmental tolerance, and to improve variety of other traits to make aquatic animals more profitable to aquaculture sector. In spite of intra-specific hybrid does not exhibit heterosis for traits in every case, it is still important for aquaculture as it expresses a good combination of valuable traits from both parent species. The majority of produced hybrids did not prove advantageous for aquaculture. However, a small number of valuable fresh and marine water hybrids were exploited.

Crosses among cyprinids were the most suitable for aquaculture. Crosses between silver carp (*Hypophthalmichthys molitrix* Val.) and bighead carp (*Aristichthys nobilis* Rich.) exhibited higher survival and yield than parental line. This hybrid was commercially cultured during the 1970s to 1980s (Issa et al. 1986). Good growth and improved general performance were also achieved from crosses of common carp (*Cyprinus carpio communis* L.) with rohu carp (*Labeo rohita* Ham.), mrigal carp (*Cirrhinus mrigata* Ham.), and catla carp (*Catla catla* Ham.) in monoculture in India. In addition to improve growth and performance in these hybrids, they were triploid and sterile and exhibited higher flesh content, and showed lower seine escapability than the maternal parent (Reddy et al. 1990). The rohu x catla hybrid grow almost as fast as pure catla, but had the small head of the rohu and was consequently useful in India aquaculture. Another triploid hybrid produced from crosses among female grass carp (*Ctenopharyngodon idella* Val.) and male bighead carp (*Aristichthys nobilis* Rich.) (Bakos et al. 1978). The triploid offspring of the cross appear to be sterile, therefore reducing the likelihood of unwanted reproduction, and attracted US fisheries biologists looking for an efficient herbivorous fish to control aquatic vegetation (Sutton et al. 1981).

Culturists produced hybrid among female channel catfish *Ictalurus punctatus*, and male blue catfish *Ictalurus furcatus*, wished to combine the good culture characters of channel catfish with the simplicity of harvesting characters of blue catfish such as increased catch ability. This hybrid was useful in culture as it was better performance than channel catfish for growth rate, low dissolved oxygen tolerance, uniformity in body shape and disease resistance (Smitherman & Dunham 1985). However, only the first generation of channel-blue hybrids increased processing yields in comparison to the second generation (Argue et al. 2003).

The catfish aquaculture in Thailand is depending on the hybrid between the African catfish (*Clarias gariepinus* Burchell) and the Thai catfish (*C. macrocephalus* Günther). This hybrid combines the desirable flesh characters of the Thai with fast growth rate of the African catfish. In addition, catfish hybrids among two African catfishes (*Clarias gariepinus*) and (*Heterobranchus longifilis*) were reported to grow faster. Hybrids between catfish species *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum* were produced in several Brazilian hatcheries to obtain individual with beneficial characters and useful for fish farm (Pardo et al. 2011). Hybridization between some species of tilapia such as blue tilapia (*Oreochromis aureus*) and Nile tilapia (*Oreochromis niloticus*) resulted in the production of all male tilapia that reduced unwanted reproduction. The production of all male tilapia in this cross was as a result of different sex determination in the two species: blue tilapia has ZZ/ZW system with the female being heterogametic, while Nile tilapia has XX/XY with the heterogametic genotype being male. Other tilapia crosses produced all male tilapia were the cross of Nile tilapia (*O. niloticus*) with Wami tilapia (*O. hornorum*), and the cross of Mossambique tilapia (*O. mossambicus*) with Wami tilapia (*O. hornorum*). (Wohlfarth 1994). All male tilapia hybrids were suppressed by hormonal sex inversion and breeding sex-inversed neo-male, as alternative methods of producing all male tilapia.

Tilapia hybrids were also produced to increase environmental tolerance. Several experiments were conducted, involving Mossambique tilapia, blue tilapia and their F1 and F2 hybrids to study genetic basis of cold tolerance in tilapia fish. These experiments showed that Mossambique tilapia was the most cold sensitive group, followed by F2, and the F1 which was similar to blue tilapia. Genetic variation in cold tolerance had a large dominance component, based on the similarity of the F1 hybrid to the blue tilapia parent (Cnaani et al. 2000). Florida red strain hybrids (*O. mossambicus* x *O. urolepis hornorum*) can reproduce in salinities of 19 ppt (Ernst et al. 1991), and hybrids (*O. niloticus* x *O. aureus*) also showed enhanced salinity tolerance (Lahav & Lahav 1990; Wohlfarth 1994).

The sun shine bass, a crossbred among white bass female *Morone chrysops* and striped bass male *M. saxatilis* exhibited fast growth performance, and has better overall culture characteristics under different commercial culture conditions in ponds, tanks and cages. This hybrid is commercially cultured mainly in the USA, and introduced to other countries such as Israel and Taiwan. Crosses between bass species were also resulted in the production of mono-sex population, as the hybrids among striped bass and yellow bass (*M. mississippiensis*) produced 100 percent female. This can be desirable for culture purposes where the growth performance differ between sexes (Wolters & Demay 1996). Seabream hybrids (*Sparus aurata* x *Pagrus major*) resulted in improved overall performance for aquaculture systems (Knibb 2000).

Hybridization of coho salmon (*Oncorhynchus kisutch*), which is considered resistant to several salmonid viruses. The hybrids exhibited higher disease resistance, but overall viability was poor. Viability increased when hybridization was followed with triploidization. Triploid hybrids from rainbow trout and char (*Salvelinus* spp.) were resistant to several salmonid viruses, but grew more slowly than their diploid. Similar results were found with rainbow trout and coho crosses (Dorson et al. 1991). Triploid pacific salmon hybrids showed earlier seawater acclimation (Seeb et al. 1993). Although hybrid salmonids were not cultured commercially in significant quantities, the widely used triploidization in the salmon industry involved with genetic improvement programs in salmonids (Galbreath, & Thorgaard 1996).

### **Chromosome Set Manipulation Technologies**

Chromosome manipulation technique has been applied to various aquatic species over the last decades. Including androgenesis, gynogenesis and induced polyploidization techniques to prevent uncontrolled reproduction, and to increase

culture performance of the sexes. These techniques produce mono-sex gametes that involve the production of mono-sex diploid or triploid stocks.

### Gynogenesis

Gynogenesis produces an organism whose entire genetic material has been inherited from the mother. Gynogenetic development is induced by fertilization of spawn with irradiated sperm, by inhibiting of either the second polar body extrusion or the first cleavage. The former type of gynogenetic diploid is termed meiotic gynogenetic diploid, polar body gynogen or meiogynogenesis (Figure 1). while, the latter is termed mitotic gynogenesis diploid, cleavage gynogen, or mitogynogenesis (Figure 2) (Arai & Mukaino 1997; Pandian & Koteeswaran 1998). Gynogenesis technique has been carried out in several species such as, common carp, Tilapia rainbow trout, Atlantic halibut, European seabass and more than 100 other fish species (Komen & Thorgaard 2007; Liu & Yang 2009; Liu, 2010; Felip 2001). However, Gynogenetic strains of rainbow trout, red seabream, olive flounder have been used in commercial production (Komen et al. 1992; Fopp-Bayat et al. 2007). Gynogenesis technique involves the production of all-females and sterile triploidy. In salmonids, all-female are produced by gynogenesis technique, while sterile (monosex female triploid) are produced by gynogenesis followed by the use of pressure or treatment shock to induce triploidy (Donaldson 1996). These techniques are often used in combination with other technique such as hormonal- sex reversal. The combination of sex-reversal of gynogenetic progeny to produce neomales or neofemales, or triploidization of gynogenetic females to produce XXX sterile females, which were intensively investigated for many decades have been commercially applied in several aquacultured species over the world.

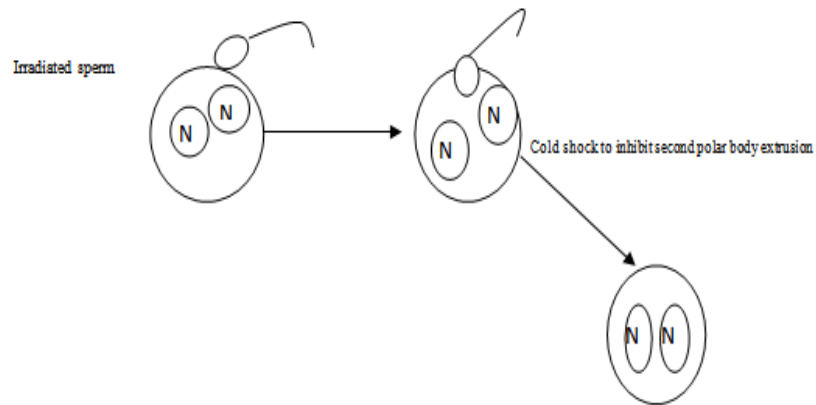
During 1980s, all females diploids were used in many farms, over half of the production of trout farms was from all female stocks, and the use of sterile triploids trout, in particular for sport fisheries was increased (Bye & Lincoln 1986). Gynogenesis and triploidy techniques to produce all-female and all-female sterile stocks were widely cultured in Canada Hulata (2001), and in private trout farms in Japan (Arai 2001). These triploids were produced either by triploidization of gynogenetic females, or by breeding among tetraploid XXXX females and diploid XX males (Arai 2001).

All female monosex populations were also used in coho salmon *Oncorhynchus kisutch* and Chinook salmon *Oncorhynchus tshawytscha* farms, to prevent losses arising from early maturation of males, and for the desirable of the culture of all female populations (Devlin et al. 1991, 1994). Female triploidy was being discovered by the Canadian salmon industry for containment purposes, especially for the culture of non native salmonid species. Unfortunately the performance of the triploid salmonids was not as good as that of diploids (Davis & Hetzel 2000).

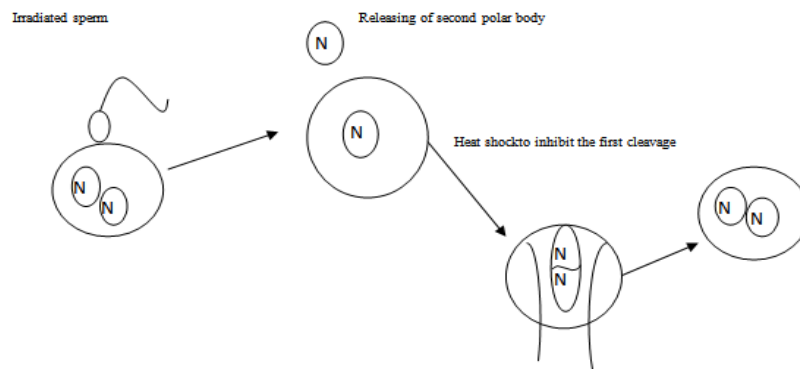
In Japan, the commercial culture of all female diploid or triploid salmonids (rainbow trout, coho, masu and yamame salmons) were regulated by governmental Fisheries Agency (Yamamoto 1999). In the USA, triploidy was applied in grass carp species to prevent aquatic plants. Triploid grass carp was commercial valuable and cultured in many farms. Combined technology of gynogenesis and hormonal sex- reversal is being developed for the production of all female triploid black carp and grass carp that were completely sterile (Rothbard et al. 1997). (Zhang et al. 2010) have developed disease resistant gynogenetic strains of grass carp by the activation of inactivated sperm of tetraploids. These gynogenetic grass carp reveal that gynogenesis can be used to obtain all-female individuals that have desirable genetic traits.

Research suggests that using sperm from distant fish can improve the survival rate of gynogenetic fish, and simplify their identification. Gynogenetic crucian carp was obtained using sperm from blunt snout bream. This variety has higher survival rate than gynogenetic crucian carp created using common carp sperm, and is more easily identified (Sun et al. 2007).

Gynogenetic strains has been developed from natural origin red crucian carp *Carassius auratus*, gold fish, common carp, orange ornamental carp *Cyprinus carpio*, grass carp *Erythroculter ilishaeformis*, and artificially cultivated strains including tetraploid hybrids of common carp with red crucian carp and tetraploid hybrids of red crucian carp with snout bream (Sun et al. 2007; Xiao et al. 2011). Gynogenetic strains also were developed from red crucian carp, Japanese crucian carp and gold fish. These gynogenesis strains have a higher survival rate and are easily identified (Xu et al. 2015).



**Figure 1: The Induction of Meiotic Gynogenesis in Fish.**



**Figure 2: The Induction of Mitotic Gynogenesis in Fish.**

### Androgenesis

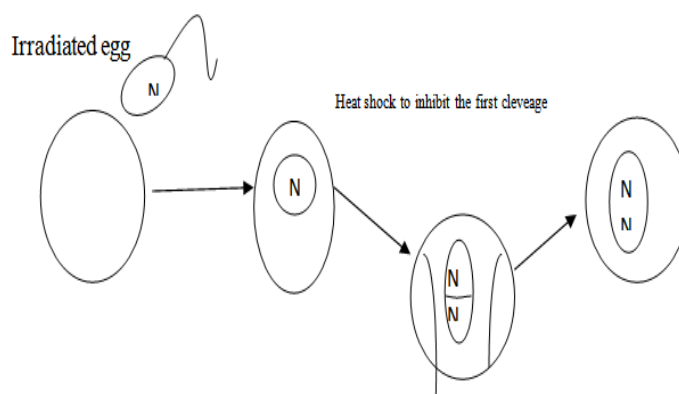
Androgenesis is the technique by which offspring is produced by the male parent with no genetic contribution from female. This is the production of viable progeny with all

paternal inheritance involved the fertilization of inactivated eggs using active sperm (Figure 3). The induction of androgenesis can produce all-male population in fish which would have commercial application in aquaculture sector (Nwokwa 2012). It can also be used in the recovery of lost genotypes from the preserved sperms, and in generating homozygous lines of fish (Xu et al. 2015).

Androgenetic individuals have been produced in a few species of cyprinids, cichlids, and salmonids (Komen & Thorgaard 2007; Arai et al. 1979; Araki et al. 1995; Babiak et al. 2002). It is worth noting that because male tilapia grows more rapidly than female tilapia, androgenesis could be used to obtain super male individuals YY. That can be crossed to normal female XX to produce all male tilapia XY (Xu et al. 2015). Their all male progeny XY known as genetically male tilapia GMT, which has been widely practiced in the Philippines, Thailand, China, and USA (Hulata 2001).



Haploid sperm has been rarely used to produce artificial androgenesis in other fish species for commercial industry. This is as a result of the difficulty in inactivating the DNA in the egg, and the negative impact on the sperm nucleus genome that caused from artificial doubling of androgenesis individual embryos, and later life activities that lead to a low rate of individual survival in androgenesis. The use of tetraploid fish with two sets of chromosomes in the diploid sperm for artificial androgenesis would yield diploid androgenetic progeny without using chromosome doubling treatment. Androgenesis techniques have been carried out using diploid sperm, that was produced by artificial tetraploid rainbow trout and natural tetraploid loach without chromosome doubling treatment. This produced diploid androgenetic offspring that have higher survival rate in comparison with offspring that produced using haploid sperm (Thorgaard et al. 1990; Arai et al. 1995; Fujimoto et al. 2010).



**Figure 3: The Induction of Androgenesis in Fish.**

### **Polyplody**

Polyplody technology has been commercially applied in several cultured fish species. This technology induced polyplody (triploidy and tetraploidy), which contain extra sets of chromosomes (Pandian & Koteeswaran 1998). The normal and most common chromosome complement is two sets that known as diploidy. Triploidy refers to individuals with three sets of chromosomes and tetraploidy refers to individuals with four sets. These techniques are important in the improvement of fish breeding as they provide a rapid approach for gonadal sterilization, sex control, and cloning (Lakran & Ayyappan 2003). Polyplody individuals are produced by doubling cell genomes using artificial or natural mutagenesis. Fish genomes have greater plasticity, and could be doubled easily. This encourage researchers to produce fish polyplody (Song et al. 2012).

Polyplody is induced by different methods, physical, biological, and chemical.

Physical methods include temperature shock, hydrostatic pressure. Biological methods include hybridization, nuclear transfer, and cell fusion. Chemical methods involve the use of different chemicals such as, cytochalasin, polyethylene glycol, colchicine to induce embryonic polyplody. Physical and chemical methods induce polyplody individuals by inhibiting the exclusion of the second polar body in the oocytes or inhibiting the first cleavage of zygotes (Song et al. 2012).

### **Triploidy**

Triploidy technique is generally accepted as the most efficient methods for producing sterile fish for aquaculture and fisheries management. Triploids can be induced by blocking the second polar body release with heat shock or high

hydrostatic pressure shock, just after fertilization with normal spermatozoa (Pandian & Koteeswaran 1998). Culture of triploid fish have higher economic value, including rapid growth, higher flesh quality, strong disease resistance, and higher survival rates. In addition, the majority of triploid fish are sterile that can improve the growth rate and reduce interbreeding with wild stocks when the cultured fish escaping in to the wild (Benfey 1999). Methods of triploidy induction include temperature shock (cold or hot), hydrostatic pressure shock, chemical (colchicines, cytochalasin-B or nitrous oxide), and the crossing of tetraploids XXXX females with diploids XX males. Hydrostatic pressure was used to induce triploid of different species such as, *Carassius auratus* (Gui et al. 1995), and *Cynoglossus semilaevis* (Li et al. 2011). The hydrostatic pressure was applied to prevent loss of the second polar body of zygotes. Hybridization was used to induce triploidy in fish. Crossed the female descendants of *Cyprinus carpio* female x *Carassius auratus* male with male mirror carp *Cyprinus carpio* obtained triploid carp (Wu et al. 1993).

### **Tetraploidy**

Tetraploid individuals have a balanced set of chromosomes, which can result in viable and fertile individuals. Tetraploids can be induced by disrupting the first cleavage using hydrostatic pressure or temperature shocks in spawn fertilized with normal sperm. This method resulted in viable tetraploids in several aquacultured species (Pandian & Koteeswaran 1998). The production of tetraploid individuals offered a convenient way to produce a majority of sterile triploid individuals through hybridization among tetraploid and diploid (Guo et al. 1996). The success of treatments to induce tetraploidy depends on the time of beginning of the shock, the magnitude of the shock, duration of the shock, quality of the gametes. The methods of heat shock have been used to obtain tetraploid rainbow trout, channel catfish, and loach, and the combination of hydrostatic pressure and cold shock was used to obtain tetraploid *Oreochromis* (Myers 1986).

### **Modification Breeding Technologies**

Transgenic or genetically modified organisms (GMOs) are a category of animals that were developed using gene transfer technology whereby a foreign gene is integrated into the genome of the recipient animal, and it can be passed onto future generations (El-Zaeem 2004). Fish species tend to be relatively tolerant to artificial manipulation of their genes during early development, making them ideal subjects for genetic modification (Foresti 2000). The traditional genetic breeding requires repeated breeding over much generation, while transgenic technology can reduce the time of evolution and generate new brood stock or strains during a short time. The application of fish transgenic technology has been focused on three needs. The first is the development of growth rate and food conversion rate. The second is the cultivation resistant strains such as (disease resistance or cold resistance). The third is the fundamental study of the mechanisms of animal growth, development, and reproduction (Xu et al. 2015).

The first successful case of transgenic fish was reported in 1985, when Chinese researchers microinjected the human growth hormone hGH gene into the fertilized eggs of gold fish *Carassius auratus* (Zhu et al. 1985). This was followed by successful introduction of hGH gene in the genome of the loach *Misgurnus anguillicaudatus*, with resulting of transgenic fish that grew faster than the control (Zhu et al. 1986). Since then, the Chinese researchers have developed variety of transgenic fish, and have set theoretical foundation for fish gene transfer research and development. Consequently several countries have experimented gene transfer in many aqua cultured species, including (Atlantic, Coho, and Chinook salmon, rainbow and cutthroat trout, tilapia, striped bass, mud loach, channel catfish, common carp, gold fish, red and silver sea bream) (Devlin 1998; Maclean & Laight 2000). Recently, extensive research has been focused on the use

of GH genes originating from fish rather than human, with the hope of increasing consumer acceptability (Levy et al. 2000). Negative perceptions associated with the use of viral promoters to express trans genes have also encourage many researchers to substitute them with fish based promoters for the creation of all-fish trans gene constructs. These all-fish GH transgenic strains have been developed in several species including red sea bream *Pagrosomus major*, silver sea bream *Sparussarba*, common carp *Cyprinus carpio*, tilapia *Oreochromis niloticus*, and Atlantic salmon *Salmo salar*. All-fish gene construct consisting of ocean pout antifreeze protein (AFP) promoter fused to Chinook salmon GHc DNA, were injected in to salmonid embryos (Shears et al. 1991). This construct showed suitable pattern of tissue expression. Such studies have proved enhancement of growth in salmon to an average of 3-5 times in comparison to the non transgenic control. Some individuals showed an increase in body weight reached 10-30 times during the first few months of growth (Du et al. 1992; Devlin et al. 1994). The Aqua Bounty company is seeking approval for commercial use of this transgenic fish, and the application has been under evaluation in almost 10 years by the US Food and Drug Administration FDA (Olesen et al. 2011). In spite of using transgenic technology to accelerate production of new variant strains, worries over bio safety / security have frustrated these strains from entering the commercial use stage (Maclean & Laight 2000). To deal with these worries, researches will be carried out to produce transgenic sterile fish that can reduce the breeding with wild fish. In addition, the application of all-fish genes as exogenous genes for trans genesis would reduce the food safety risks of transgenic fish (Xu et al. 2015).

## CONCLUSIONS

The range of aquaculture production has been increased over the world. However, the issue remains whether the industry can continue to grow in a sustainable way to meet the future aquatic animals demand. The traditional aquaculture practices alone cannot improve the production quality, to meet a rise of aquatic animal demand. Thus, There is a need to implement biotechnological practices to increase the aquatic animal production, and to meet the world food security.

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