



## Review Article

# Biotechnology as an Important Tool for Improving Fish Productivity

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**Abstract:** Due to the increase in growth rate of the world population, the demand for fish is soaring worldwide and it appears unlikely that the increasing demand can be met through increased natural harvests as many of the oceans and natural freshwater fisheries are being harvested to their limit. Aquaculture, therefore, remains the last hope for providing enough fish for the world, but with limited land and water space. Aquaculture biotechnology, therefore, has come to have a key role to play as it can make a great contribution to improving aquaculture yields. The application of biotechnology to various production systems does not come without its negative impacts but even still, the merits far outweigh the associated concerns because the techniques are constantly being developed thereby reducing the negative impacts thereof. Therefore, there is need to adopt biotechnological practices if the world is to stand any chance of achieving food security.

**Keywords:** Population, Aquaculture, Biotechnology, Food Security

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## 1. Introduction

The world fisheries are in a period of crisis. Many major fish stocks are showing precipitous declines in productivity due to overfishing and further increases are not anticipated under the current global conditions and environment [1]. Aquaculture remains the last hope for providing enough fish for the world, being the cheapest source of animal protein [2]. But the aquaculture industry is currently faced with solving the simultaneous problems of developing economically viable production systems, reducing the impact on the environment and improving public perception while utilizing a lesser amount of land space.

With increased demand for aqua-cultured foods has come a need for more efficient production systems than the traditional systems faced with impediments to sustainability, such as slow growth of fish, inefficient feed conversion, heavy mortality from disease and the associated use of chemicals, loss of fish from low oxygen levels, inefficient harvest, and poor fecundity and reproduction [3]. The development of improved fish seed stocks that can contribute to increased fish production is seen as one of the key solutions to meeting the

future food demands of the growing world population [4]. Biotechnology has opened a new window for development of genetic resources in aquaculture. Genetic technologies can be utilized in aquaculture for a variety of reasons, not just to improve production but also marketability, culturability and the conservation of natural resources [5].

## 2. Biotechnology Techniques

Since the early 1980s, research in aquaculture and fisheries genetic biotechnology has steadily grown, and now research in this area is extremely active. Cultured fish are being improved for a multitude of traits, including growth rate, feed conversion efficiency, disease resistance, tolerance of low water quality, cold tolerance, body shape, dress-out percentage, carcass quality, fish quality, fertility and reproduction and harvestability [3]. The main vision of aquaculture biotechnology is to achieve improvements of aquaculture stock, preservation of genetic resources, disease diagnosis, and control of microbial/microalgal genetic engineering [6].

In broad terms, biotechnology can be defined as any

technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use [7]. This ranges from the use of synthetic hormones in induced breeding, hybridization, production of monosex, uniparental and polyploid population, molecular biology, transgenic fish to gene banking [8]. Biotechnology has the potential to enhance reproduction and the early developmental success of culture organism. The technology is used in several different ways in aquaculture and its application benefits both producers and consumers of aquaculture products [2].

### 2.1. Induced Breeding

Artificial propagation methods constitute a major practicable means of providing enough quality seed for rearing in confined enclosure such a fish ponds, reservoirs and lakes [9]. Fish culture today is hardly possible without the artificial propagation of fish seeds of preferred cultivable fish species. Apart from being able to obtain quality seed, the artificial propagation technique can also be used to develop strains superior to their ancestors by the methods of selective breeding and hybridization [10].

The most successful method of artificial reproduction in catfish is by induced breeding through hormone treatment followed by artificial fertilization and incubation of fertilized eggs and the subsequent rearing to fingerlings [11]. Hormonal stimulation allows year-round production of gametes and fry of economically valuable species. Hormone therapy is applied to improve and control of reproductive cycles during the domestication [12].

The induced breeding of fish is now successfully achieved by the development of Gonadotropin releasing hormone (GnRH) technology [13]. GnRH is the key regulator and central initiator of reproductive cascade in all vertebrates [14]. It is a decapeptide with the ability to induce pituitary release of luteinising hormone (LH) and follicle stimulating hormone (FSH) [15].

Salmon GnRH is now profusely used in fish breeding and marked commercially under the name of "Ovaprim" throughout the world. In fact, most of the economically important culturable fish, especially catfishes, in land locked water do not breed until the hormone induces them. Ovaprim is administered intramuscularly at 0.5ml per kg of fish body weight [11].

### 2.2. Monosex Fish Culture

Monosex populations of fish are desirable in aquaculture for a variety of reasons. Some species of fish mature at small sizes and young ages prior to the desired time of harvest. This can decrease production because unwanted reproduction results in crowding of the fish and higher densities than intended in the culture pond as well as wasted energy from the sexual activity of the stocked fish [3]. This is a major problem particularly in tilapia which can decrease production because precocious maturity always results in overcrowding and stunted growth in the culture pond.

In aquaculture, one sex is often more desirable for the market than the other due to sexual dimorphism. Sexual dimorphism for growth occurs in most cultured fishes for flesh quality and carcass yield [16]. [17] demonstrated that male *Oreochromis niloticus* grows 2.5 and 2.2 times faster than females when grown in cages mixed and separately respectively. All-male progeny would be beneficial for catfish culture since they grow 10–30% faster than females, depending upon strain of catfish [18]. All-female populations are desirable in salmonids because of the more rapid growth of females, the early sexual maturity and associated slow growth of males and the poor flesh quality of males, especially early maturing males [19].

### 2.3. Hybridization

Increased heterozygosity from hybridization has resulted in improved growth and other desirable characters such as developmental compatibility, food conversion efficiency, and oxygen metabolism in a variety of species [20]. Hybridization attempts to produce fish that combines valuable traits from more than one species or high heterosis (hybrid vigour) [16]. Hybridization is aimed to evolve a hybrid or strain of superior quality than the parent species. In Nigeria, *Clarias gariepinus* and *Heterobranchus bidorsalis* have been crossed to produce a sterile hybrid which possessed the hardiness of *Clarias* and the fast growth of *Heterobranchus*.

Hybridization can also be used to produce single sex groups of fish when the sex determining mechanisms in the parental lines are different. An example is the hybridization of Nile tilapia, *Oreochromis niloticus* and the blue tilapia, *Oreochromis aureus* produces an all-male progeny which controls unwanted reproduction [2]. The hybrid between striped bass (*Morone saxatilis*) and yellow bass (*Morone mississippiensis*) produced 100% females with excellent survival and growth [21].

Many inter-specific hybridizations result in sterility thus functioning as reproductive isolating mechanism to prevent the permanent mixing of genes from two species. The more distantly related the two species, the greater the likelihood their hybrid being sterile [16]. Production of sterile animals may be advantageous to diminish unwanted reproduction or to improve growth rate and avoiding energy loss due to prolific breeding [22].

### 2.4. Hormonal Sex Reversal

The production of single sex groups of fish can be accomplished by manipulation of the developing gametes and embryo [23]. The use of sex control techniques to influence characteristics of economically desirable teleost species is becoming an important management tool to increase aquaculture production [13]. The principle behind this method lies on the fact that at the stage when the fish larvae are said to be sexually undifferentiated (right after hatching up to about 2 weeks or up to the swim-up stage), the extent of the androgen (male hormone) and the oestrogen (female hormone) pre-sent in a fish is equal [24]. Although the male or female genotype is

established at fertilization, phenotypic sex determination occurs later in development. The artificial elevation of the appropriate sex hormone is sufficient to overcome the natural hormone or gene product during the period of sexual differentiation and to dictate the sex of the individual [3].

Sex reversal can be accomplished by administering the exogenous hormones by bath or mixing hormone with culture water, in feed, and through placing of implants in fish. Different steroids have been used over the years to induce sex reversal although  $17\alpha$ -methyltestosterone is the most commonly used [25].

The genetic male tilapia, that are physically female, can be mated with normal males to produce a group of all-male tilapia that grow faster and have less unwanted matings than a group of mixed-sex tilapia [3]. The all-male offspring of this process have two male chromosomes and these fish can be used in turn as brood stock for subsequent generations. A big advantage of this technique is that all-male populations can be produced in those subsequent generations without the use of any hormones [23].

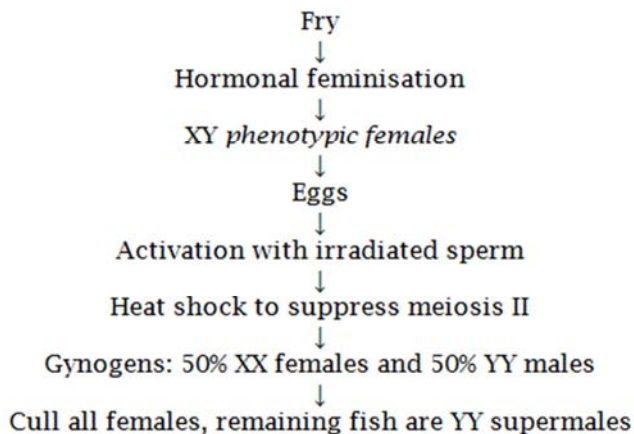


Figure 1. Method for the production of YY super-male tilapia.

## 2.5. Ploidy Manipulation

Chromosome sex manipulation techniques to induce polyploidy (triploidy and tetraploidy) have been applied extensively in cultured fish species [8]. These techniques are important in the improvement of fish breeding as they provide a rapid approach for gonadal sterilization, sex control, improvement of hybrid viability and clonation [13]. By manipulating ploidy, sterile, unisex or highly homozygous cohorts of animals can be produced [26].

The polyploid state refers to individuals with extra sets of chromosomes. The normal and most common chromosome complement is two sets (diploid). Triploidy refers to individuals with three sets of chromosomes and tetraploidy refers to individuals with four sets.

### 2.5.1. Triploidy

Induced triploidy is widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management [13]. Inducing triploidy is the only practical

means in which to sterilize large numbers of fish without using of potentially harmful chemicals or radiation [27]. It is through the triploidization technique that sterilization can be achieved by administration of an environment shock shortly post fertilization [28].

Culture of triploid fish can be advantageous for several reasons. The potential of increased growth, increased carcass yield, increased survival and increased flesh quality are the main culture advantages. Triploids would reach a larger size than diploids because of their larger cell size [3]. [29] reported increased growth rate in triploid fish compared to their normal diploid siblings. This increased growth rate can be a result of lack of sexual development since the growth rate of fish slows as they approach sexual maturity or increased cell size. Therefore, degradations due to sexual maturation are overcome by triploidy technique [30].

Triploid induction can also allow production of otherwise non-viable or sub-viable diploid hybrids probably because of the presence of a balanced maternal chromosome set in triploids that is not present in diploid hybrids. Diploid *Oreochromis niloticus* female  $\times$  *Tilapia rendalli* male hybrid embryos experience near 100% mortality. However, this hybrid combination is viable when triploidy is induced [31].

Methods of triploidy induction include: temperature shock (hot or cold), hydrostatic pressure shock, chemicals (such as colchicine, cytochalasin-B or nitrous oxide), and the crossing of tetraploids with diploids.

### 2.5.2. Tetraploidy

Tetraploids have a balanced set of chromosomes, which can result in viability and fertility. Tetraploidy in fish is commonly produced by disrupting the first cleavage with thermal or hydrostatic pressure shocks in eggs fertilized with normal sperm. Viable tetraploids have been produced by these methods in a number of fish species [8].

Tetraploid breeding lines are of potential benefit to aquaculture by providing a convenient way to produce large numbers of sterile triploid fish through simple crosses between tetraploids and diploids [32]. The success of treatments to induce polyploidy depends on the time of initiation of the shock, the magnitude of the shock, duration of the shock, genetics and quality of the gametes

## 2.6. Uniparental Fish Production

The production of fish with uniparental genetic material is also becoming common in biotechnology. This system operates on the same principle as monosex culture where the traits of one parent are preferred over the other parent's.

### 2.6.1. Androgenesis

Androgenesis is the process by which a progeny is produced by the male parent with no genetic contribution from female. This is the production of viable progeny with all paternal inheritance as the DNA of the egg is inactivated by radiation [6]. Induction of androgenesis can produce all-male population in fish which would have commercial application in aquaculture. Androgenetic individuals have been produced

in a few species of cyprinids, cichlids and salmonids [33].

Androgenesis is a developmental process, facilitating the inheritance of an exclusively paternal genome. It involves two steps: the first treatment is the deactivation of the female genome by UV or gamma rays. Egg activation with untreated spermatozoa then requires diploidization of the haploid zygote by some form of shock to interrupt the first mitotic division [34]. Otherwise, a diploid sperm - the gonad product of a tetraploid male - is needed to fertilise the irradiated egg and produce diploid embryos without further treatment [26].

Due to irradiation damage suffered by eggs, homozygous expression of lethal genes and damage inflicted by thermal shock treatment to suppress the first mitotic cleavage, survival of the androgenotes is very low. However, efforts have been made to improve the survival of the intraspecific androgenotes [35]. For instance, [36] excluded the thermal shock treatment for restoration of diploidy in genome-eliminated eggs of rainbow trout, *Oncorhynchus mykiss*, by using diploid sperm for activation, and significantly improved the survival of the androgenotes.

### 2.6.2. Gynogenesis

Gynogenesis is the process of animal development with exclusive maternal inheritance. Gynogenesis involves the parthenogenetic development of an egg or the stimulation of an egg by a genetically inactive spermatozoon. All-female inheritance is accomplished by activating cell division with irradiated sperm and then restoring diploidy to the developing zygote [16]. Retention of the polar body is accomplished with temperature shocks or pressure treatments. The treatment is applied shortly after sperm penetration prior to extrusion of the polar body. The most effective time for these shocks varies among species [37].

If the first cell division is blocked, a single diploid cell results. Gynogens produced by this technique - mitotic gynogens or mitogynotes - are 100% homozygous since a single set of chromosomes is duplicated [3]. A way of producing diploid gynogens is to block the extrusion of the second polar body; then the diploid gynogen has two sets of chromosomes, both of maternal origin. This type of gynogen is referred to as a meiotic gynogen, or meigen, since it was produced by blocking the second meiotic division.

### 2.7. Transgenics

Transgenics involve the transfer of certain preferable traits from one species into another species, in this case, fish. These traits may include improvement of growth rates, larger size, more efficient feed conversion and control of sexual maturation [38]. Transgenic technology provides a means by which such a quantum leap in production is possible [39].

Transgenics may be defined as the introduction of exogenous gene/DNA into host genome resulting in its stable maintenance, transmission and expression. The technology offers an excellent opportunity for modifying or improving the genetic traits of commercially important fishes, molluscs and crustaceans for aquaculture [13]. It is a short cut to achieving genetic change for fast growth, disease resistance, tolerant to

low level of dissolved oxygen in the water and fish resistant to freezing temperature [40].

Some studies have revealed enhancement of growth in adult salmon to an average of 3-5 times the size of non-transgenic controls, with some individuals, especially during the first few months of growth, reaching as much as 10-30 times the size of the controls [41, 42]. Therefore, researchers have developed new strains of transgenic fish which naturally produce just the right amount of growth hormone to speed their growth [3].

A foreign gene can be transferred into fish *in vivo* by introducing DNA either into embryos or directly into somatic tissues of adults [43]. Direct delivery of DNA into fish tissues is a simple approach, providing fast results and eliminating the need for screening transgenic individuals and selecting germ line carriers. Gene transfer and expression following intramuscular direct injection of foreign DNA into skeletal muscles of fish has been achieved [44].

By microinjecting a fish growth hormone gene linked to a suitable fish promoter into freshly fertilized eggs, transgenic fish with remarkable growth rates have been obtained [39]. Electroporation involves the use of series short electrical pulses to permeate cell membranes, thereby permitting the entry of DNA molecules into embryos [6]. The overall rate of DNA integration in electroporation may be equal or slightly above that of microinjection and the amount of time required to handle large numbers of eggs in electroporation is way less than needed in microinjection [45]. In recent research, gene has been transferred by electroporation of the sperm rather than the embryo. Electroporation is, therefore, considered as an efficient and versatile massive gene transfer technology.

An increased resistance of fish to cold temperatures has been another subject of research in fish transgenics for the past several years [46]. Coldwater temperature is a stressor to many fishes and few are able to survive water temperatures much below 0-1°C. This is often a major problem in aquaculture in cold climates. Interestingly, some marine teleosts have high levels (10-25 mg/ml) of serum antifreeze proteins (AFP) or glycoproteins (AFGP) which effectively reduce the freezing temperature by preventing ice-crystal growth [13]. The isolation, characterization and regulation of these antifreeze proteins particularly of the winter flounder, *Pleuronectes americanus*, has been the subject of research for a considerable period in Canada. The introduction of AFPs to gold fish also increased their cold tolerance, to temperatures at which all the control fish died [47].

Similarly, injection or oral administration of AFPs to juvenile milkfish and tilapia led to an increase in resistance to a 26 to 13°C drop in temperature [48]. The development of stocks harbouring this gene would be a major benefit in commercial aquaculture in countries where winter temperatures often border the physiological limits of these species [13].

## 3. Conclusion

The rate of aquaculture production is increasing globally, but the question remains whether the industry can continue to grow in a sustainable manner and fast enough to meet the

future projected fish demand. Local aquaculture practices alone cannot meet the ever widening demand-supply deficit resulting from the exponential increase in human population, therefore, there is need for new and improved scientific methods. Aquaculture biotechnology has a major role to play to ensure the continued expansion and intensification of aquaculture to meet the growing demand.

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