**Review Article**

# EXPLOITATION OF BIOTECHNOLOGY IN FISHERIES: A REVIEW

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## Introduction

The Global demand for fish is growing as per increase in population growth. However, the farmed fishes are facing barriers to sustainability, such as slow growth rate, inefficient harvest, high mortality due to low temperature, low dissolved oxygen levels and disease, poor fecundity and reproduction (Dunham, R.A. 2004). Biotechnology provides powerful tools for increasing the growth rate, producing disease resistance and cold tolerance species. Biotechnology benefits in induced breeding, chromosome engineering, production of monosex fish, sex reversal, sterile hybrids and transgenic fish. Biotechnology has a great potential for sustainable development in fisheries. This article outlines the basic biotechnology techniques and some instances of their use on fishes.

## Biotechnology techniques

In the fisheries and aquaculture, many techniques are in used to increase the growth rate, production, disease resistance etc. Some of biotechnological techniques are listed and describe in brief as follows.

## Monosex fish culture

In this technique, either only males or only females are produced. It can be achieved by sex reversal hormones and hybridization. Due to sexual dimorphism, one sex is more preferred to the other in the market. Monosex culture is practiced to reduce the prolific breeding and overcrowding in ponds. For example: Tilapia. Male Tilapia grows approximately 2.5 times faster than the female (Stone, 1981). Similarly, male catfish grows 10-30% faster than the females (Smitherman, 1985). In contrast, female populations are desirable in trouts, salmons and common carps because of their early growth and sexual maturity (Hulata, 2001).

## Sex Reversal

Developing gametes and embryo are manipulated to produce single sex groups of fish (FAO 2014). Right after hatching up to about 2 weeks or up to the swim-up stage, the fish larvae are sexually undifferentiated i.e. androgen and oestrogen are present in equal amount (Fuentes-Silva et.al., 2013). Phenotypic sex determination occurs later in the development although the male/ female genotype is established at fertilization. Appropriate increment in the artificially induced sex hormone overcomes the natural hormone/gene product and dictates the sex of individual (Dunham, 2004). 17α-methyltestosterone and estradiol-17β are the most commonly used steroids to induce the masculinity and feminity respectively (Pandian and Varadaraj1990). The exogenous hormones are administered by bath or mixing hormone with culture water, in feed, and through placing of implants in fish.

The genetically male Tilapia, that is physically female, when mated with normal males, results in all-male progeny (Dunham, 2004). The offsprings grow faster and all-male progeny controls the unwanted reproduction. They can be used as brood stock and all-male progenies can be produced without inducing any hormones in the subsequent generations (FAO, 2014).

## Hybridization

Hybridization refers to the breeding between two species/genera which are not interbred ordinarily. In Nigeria, cross between C. gariepinus and Heterobranchus bidorsalis produces sterile hybrid of superior quality. The hybrid possesses the hardiness of Clarias and fast growth of Heterobranchus. More distantly the species are related, more is the possibility of hybrids being sterile (Aluko, 1993). Hybridization of Nile Tilapia, Oreochromis niloticus and Blue Tilapia, O. aureus produces predominantly male progeny (95-98%) (Ayoolaand Idowu2008). The single sex group controls unwanted reproduction.

Cross between Stripped bass, Morone saxatilis and Yellow bass, M. mississipiensis results in the production of 100% female progeny with excellent survival and growth (Woltersand DeMay1996). Hybridization is used to produce superior quality offsprings (hybrid vigor), sterile animals, manipulate sex ratios, increase growth rate, improve flesh quality, increase disease resistance and improve environmental tolerance.

## Induced breeding

It is a technology by which ripe or mature fishes breed in confined water. The most widely used technology for induced breeding is GnRH (Bhattacharya et al., 2002). It is a decapeptide, first isolated from pig and sheep hypothalamus, with the ability to induce pituitary release of LH and FSH (Schally et al., 1973). Various inducing agents used for artificial propagation are: Extract of Pituitary Gland (PG), Human Chorionic Gonadotropin (HCG), Ovaprim, Ovulin, and various gonatropin releasing hormones analogues (GnRH).

## PG extract

The pituitary gland secretes two gonadotropins, the follicle stimulating hormone (FSH) and luteinizing hormone (LH). The FSH causes spermatogenesis in the testes of male and growth and maturation of ovarian follicle in female. LH helps in promoting the production of testosterone in male and transforming the ovarian follicles into corpora lutea in female (Shrestha and Pandit, 2017). Pituitary gland can be collected form the freshly killed matured fishes. Sex and species specificity of PG homogenate is observed in fish although PG from both sexes is equally potent in carps. Common carp pituitary serves as a common donor of PG for breeding different species of cultivable carps (Shrestha and Pandit, 2017).

## Human Chorionic Gonadotropin (HCG)

This hormone is extracted from the urine of pregnant women and comprises LH and FSH. Only one injection of HCG is enough to stimulate the egg release from female brood fish.

## Luteinizing releasing hormone-analogue (LRH-a)

This is a synthetic hormone made up of different amino acid chains. It acts on the hypothalamus pituitary interface to increase the level of gonadotropin secretion. This hormone is very effective on Chinese carps.

## Ovaprim and Ovulin

Salmon GnRH is now profusely used in fish breeding and marked commercially under the name of "Ovaprim" throughout the world. In this hormone, a chemical called Domperidone or Pemozide is used along with LRH-A, to control the release of excess hormones. This hormone is very effective in Indigenous Major Carps.

## Chromosome Sex manipulation

Chromosome Sex Manipulation techniques of induced polyploidy (Triploidy and Tetraploidy) and uniparental chromosomal inheritance (Adrogenesis and Gynogenesis) have been studied extensively in fish species (Pandian and Koteeswaran, 1998; Lakra and Das, 1998).

## Induced Polyploidy

It is the production of organisms with extra set of chromosomes, usually either triploidy or tetraploidy. Induced triploidy is useful for the production of sterile offsprings, increase the hybrid survival and hybrid heterozygosity (Thorgaard, 1986). Triploidy is induced by exposing the fertilized eggs to temperature shock (hot or cold treatment), hydrostatic pressure shock or chemicals such as colchicine, cytochalasin-B or nitrous oxide. If the treatments are applied shortly after fertilization, triploids can be produced due to the retention of the second polar body of egg. If the treatments are applied shortly before the first cleavage division, tetraploids are produced. Triploids can also be produced by crossing tetraploids and diploids. Failure of homologous chromosomes to synapse correctly during the first meiotic division in triploid fish results sterility which is an advantage. Sterility in Cyprinus carpio, Tilapia helps to control unwanted reproduction. Similarly, sterility in Grass carps help to control weeds effectively. Although tetraploidy has been induced in May finfish species, the viability of tetraploids was low in most cases (Rothbard et.al., 1997). Triploids are observed to grow faster than normal diploids, as seen in Rainbow Trout (Kraznai and Marian, 1986 and Reddy et.al., 1998).

## Uniparental Chromosomal Inheritance

**Gynogenesis-** Gynogenesis is the inheritance in which all genetic material in the offspring comes from the female parent. Gynogenesis is induced by genetic inactivation or destruction of DNA content of milt in exposure to ultraviolet rays or gamma rays (Chourrout, 1982). Eggs are activated using the genetically inactivated milt. These activated eggs develop into haploid embryos which do not survive. Hereafter, gynogenetic diploids are then produced by using treatments (thermal or pressure shocks) just before the extrusion of the polar body (meiotic gynogenesis) or by blocking the first mitotic division in the activated egg (mitotic gynogenesis) (Reddy,1999). Milt from heterologous species, having low compatibility to hybridize is preferred for the easy elimination of paternal genetic input with irradiation. Gynogenesis is an effective tool for the rapid production of inbred lines in fish (Streisinger et.al., 1981; Nagy and Csanyi, 1984). Methodologies combining use of induced gynogenesis with hormonal sex inversion have been developed for several fish species (Gomelsky et.al, 2000).

**Androgenesis-** Androgenesis is the inheritance of the paternal genetic material in the offsprings. Androgenesis is induced by irradiating eggs with the gamma radiation before fertilization. Inactivated egg genome is then fertilized with normal sperm of the corresponding species. The mechanism to restore diploidy in haploid embryos is quite different from that of gynogenesis. The process probably involves dispermy or other mechanisms (Reddy, 1999). Androgenetic individuals have been produced in a few species of cyprinids, cichlids and salmonids (Bongers et.al., 1994). Attempts to induce Androgenesis in Indian Major Carps were unsuccessful due to Due to the difficulties in effective irradiation of the eggs. The eggs of IMCs have a huge mass of cytoplasm and yolk that prevents the proper exposure to UV rays. UV irradiation of eggs may need a longer duration exposure which is lethal. Irradiation under gamma rays may be effective in carps due to stronger intensity and lesser duration of exposure (Reddy, 1999). The probable application of androgenesis is in the rapid generation of inbred lines. Androgenesis could potentially be useful general approach to the recovery of genotypes from cryopreserved sperm (Parsons and Thorgaard, 1985).

**Transgenics-** Transgenics may be defined as the introduction of exogenous gene/DNA into host genome resulting in its stable maintenance, transmission and expression. It is the transfer of certain preferable traits from one to another fish species, which is not naturally present in the species. It results in the improvement of growth rate, larger size, more efficient feed conversion, disease resistance, tolerant to low level of dissolved oxygen and fish resistant to freezing temperature (El-Zaeem, 2004; Ude et.al., 2006). A foreign gene can be transferred into fish in vivo by introducing DNA either into embryos or directly into somatic tissues of adults. The most commonly used technique is microinjection as it produces the highest survival rate (Dai et.al., 2014).

Transgenic fish have increased resistance to cold temperatures. Some marine teleosts have high levels (10-25 mg/ml) of serum antifreeze proteins (AFP) or glycoproteins (AFGP) which efficiently reduce the freezing temperature by preventing ice-crystal growth (Lakranand Ayyappan, 2003). The isolation and extraction of these antifreeze proteins followed by the inoculation or oral administration to juvenile milkfish and Tilapia led to an increase in resistance to a 26 to 13°C drop in temperature (Wu et.al., 1998). The administration of AFPs to gold fish also increased their cold tolerance (Wang et.al., 1995).

Farmed fishes are more vulnerable to the contagious diseases due to their high stocking rate. Antisense and ribozyme technologies could be used to neutralize or destroy the viral RNA such as the infectious hematopoietic necrosis virus (IHNV), which causes extensive mortality in salmonids. Lysozyme has been preferred for the production of transgenic fish resistant to a wide variety of bacterial pathogens (Austin and Allen-Austin, 1985). The rainbow trout lysozyme is a potent antibacterial agent against many Gram-positive bacteria such as Vibrio anguilliarum, Aeromonas salmonicida, Yersinia ruckeri and Flavobacterium spp. (Grinde, 1989).

Genetically modified zebrafish are being discovered for solving human organ tissue diseases and failure mysteries. Research have shown that transgenic rainbow trout (Oncorhynchus mykiss) might be used as indicator of aquatic pollutants (Gabillard et.al., 2010). Modification in the expression of growth hormone (GH) has resulted in dramatic growth enhancement in several species, including Salmon (Shao; et.al., 1992), Trout (Devlin et.al, 2001) and Tilapia (Rahman et.al., 2001).

## Conclusion

Local practices in fisheries cannot meet the ever-widening demand therefore, there is need to adopt the biotechnology techniques to meet the increasing demand. Application of biotechnology is a promising area that ensures the enhancement in production and productivity in fisheries.

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