# TECHNIQUES OF PRODUCING MONOSEX OR STERILE POPULATION OF FISH FOR AQUACULTURE - A REVIEW OF SELLECTED LITERATURE

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## ABSTRACT

The need to develop techniques that can make the male grow faster in many species of fish as well as the female in some other species cannot be over-emphasized. Monosex culture of the faster growing sex can increase production if the method is reliable.

The use of such techniques as manual sexing, sterilisation, hybridization, gynogenesis, androgenesis poluploidy and sex-reversal can provide solutions or partial solutions to the problems associated with sexual difference, sexual maturation and unwanted reproduction.

## INTRODUCTION

Genetic techniques have been applied to other animals to improve their quality and quantity but that of fish is not yet fully exploited. These techniques depend on interactive research for the development of better breeds of farmed aquatic organisms and better farmed environment.

Monosex populations of fish are desirable in aquaculture for a variety of reasons. Males have greater growth potential than females in some species and vice versa (Kirk, 1972; Dunham, 1990). Thus, monosex culture of the faster growing sex can increase production. Some species of fish attain maturity within three months and at small sizes This is a major problem particularly in Tilapia which can decrease production because precocious maturity always result in overcrowding and stunted growth in the culture pond. Moreover, sexual dimorphism for growth occurs in most cultured fishes for flesh quality and carcass yield Sexual maturity is inversely proportional to growth rate and carcass yield.

Production and dissemination of improved strains should be concurrent with the breeding programme. Effective dissemination of the genetic gain is possible only when there are organised channels for production and distribution of fish seed to farmers:

### *Techniques*

#### *Sexual Dimorphism*

In most fish species, one sex grows faster than the other, Beaver et al (1966), Stone (1981) and Hanson et al (1983) have reported that in the channel catfish, *Ictalucus punctatus* and *Tilapia* spp, the males grow faster than the females, whereas females grow faster than the males in the grass carp, *Ctenopharyngodon* idella and rainbow trout, *Salmo gairdneri* (Hickling, 1967 and Fall 1986). Faster growth of a sex probably results from hormonal, genetic factor competition, suppression of one sex and magnification of initial size difference. Stone 1981 had demonstrated that *Oreochromis niloticus* males grow 2.5 and 2.2 times faster than females when grown in cages mixed and resparately respectively.

Lovshin and Da Silva (1976) and Popma (1987) have applied the technique of manual separation effectively. This approach takes advantage of sexually dimorphic rates and growth for monosex culture. Another approach - mechanical separation grades the fish on the basis of size and and thus most of the fish can be separated by sex.

However, both the manual and mechnical separation techniques result in almost half the fingerling production being wasted.

## STERILIZATION THROUGH NON-GENETIC APPROACH

It is an approach that prevents unwanted reproduction in ponds as well as promoting growth of the fish. This is usually accomplished through surgery, immunology, radiation and chemicals.

In surgical sterilization, the entire gonad has to be removed including surrounding mesentery. This is referred to as gonadectomy. Donaldson and Hunter (1982) has indicated that surgical sterilization was successful in salmonids. However, Laird *et al* (1978) reported that the problems associated with this method include labour and the necessity of complete testicular removal to prevent development of secondary sexual characteristics.

Research efforts on immunlogical sterilization of fish are limited to a few authors. Laird *et al* (1978, 1981) injected juvenile atlantic salmon with a homogenate of homologous gonad tissue with fraund's adjuvent and obtained some inhibition of scandal development.

The use of X - rays and Y - rays to sterilize fish have not been very successful (Kobayashi and Mogami, 1958 and Al - Daham 1970). Some experiments whose effects were temporary have been carried out. For instance, Boham and Donaldson (1972) reported that isotopes depressed primary sex cells of Atlantic salmon. In addition, Kobayashi and Mogami (1958) demostrated that X - rays reduced ovarian size in rainbow trout fingerlings and suppressed gametogenesis for up to 7 months as well as sterilized some pink salmon *(Oncorhynchus gorbuscha)* males (Pursov, 1975).

As with surgical sterilization and sterilization by radiation, the effects of chemosterilization are often temporary (Stanley, 1979). Hanson and Manion (1979) employed this technique to stock mating competitiveness in sea lampreys, *Petronyzon mapinus*. Yamazaki (1976) has reported that overdoses of hormones have led to sterilization in fish chemosterilization can be brought by the use of chemicals such as methallibure and cyproterone acetate. These chemosterilents cause delayed spawning, reduced mating and total fry production.

## STERILIZATION THROUGH PLOIDY MANIPULATION: HYBRIDIZATION:

This breeding technology attempts to produce fish that combines valuables traits from more than one species or high heterosis (hybrid vigor). Apart from some tilapia hybrids (Hickling, 1980, Pruginin et al, 1975, and Wohifarth and Hulata, 1983), channel female crosses with blue male catfish hybrid (Duham and Smitherman, 1984, Smitherman and Dunham, 1985 and Dunham and Smitherman, 1987) and striped bass, *Morone sexatilis*, crossed with white bass *M. chrosops* (Bayless, 1972)) that exhibited heterosis for high performance traits, beneficial sex ratios or sexual traits, majority of interspecific hybrids do not perform well, are subviable or are difficult to produce (Smitherman and Dunham, 1985: dunham and Smitherman, 1987; and Chevassus, 1983).

Many interspecific hybridizations result in sterility, thus functioning as a reproductive isolating mechanism to prevent the permanent mixing of genes from two species. The more distantly related the two species, the greater likelihood of their hybrid being subviable or sterile. Hybridzation of two species may result in monosex populations. Tilapia hybrids offer the best examples.

The genetic explanation of hybridization particularly in tilapia is the explanation of hybridization particularly in tilapia is the multiple sex determining mechanism. Chem (1969) has reported that both XY andWZ sex determining mechanisms exist. Males are the heterogametic sex XY, and females the homogametic sex, XX, for species such as *Oreochromis niloticus* and *O. mossambicus* (Wohlfarth and Hulata, 1983). Females are the heterogametic sex WZ and males the homogametic sex, ZZ in *O. aureus* and *T. hornorum*. When homogametic males of say *O. aureus* (ZZ) are hybridrized with homogametic males XX to produce all - male progeny (ZW). When heterogametic males XY are hybridized with heterogametic females WZ, four combinations of sex chromosomes, XW, XZ, YW and YZ and produced in equal proportion. The maleness chromosome from either species is dominant to the femaleness chromosome, so when heterogametic males are hybridized with heterogametic females, 75% of the progeny are males. Only the XW genotype results in females.

Hulata *et al* (1983) have reported production of nonosex male (100% all male) population of tilapia on a commercial scale in Israel. In addition, Hulata *et al* (1983) also reported that the expected 100% maleness for these hybrid combinations is not always possible. Electrophore tic explanation of this behaviour has been provided by Macaranas *et al* (1986) and Abdelhamic (1988). These authors said that one or both of the species involve in hybridization is genetically contaminated by another species. Another explanation was given in an earlier report by Avtalion and Hammerman (1978) and Hammerman and Avtalion (1979) as due to the influence of autonimal genes on sex determination. These authors said that one or more loci located on the autosomes may act as modifiers and diminish the influence of the sex chromosomes.

The main benefit of utilizing monosex hybrids is the elimination of reproduction in reproduction ponds. Other advantages include heterosis for growth rate, ability of hybrids to be more harvestable than their parent species.

## GYNOGENESIS AND ANDROGENESIS

Gynogenesis involves the stimulation by a genetically inactive spermatozoan or the parthenogenetic development of an egg. All - female inheritance is accomplished by activating cell division with irradiated sperm and then restoring diploidy to the developing zygote. Irradiation (Chourrout, 1986, Aluko and Awopetu, 1992) rather than Y-irradiation (which may produce supernumerary chromosome fragments from donor sperm) is employed to destroy the DNA in the sperm. Thus there is no paternal contribution to the zygote. Chemical mutagens such as dimethylsulphate can also inactivate large volumes of sperm. (Tsoi, 1969) and Chourrout (1986). Haploid gynogens are produced which can be diplodized by retaining the second polar body through the application of temperature shock or pressure treatments.

Gynogens produced by blockage of polar body extrusion are inbred since all genetic information is maternal. Diploid gynogen can be produced by blocking first cleavage. This could be achieved chemically be means of temperature shock or with hyderostatic pressure. Gynogens produced by blocking the first mitotic division would be more likely to die during embryonic development because of a higher frequency of deleterious genotypes found in individuals that are 100% homogenous (Scheere *et al* 1986).

Individuals that are produced when irradiated egg is fertilized by non - irradiated spermo are called androgens. Androgens a-e more difficult to produce than gynpges.

Gynogenesis and androgenesis can be used to elucidate sex - determing mechanisms in fish. If the female is the homogametic sex, all the gynogens will be XX and female. If the female is the heterogametic sex, the gynogens will be XX, XY and YY and both sexes will be found in the progeny (Parsons and Thorgaard, 1985). If the male is the homogametic sex when androgens are produced, the androgens will be 100% ZZ and all male. If the male is the heterogametic sex, XX and YY androgens will be produced resulting in both sexes.

## POLPLOIDY

Polyploidy is lethal to mammals and birds but has led to the development of many useful plant varieties (Chourrout *et al*, 1986). Many researchers in the area of triploidization of fish such as Thorgaard *et al* (1981), Wolters *et al* (1981) and Casani and Caton (1986) have reported that triploid fish are viable and usually have the attribute of sterility which is a result of lack of gonadal development. Triploids are generally expected to be sterile due to the likely failure of homologous chromosomes to pair correctly during meiosis. Valenti (1979), and Taniguchi et al (1986) have 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.

A variety of techniques have been development to induce triploid in fish (Thorgaard 1983). In Salmonids, hydrostatic pressure treatment of newly inseminated and activated eggs gave the most successful triploid production (Arai, 1984, 1986; Chourrout, 1984, Lou and Purdom, 1984) but the technique is not in wide-spread use becuase of the need for special equipment as well as the diffficulty in treating a large number of eggs. The application of thermal treatment (heat shock) is easier to administer than pressure shock and is suitable for large-scale production of triploid. Optimal heat shocks generally give sufficient yields of triploids in rainbow trout (Thorgaard *et al*, 1981); Atlantic Salmon (Johnstone, 1985); Chinook Salmon (Utter *et al*, 1983). In brown trout (Arai and Wilkins 1987) successfully induced triploidy by heat shocks.

Similar techniques for triploid induction; temperature shocks and hydrostatic pressure are used to induce tetraploidy Mosaic individuals having both 3n and 4n cells are sometimes obtained (Bidwell et al 1985), and catfish mosaics usually have compressed bodies or caudal deformities (Dunham 1990).

Shelton et al (1986) and Johnstone *et al* (1989) have also induced triploidy in rainbow trout (*Salmo gardneri*) and Atlantic baemm (*Salmon salar*) ova using anaesthetics. In this approach eggs are exposed to nitrous acid treatments. Other anaesthetics in use include freon 22 ($CHCLF_2$ chloro-difluoro-methane), cyclopropane ($CC_3H_8$), hll-thane and othrane (Johnstone et al 1989).

## SEX REVERSAL

Monosex populations can be produced through hormonal sex-reversal. The development of fish makes them conducive to the manipulation of their sex. Although male or female genotype is established at fertilization, phenotypic sex determination occurs later in development Phenotypic sex is determined prior to hatch in some salmonid species, during the first 3 to 4 weeks after hatch in channel catfish and *Oreochromis niloticus* even as late as fingerlings for grass carp. The phenotypic sex can be altered by adminstration of estrogen or androgens at the critical period of sex determination to produce or all-female and male populations. The hormones commonly in use to produce all-male include 17 & - methyltestosterone, 19-norethylntesterone and 11-ketotestostorone (Yamazaki, 1983) Thyroid hormones have also been used to alter sex or growth rate (Howerton et al 1988). Several estrogens are commonly used to produce monosex female populations, such as B-Estradiol. Esterone and ethynylestradiol (Yamazaki, 1983).

The artificial hormones are administered by bath (Yamasaki 1969), in feed (Shelton et al 1979) or through implants (Boney et al 1984) depending on the development characteristics of the species.

The administration of male hormone can result in an anabolic, catabolic or no effect on the subsequent growth of the fry after eassation of treatment. Estrogeus also cause catabolic effects.

In the US it is illegal to sell animals that have been treated with hormones or drugs unless it is proved that there are no risks to human health from consuming these animals. This poses a problem for aquaculturist desiring to market fish that have been treated with sex hormones in countries with these regulations.

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