Techniques to Produce 100% Male Tilapia

*T.J. PANDIAN** and *K. VARADARAJ*
School of Biological Sciences
Madurai Kamaraj University
Madurai 625021
Tamil Nadu
India

For a given unit of food energy, tilapia are known to produce the maximum protein with high quality flesh. The major drawback in tilapia culture is their ability to quickly overpopulate aquatic systems. The most widely used technique to eliminate undesirable reproduction is to produce monosex populations. All-male tilapia populations are often preferred, as they are generally known to grow faster than the female.

Monosex Populations

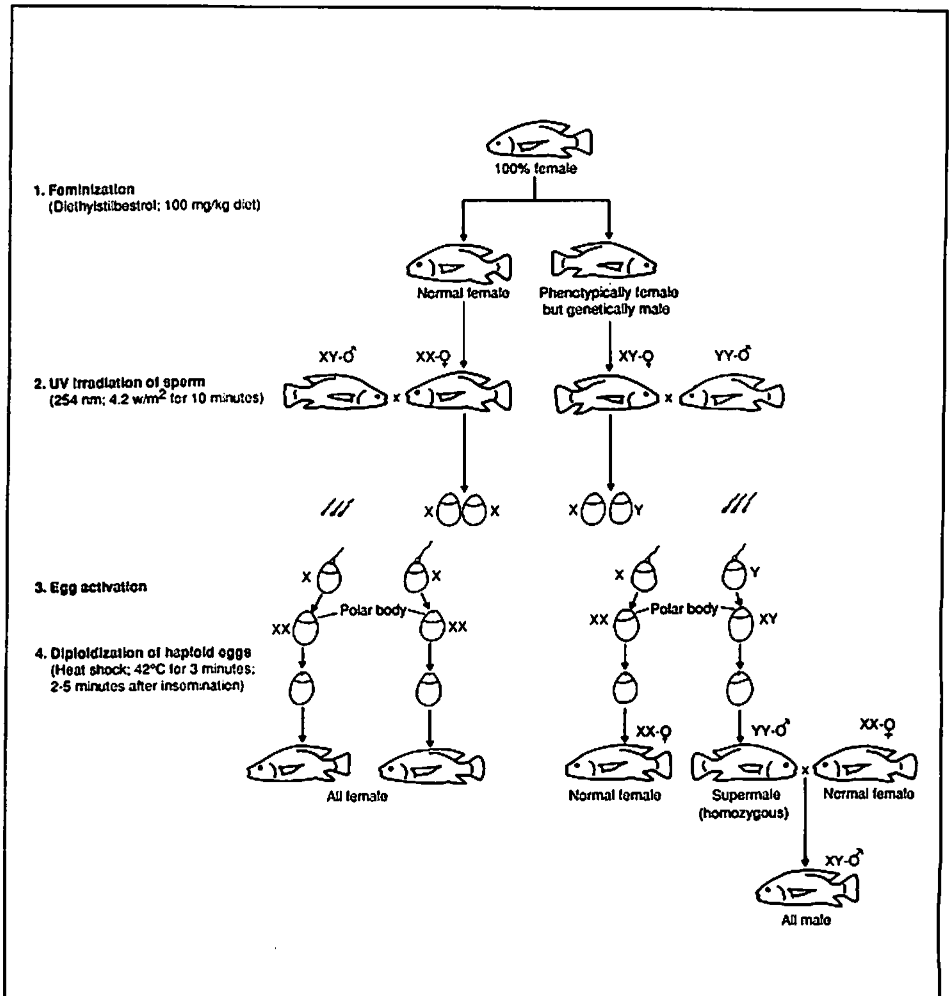
Monosex populations may be obtained by (i) manual sexing of fingerlings and separating the sexes; (ii) hybridization; (iii) sex reversing by hormone treatment; and (iv) chromosome manipulation. Manual sexing is laborious and requires skill; the major disadvantages of this method are human error in sexing and the wastage of females. Interspecific and intergeneric hybridizations are known to produce all-male progeny. However, difficulty in maintaining pure parental stocks that consistently produce 100% male offspring, poor spawning success, and incompatibility of breeders resulting in low fertility have limited the scope and use of the technique.

Our presentation is restricted to endocrine sex reversal and chromosome manipulation techniques for producing 100% male tilapia. In a series of publications, we have described different techniques for the production of 100% male tilapia. In this article we summarize all available information and recommend a procedure for the establishment of broodstock, from which unskilled fish farmers can mass-produce 100% male tilapia consistently.

*Present address: c/o Dr. M. Schartl, Max Planck Institut für Biochemie, 8033 Martinsried, bei Munchen, Germany.

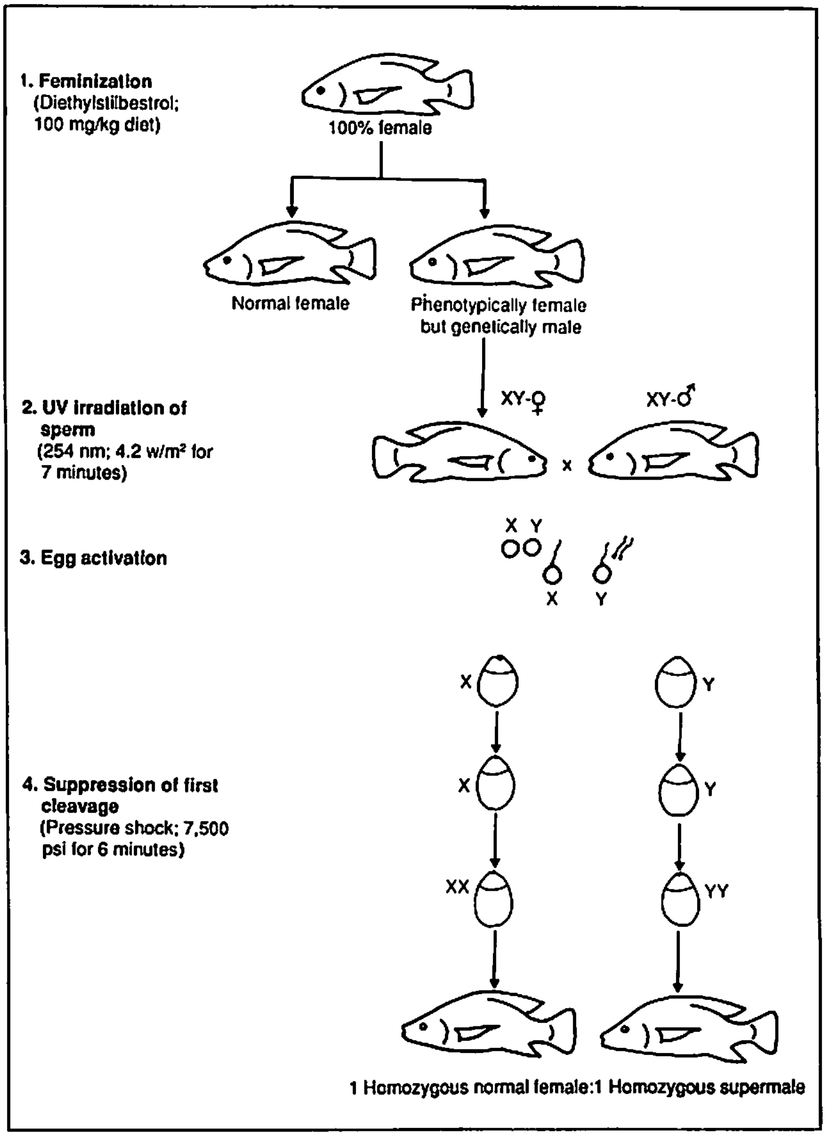
Administration of Steroids

In many fish it is possible to reverse the sex by administering one or other androgenic or estrogenic steroids through diet or water. The theoretical minimum dosage and treatment duration for endocrine sex reversal varies from species to species and in some cases, strain to strain. Several researchers have attempted to determine the labile period during which the fish is amenable for endocrine sex reversal. The fact that the residual level of the administered steroids can be carcinogenic



Schematic diagram of supermale tilapia production by integrating endocrine and meiotic gynogenesis techniques.

and/or interfere with consumer's sex has led research workers to determine the period to a critical minimum duration. Various reports in the literature show that the difference in the labile period is very wide, ranging from 11 to 69 days for *Oreochromis mossambicus*, 18 to 32 days for *O. aureus*, and 25 to 59 days for *O. niloticus*. Through a series of experiments, we were able to fix the critical minimum period for *O. mossambicus* as 11 days from the 10th day after hatching. Also, in other commercially important tilapia we found that this period is short and terminates at a very early age when the fish is not more than 50 g. Conversely, the period for carp and salmonid lasts a long time; for instance, 130 days or longer in *Cyprinus carpio*, when the fish has already attained a harvestable body weight of 300 g. The fact that the labile period in tilapia is short and is terminated long before the fish attains a harvestable body weight confers two advantages: first, the hormone treatment cost is considerably reduced, and secondly, the residual level of the administered steroid may be so little that it may not evoke any concern from the consumers.



Schematic diagram of homozygous androgenetic tilapia (androgynogenesis) production by integrating endocrine and using mitotic gynogenesis techniques.

The recommended dosage to ensure all-male tilapia populations has varied widely; for instance, it ranges from 5 to 1,000 mg methyltestosterone (MT)/kg diet for *O. mossambicus*, 30-60 mg MT/kg diet for *O. aureus* and 5 to 60 mg MT/kg diet for *O. niloticus*. Apart from such wide variations in the administered dosage, many researchers secured only 47-98% males, despite using a high dosage (20-50 mg MT/kg diet). However, in 1987 we succeeded in producing 100% male populations by administering the lowest dosage of 5 mg MT/kg diet during the critical labile period of 10 to 20 days after hatching.

The major drawback in tilapia culture is their ability to quickly overpopulate aquatic systems. The most widely used technique to eliminate undesirable reproduction is to produce monosex populations.

With regard to dosage, available information can be summarized as follows:

1. Among the tested steroids, 19α-norethisterone acetate is nearly twice as potent as either 17α-methyltestosterone or 17α-ethynyltestosterone (ET).
2. There is considerable variation in the sex reversing potency of 17α-methyltestosterone when it is tested on *O. mossambicus* by different authors (differences in quality of the MT from different sources may explain some of these variations).

Any endocrine treatment which entails variations in dosage and treatment duration from those fixed as critical minima may lead not only to the decreased sex-reversing potency of the administered hormone but also to the production of intersex and/or female fish. Other reasons for reducing the sex-reversing potency of the steroid are temperature changes and establishment of size hierarchy among the treated fry. In *O. mossambicus* fry, size hierarchy becomes apparent even on the third day of the treatment; subsequently, the dominant fry acquire more food and hormone than others, which as a result turn out later to be intersex or female. As oral administration of steroids led to this problem, alternative techniques were sought.

Immersion Method

One such alternative was the administration of hormone through water. This kind of "immersion method" eliminated the problem incurred in the oral administration. As many of the steroids are not soluble in water, dimethylsulphoxide (2.5 ppt) can be added to promote their solubility. By this method we have succeeded in securing 100% sex reversal by immersing *O. mossambicus* fry in water containing 5 mg ET/1. Many others could secure a maximum of 60% males only; on increasing dosage, for instance, the hormone MT paradoxically induced feminization rather than masculinization. Therefore, the administration of hormone through either diet or water has inherent problems.

Identification of sex in tilapia cannot be made instantaneously and it involves skilled labor. Hence, sex reversing can be widely practised only when it ensures 100% masculinization. One such technique appears to be the production of monosex populations by manipulation chromosome set.

Ploidy Induction

Techniques to control the sex of fish by ploidy induction through radiation or physical stock (pressure or thermal) are practicable, because of the ease with which fish gametes can be handled and fertilized *in vitro*.

Three broad classes of ploidy induction have been recognized. The first is induced gynogenesis, in which the paternal chromosomes of the sperm are inactivated. Two kinds of gynogenesis are possible: meiotic gynogenesis, in which the extrusion of the second polar body is prevented; and mitotic gynogenesis, in which the first mitotic division of the zygote is prevented. The second class is androgenesis, the process of inducing all-paternal inheritance. The third is induced polyploidy, in which early cell division in the fertilized egg is blocked to produce triploidy.

Induction of gynogenesis has been reported for several fish species. However, it has not been possible to induce androgenesis, as the irradiation process of the egg to make it genetically impotent is rather difficult; the irradiation destroys the structural organization of cytoplasmic organelles, too. However, it is now known that androgenesis can be induced successfully in rainbow trout.

Supermales

In our laboratory we have combined endocrine sex reversal and gynogenetic techniques to produce what is called supermales (YY). The figures on pages 3-4 illustrate the processes through which supermales can be produced. The difference between the two processes is that the one involves meiotic gynogenesis and retention of the second polar body, and the other involves mitotic gynogenesis and prevention of the first cleavage. Mitotic gynogenesis is 100% homozygous, but the homozygosity of the meiotic gynogens is likely to be less than 100%. Hence the production of all male populations through mitotic gynogenesis is preferable.

The fact that supermale *O. mossambicus* are viable and produce fertile sperm has helped us to produce consistently 100% males. As the genome of female could not directly be inactivated, it has been possible to indirectly inherit the genome of males through a phenotypic female. Hence, this process is termed gynandrogenesis.

The gynandrogenetic technique can be accomplished only by skilled technicians in the laboratory. With a view to helping fish farmers mass produce 100% males consistently, we are also endeavoring to reverse the sex of YY supermales endocrinologically. When such females are achieved, it may be possible for mating partners (YY♂ and YY♀) to continuously produce supermales. In the not too distant future, it may be possible for us to produce commercially males and females possessing YY chromosomes.

Incidentally, this technique involving gynogenetic method of generating YY males may or may not suffer from deleterious effects of inbreeding and an alternative method is to produce or use naturally occurring XY females, and to mate them with normal males for generating supermales, which can be distinguished from normal males by progeny testing.

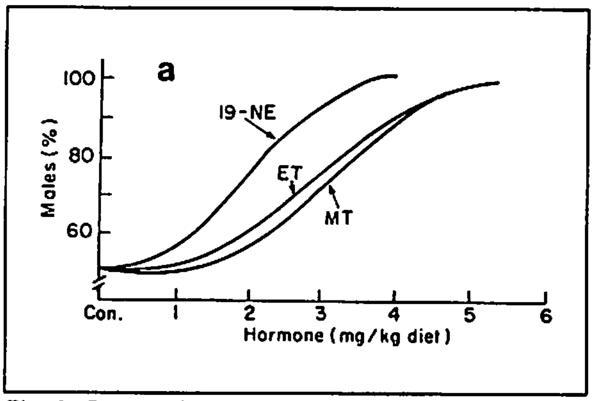


Fig. 1. Sex-reversing potency of different androgens on O. mossambicus: *19-NE: 19-norethisterone; ET: 17α-ethynyltestosterone; MT: 17α-methyltestosterone.*

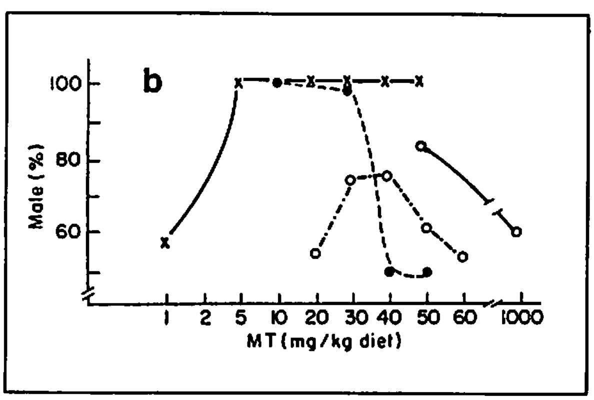


Fig. 2. Variations in the sex-reversing potency of 17α-methyltestosterone administered through diet by different authors o- - -o, *Clemens and Inslee (1968);* o——o, *Nakamura (1975);* •·······•, *Das et al. (1987);* x——x, *Pandian and Varadaraj (1988).*

Further Reading

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