INDUCED ANDROGENIC SEX REVERSAL AS A POPULATION CONTROL METHOD FOR TILAPIAS

By Rafael D. Guerrero III, Ph.D.

Abstract

Tilapias are important foodfishes with vast potentials as a source of animal protein for human nutrition in developing countries. The major drawback of tilapias is their prolific breeding habit that often results in overpopulation and stunted growth in ponds. One method that has promise for controlling populations of the species in commercial culture is induced androgenic sex reversal.

Application of the sex reversal method for production of all-male progenies in three species of tilapia is reviewed. The method is considered effective, easy to apply and economical for aquaculture application in developing countries. Androgen-treated fish are safe for human consumption.

Introduction

Tilapias are important foodfishes commercially raised as a source of animal protein for human nutrition in many developing countries including the Philippines. The advantages of tilapias as cultured species include their fast growth, ease of breeding and resistance to disease.

The major drawback of tilapias is their tendency to breed excessively and overpopulate ponds. With crowding, growth is hampered and yields of harvestable-size fish are low.

To control tilapia populations, various methods have been tried. Hybridization, manual sexing, use of predaceous fishes and induced sex reversal are among the population control methods applied for commercial production of tilapias.

This paper reviews the hormone theory of sex reversal and the application of induced androgenic sex reversal for population control of tilapias. It will also discuss the significance of the method to aquaculture.

Hormone Theory of Sex Reversal

The theory that differentiation of the genital structures of the embryo is controlled by hormone or hormones produced by the embryonic gonads is currently accepted (Burns, 1961). This hormone theory of sex differentiation in vertebrates was first proposed by Bouin and Ancel (1903, cited by Burns, 1961) and later formulated by Lillie (1916, 1917).

Development of the gonad in the vertebrate embryo takes place in the dorsolateral lining of the peritoneal cavity. Unlike most vertebrates, however, the gonads of cyclostomes and teleosts arise from the cortical portion of the peritoneal wall (Hoar, 1969). The cortex develops into the indifferent protogonia and later differentiates into either the male or female gonad depending on the genotype of the embryo.

Hormones or hormone-like substances (sex inductors) are believed to be produced by the embryonic gonad of fishes. This assumption is supported by mounting evidence that administration of androgens and estrogens to sexually undifferentiated fry can modify the direction of sex in either direction by overriding genetic regulation of sex differentiation (Yamamoto, 1969). The mechanism of action of sex hormones on the undifferentiated gonad, however, is not well understood. Furthermore, the nature and site of production of sex inductors (androtermone for the male and gynotermone for the female) have not been established.

Induced Androgenic Sex Reversal of Tilapias

Yamamoto (1958) was the first to achieve functional sex reversal in a female fish (Oryzias latipes) using methyltestosterone at dosage levels of 1 and 2 I.U./g (25 and 50 mg/kg) of diet. Yamamoto (1969) stated that two conditions should be fulfilled for achieving sex reversal in differentiated gonochorists: (1) the heterologous sex hormone should be administered starting with the age of indifferent gonad and continued throughout the stage of sex differentiation and (2) adequate dosage levels of hormones should be used.

The males of tilapias grow faster than the females. Hickling (1960) indicated that growth rate of female tilapias is greatly reduced upon attaining sexual maturity. Van Someren and Whitehead (1960) compared the growth rates of all-male and all female *Tilapia nigra* populations and found that separation of sexes did not affect the faster growth of the males. Moreover, growth rate was significantly lower and mortality was higher in the all female population than in the all-male population. Fryer and Iles (1972) concluded that although male growth superiority in tilapias may be modified by environmental factors, it has a genetic basis and is not associated with the reproductive process.

Culture of all-male tilapia is preferred to the all-female population. With no females in the population, reproduction is not possible and overpopulation is averted.

Phenotypic sex reversal of genotypic females of three tilapia species, using methyltestosterone and ethynyltestosterone, has been achieved (Table 1).

Table 1. Tilapia Species Treated with Androgens for Sex Reversal

Species	*Androgen*	*Dosage (mg/kg diet)*	*Duration (days)*	*% Males*	*Reference*
T. mossambica	M	10-30	60	95-100	Clemens & Inslee (1968)
	E	50	40	100	Guerrero (1976a)
	M	30	21-28	93-98	Guerrero (1976b)
T. nilotica	M	30	60	100	Jalabert *et al.* (1974)
	M	15-50	42	96-98	Guerrero & Abella (1976)
	M	30-60	25-59	99-100	Tayamen & Shelton (1978)
	E	30-60	25-59	98-100	Tayamen & Shelton (1978)
T. aurea	M	15-60	18	84-98	Guerrero (1975)
	E	15-60	18	85-100	Guerrero (1975)
	E	30	22	90-100	Sanico (1975)

M = methyltestosterone
E = ethylnyltestosterone

According to Yamamoto (1969), effectiveness of the sex reversal treatment depends on the kind and dosage of steroid used, the method of administration, time and duration of treatment, and on the species to be treated.

For the three tilapia species in Table I, methyltestosterone and ethynyltestosterone were found effective for inducing sex reversal at dosages of 15-60 mg/kg diet. Guerrero (1975) found ethynyltestosterone more potent than methyltestosterone for sex inversion of *T. aurea*, particularly at the dosage level of 60 mg/kg diet. Clemens and Inslee (1968) found 40 mg/kg and 50 mg/kg diet of methyltestosterone less effective than 30 mg/kg diet for inducing sex reversal in *T. mossambica.* The refractoriness of methyltestosterone at high concentrations was also reported by Yamamoto (1958).

The route of administration affects the activity of androgens (Dorfman, 1969). Synthetic androgens such as ethynyltestosterone and methyltestosterone are orally active while naturally occuring androgens like testosterone, androsterone and androstenedione are more potent when injected.

Clemens and Inslee (1968) suggested that androgen treatment of *Tilapia mossambica* fry when 30-50 days of age (an interval of about 3 weeks) which includes the indifferent gonad stage and period of differentiation is sufficient to influence sex direction. Ekstein and Spira (1965) indicated that gonadal differentiation in *T. aurea* takes place when the fry are 18-22 mm in total length or about 7-8 weeks of age.

Induced sex reversal of other tilapia species has not succeeded. Using oral administration of methyltestosterone at 50-200 mg/kg diet for 10-30 days after hatching, Yoshikawa and Oguri (1978) did not achieve sex reversal in fry of *Tilapia zillii.* Similarly, Jalabert *et al.* (1974) obtained negative results with *T. macrochir.*

Culture conditions during hormone treatment may also affect effectiveness of sex reversal. Yamamoto and Kajishima (1968) indicated that variations in feeding, water temperature and degree of crowding greatly affect the growth of goldfish under treatment for sex reversal.

In the androgen treatment of *T. aurea*, Shelton *et al.* (1981) reported that temperature, stocking density and feed regime appeared to have no influence on the effectiveness of the sex reversal treatment. For standard application, oral treatment of 9.11 mm *T. aurea* fry at a density of 2,600/m^2 or less with ethynyltestosterone dosage of 60 mg/kg diet for six weeks at 25-29°C is recommended for production of all-male populations (Shelton *et al.*, 1978).

Sex reversal in the species is ascertained by comparing the sex ratio of androgen-treated fish with that of the controls (untreated fish) and/or by progeny-testing. Treated fish with a percentage of males significantly higher than that of the controls are considered to have been masculinized. Sexing of fish can be done by gonadal examination in juveniles and by examination of the genital papilla in adults.

Sex reversal is further verified by mating suspect genotypic females with male phenotype with untreated females and sexing the offspring. A homogametic sex reversed female will produce all-female progenies when mated with a normal female.

Significance of the Sex Reversal Method of Aquaculture

For any method of population control for tilapias to be practical for fish culture, it must be effective, easy to apply and economical.

Results of various studies have shown that 100% males can be achieved with treatment of ethynyltestosterone and methyltestosterone at 60 mg/kg and 30 mg/kg diet, respectively.

The administration of oral androgens for sex reversal of tilapias is relatively easy. The method involves three essential steps:

1. Collection of newly-released fry measuring 9-11 mm total length from the brood pond,
2. Rearing of fry in suitable indoor tanks where they can be fed adequate amounts of the androgen treated feed throughout the necessary treatment period, and
3. Stocking of androgen treated fry in production ponds devoid of females.

It has been estimated that the cost of the hormone and feed for treating 1,000 fry with methyltestosterone at 30 mg/kg diet for 4 weeks is approximately ₱7 (Guerrero, 1976b). On a commercial basis, the production of 1 million fry annually can be done by one operator working halftime (Hida et al., 1960). Thus, the application of induced androgenic sex reversal is economical even in developing countries.

The use of synthetic androgens for the production of all-male tilapia has the following advantages:

1. Reproduction of tilapia in ponds is minimized or eliminated.
2. Females are not wasted as in the manual sexing method.
3. Higher yield of fish is obtained due to the faster growth of males.
4. High stocking densities of treated fish can be used.
5. The technique is not too laborious or expensive.

Like the other population control methods, sex reversal has its disadvantages. Hatchery facilities and skilled workers are required for effective treatment of fry.

The question of whether the androgen treated fish are safe for human consumption has often been raised for obvious reasons.

As far as the present knowledge on the action of androgens is concerned, androgens are not carcinogenic in humans. Synthetic androgens are widely used in human medicine for therapy of certain tumors. Ethynyltestosterone, for instance, has antifibromatogenic activity. Likewise, methyltestosterone is used for long-term therapy of disorders such as hypogonadism and pituitary dwarfism in humans (Dorfman and Shipley, 1956).

Rongone and Segaloff (1962) isolated urinary metabolites of methyltestosterone catabolyzed in the human body. Ralph Dorman of Syntex Research, Palo Alto, California (letter dated 17 December 1973) believes that the metabolites of ethynyltestosterone and methyltestosterone are safe for human consumption.

More recently, experiments on metabolic clearance of methyltestosterone from tilapia conducted by Donald Macintosh to the University of Stirling, Scotland have provided interesting findings (letter dated 15 January 1982). I quote: " . . . the levels of hormone fed to juvenile tilapia (rate 40 ppm for 40 days) fall to undetectable levels within 133 hours after the termination of hormone feeding. Thus, eating fish five days or more after treatment involves no significant intake of hormone.

With the low dosage levels used and an interval of about 90 to 120 days between treatment and consumption, it can be categorically stated that fish treated with synthetic androgens for sex reversal are not hazardous to human health.

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DISCUSSION ON INDUCED ANDROGENIC SEX REVERSAL AS A POPULATION CONTROL METHOD FOR TILAPIAS

Jose A. Carreon, Ph. D., Discussant

The focus of Dr. Guerrero's paper centers on the significance of population control for more productive culture of the tilapias.

Tilapias, the world's most studied group of fish for aquaculture, are well noted for their rapid multiplication under normal conditions with males and females together. And over population is totally undesirable in grow-out ponds where large individual fish is desired rather than large total harvest of undersized fish.

The working objective of tilapia sex-reversal is to produce /attain one-sex-stock in a culture system. Is the method of using androgenic hormone the only way to attain monosex stock of fish? Certainly not. Is one-sex-stock of fish best easily attained by the use of sex hormone through oral administration? May be yes, may be not. In any case, hormone-induced-sex-reversal technique is the handier approach in producing "all-male" brood compared to the hybridization method, e.g., *Tilapia nilotica* vs *Tilapia aurea*, since the latter has a very tough requirement of keeping pure genes in the parent fish stocks.

Yes, indeed, hormone-induced-sex-reversal technique is the most promising for an effective control of tilapia populations in culture ponds. I for one do agree; however, there are a few things that must be given a second look about this approach to tilapia aquaculture as well as those interests that are incidentally related to it, as follows:

1. Culture period for larger fish, as is presently the trend, vary from 5 to 8 months or longer within which sex-reversed tilapia broods that are lot-stocked in ponds may likely produce substantial number of spawns which will, in effect, result into overcrowding--hence small fish sizes at harvest — since sex-reversal technique is not guaranteed 100% to attain monosex broods. In the species *Tilapia nilotica* for example, even the best results of sex-reversal, as shown in Table 1 of the paper in review, indicate a percentage range of uncertainty from 4% to 1% or an average uncertainty of 2.33%. For every lot of, say, 300 hormone-treated broods per parent female, therefore, we may expect about 7 females. Even if this is reduced to 50% by mortality upon stocking in ponds, there would still be about 3 or 4 female fish larvae per

brood lot per parent female. Since tilapias generally attain sexual maturity at very early age, the inherent error of missing to sex-reverse a few post-larvae per parent female would give significant amounts of spawn during the length of culture period of 5 to 8 months.

2. While it is true that in general, male tilapias grow faster than females, however, the belief that the "all-male" broods produced by sex-reversal will all grow faster and bigger than an "all-female" broods may not be entirely true. Pruginin (1975)[1] observed that *not* all of his "all-male" hybrid tilapia populations that were obtained by crossing *Tilapia nilotica* vs *Tilapia aurea* have grown well as may be expected, and I quote: "All-male hybrid populations in this study segregated into one class of larger sexually well developed males and a second class of smaller sexually underdeveloped males; this second class may hint at the possibility that it is composed of 'genetic females' that sex-reversed to phenotypic males." The mean weight of the first class males referred to here was 323 g, and that of the second class males was 182 g. The same suspicion will prevail even if the broods were sex-directed to be all females!

3. If all "all-female" broods are produced by sex-reversal, will it be possible that some degree of growth heterosis may be observed? Have we really tried sex-reversed "all-female" broods? Under Philippine conditions there has been no credible research yet conducted to prove that an "all-female" tilapia sex-reversed would perform inferior to an "all-male" stocks regardless of lengths of culture period, type of nutrition and other controllable factors.

4. The method of sex-directing the developing fish larvae is not really that simple, particularly to fishfarmers in general who do not have the basic knowledge on the rudiments of this technique. Not all farmers can easily apply this method of sex-reversing the tilapia broods. Only the well-informed and better educated fishfarmers may feel at ease in using this technique. For industrial practice, this would probably need the establishment of specialized centers for the dispersal of sex-reversed tilapia seedlings.

5. The stigma of the prejudice against consuming androgenic-hormone-treated-fish is something that must be overcome by medical research. Although there are scientific researches in biology and aquaculture that tend to assure no harm in eating hormone-treated fish, the concern will always be there for as long as there is no study on humans that would guarantee otherwise.

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[1]Pruginin et. al. (1975) all male broods of *tilapia nilotica* x *tilapia aurea* hybrids. Aquaculture (6) 11-21.

Notwithstanding all the concerns above enumerated, I believe, and I support, all the good intentions of Raffy Guerrero's paper. Indeed, the tilapias are the most promising group of fishes for culture in freshwater production systems for as long as we are able to effectively manipulate their populations, improve their stocks, develop their appropriate feeds, improve their market and find more and better ways of consuming them.

E. M. Rigor, Ph. D., Discussant

From this paper two important aspects are mentioned; 1) The prolific breeding habits of tilapias resulting in overpopulation, competition for nutrients and subsequently stunted growth; 2) Because of the latter, induced androgenic sex reversal has been attempted resulting in all male populations and hence the maintenance of a constant or reduced population in a given volume of environment.

It is almost tempting to extrapolate from mammals to fishes although this is not the intention of this discussion. Certainly, similarities are obvious. In mammals the masculine characteristics of the body have to be imposed in males by fetal testicular hormones against a basic feminine trend of the mammalian body. Female organogenesis results from the mere absence of the testes; the presence or absence of the ovaries being unimportant. Hence, the fetal testis is a remarkably important endocrine organ and there is no symmetry in male or female development. Femaleness corresponds to an intrinsic program of the primordia, it is obtained in vitro in the absence of male hormones. Masculine differentiation is actively imposed on the system at an early stage, no possibility being left for further feminine differentiation. This can be summarized in a simple scheme showing that every structure would become feminine if not prevented from doing so by testicular hormones.

body sex
testicular hormones ——> male
female

The developmental history of the sex glands also shows a striking chronological assymetry in both sexes. Testes differentiate early when the primordial germ cells become encompassed in seminiferous cords and when testicular interstitial cells are formed. For a prolonged period of time presumptive ovaries are characterized mainly by the fact that they do not become testes. They actually remain undifferentiated. During testicular differentiation the germ cells are rapidly enclosed in seminiferous tubules and do not enter meiosis; during ovarian development the germ cells start the meiotic process before being tightly surrounded by follicular cells. It is even more likely that when follicles develop, the follicular cells stop the meiotic process. The reason why meiosis begins in oocytes prior to true ovarian organogenesis is not understood.

The evidence of the freemartin suggests that presumptive ovaries, if submitted to an appropriate influence, probably the two kinds of fetal testicular hormones still may develop testis-like structures in place of ovarian follicles. The concept then, is introduced that the fetal testis produces at least two or two kinds of morphogenetic secretions, one (or more masculinizing hormone(s) whose action is duplicated by androgens and a Mullerian inhibitor (anti mullerian hormone, AMH) the action of which is not duplicated by steroidal androgens. AMH is synthesized by fetal Sertoli cells very early in life, as soon as seminiferous tubules are recognizable under the light microscope and before the appearance of fetal Leydig cells. AMH production ceases in the perinatal period and is not resumed at puberty. AMH is probably a glycoprotein.

Since androgen induced sex reversal in tilapias is economical, wherein only minute or physiological amounts (as against pharmacological) of androgen are required, one wonders if this practice has found widespread application. Since hatchery facilities and skilled workers are required for the effective treatment of fry, has something been done about the problem?

While the paper is a review, it would have been more meaningful if experiments were presented comparing sex reversal as against a control tilapia population in regard to yields of harvestable fish (percent?), weights of each harvestable fish, feed efficiency as well as the most desirable stocking rate per unit of volume.

Herminio R. Rapanal, Ph. D., Discussant

1. I would like to commend Dr. Rafael Guerrero for taking up this research work on tilapia which can have great scientific as well as economic significance. It is a problem based on sound and logical scientific principle and the results thus far obtained are very encouraging.

2. If I recall correctly, Dr. Guerrero's first paper enunciating this technique of sex reversal in tilapia prominently mentioned as one of the significant contributions to the FAO World Conference on Aquaculture held in Kyoto, Japan in 1975. Of course, the actual research work must have antedated this named date by a

number of years. Subsequently, Dr. Guerrero has been invited and contributed papers related to this subject to meetings and during travel that brought him to Italy, Africa, and Latin America. Between 1975 to this year (1982), a period of seven years, it would be of interest to assess to what extent the method or research results been put forward or adopted by tilapia culturists on the aquaculture industry.

3. A cursory look at the table of results of this work by different researchers (Table 1 of paper) including Dr. Guerrero and associates, and other workers, reveal that there are only two cases out of ten where 100% sex reversal was attained (Jalabert, *et al.*, 1974 for *T. nilotica* and Guerrero 1976a for *T. mossambica*). The other eight cited results give varied percentages ranging from as low as 84% to 98%, even 100%, for experiments on *T. mossambica*, *T. nilotica* and *T. aurea*. I believe that in this regard, the attainment of 100%, sex reversal is highly important. The presence of even a very small percentage of unreversed females in a treated population could wreck havoc in that particular population considering the very high fecundity or reproductive capacity of this species. What could happen is that an extremely high reproduction of the unreversed females may occur to fill up what seems to be a vacuum.

4. Corollary to the problem presented in paragraph 3 above is the inherent difficulty of screening out or eradicating tilapia in their natural environment. Tilapia eggs have been suspected of passing through the finest gate screens and tilapia eggs or fry are likewise suspected or perhaps even observed to be able to survive the smallest pools left by the footprints of the fish farmers. Again surviving females from this undetected sources can destroy management plans for a productive monosex culture of tilapia.

5. Dr. Guerrero's paper appears to be very convincing on the economic feasibility of carrying out sex reversal management in tilapia culture. This is borne out by the fact that the operational cost for feed and androgen is about 0.7 centavos per fry or about ₱7,000 per million fry. The paper however did not state the investment or capital costs for the construction and setting up of tanks and other container facilities, the feed making facilities, cost of the site, depreciation of facilities, etc. The amount of skilled as well as unskilled labour man-hours has to be estimated also to evaluate manpower costs. All such costs should be accounted for if a farmer envisions to establish a set-up in this work. Likewise, the sources of the chemicals to be used, their availability and costs of such substances and other ingredients needed, and the methodology of their preparations are inadequately explained in the present paper. If these have not been explained in previous public-

ations, perhaps a further enlightenment on these aspects will be required.

6. The paper does not mention if there are any post-treatment re-reversal in sex of the treated tilapia after a period of time. But the paper stated that if the sex reversed females that have not become males are mated with other females, the progenies would all be females. Considering that females are much smaller than the males would such an occurrence then result in the possibility of culturing a relatively dwarf population of tilapia.

7. We have great faith and confidence in the reliability of the experiments on sex reversal in tilapia by androgenic treatment. Since this idea has been enunciated for over seven years now, and considering that practise of these scientific results can have valuable economic and food production significance, I believe the time is now appropriate for the setting up of practical demonstrations or pilot projects to propagate the techniques for the benefit of the industry. Training programmes with extension workers and fishfarmers as the recipients should be put up. The practical demonstration or pilot projects should clearly illustrate farm and facilities design and the kinds and quantities of supplies and equipment required. With the encouraging prices now being paid for tilapia in the market, we look forward to the time when this research result is adopted as a general practice by fishfarmers.