SEX REVERSAL OF TILAPIA

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ABSTRACT

Early maturation and frequent spawning are management challenges when working with tilapia. Male tilapia are preferred for culture because of their faster growth. Of the various techniques that have been developed to provide male tilapia for culture, sex reversal is the most commonly used procedure. Recently hatched tilapia fry do not have developed gonads. It is possible to intervene at this early point in the life history and direct gonadal development to produce monosex populations. Exogenous steroids given during the gonadal development period can control the phenotype overriding the expression of the genotypically determined sex. This process is commonly referred to as sex reversal. Androgens direct the development to males and estrogens to females. Methyltestosterone is the most commonly used androgen to direct the sex of tilapia. Various protocols regarding dose rate and treatment duration have been evaluated. All depend on hormonal treatment with sexually undifferentiated fry. Fry may be obtained by partial or complete harvests of spawning containers. Containers used for tilapia spawning include indoor and outdoor tanks, earthen ponds and fine mesh net enclosures (*hapas*). When fish are treated from the beginning to end of the gonadal differentiation period with a proper dose of androgen the resultant fish population will be highly skewed to males.

INTRODUCTION

Tilapia are a paradox in reproduction. The relative fecundity of the *Oreochromis* species is low, 6,000–13,000 eggs/kg/spawn (Siraj et al. 1983). This is compensated for the high survival of fry as the result of the large size at hatching with a large yolk reserve and the mouth brooding maternal care given until the hatchlings are 10 mm or larger. The low fecundity is also compensated for by frequent spawning of these asynchronous species where the low fecundity per spawn may be parlayed into a yearly quantity of eggs/kg equal to many group synchronous spawning species.

Ideally a fish species used in aquaculture will not reproduce in the culture environment before reaching market size. From this perspective tilapia present some challenges to the fish culturist. Most species of tilapia under favorable growth conditions will reach maturity within 6–8 mo of hatching at a size often less than 100 g. Under favorable conditions they will continue to reproduce, the offspring competing with the initial stock for food, resulting in stunted growth and unmarketable fish. Swingle (1960) found that in 169–196 d culture cycles of mixed sex Mosambique tilapia, *Oreochromis mossambicus*, production exceeded 3,000 kg/ha but >90% of the harvest was composed of fish <100g Verani et al. (1983) produced 4,944 kg/ha of mixed sex Nile tilapia (*O. niloticus*) in 11 mo but the average weight harvested was <100g. High yields and efficient nutrient utilizations are meaningless unless a significant portion of the production is marketable. Tilapia have numerous advantages as an aquaculture species (Teichert-Coddington et al. 1997) but the ability to reproduce in the production setting has resulted in various techniques being developed to control unwanted reproduction.

Different techniques including stock manipulation (Swingle 1960), polyculture of tilapia with

predatory fish (Lovshin 1975) and monosex culture (Shell 1968) have been described to control tilapia overpopulation. The use of a predator does not prevent reproduction but can prevent recruitment. Tilapia yields are often low, reduced by the slower growing females, and often because the predator has lower tolerance to poor water quality than tilapia, forcing the producer to limit nutrient input in order to maintain adequate water quality for the predator species.

All male culture of tilapia is preferred because of their faster growth (Guerrero 1975; Shelton et al. 1978). Several techniques have been adopted for production of monosex (all male) tilapia: manual sexing (Guerrero 1982); hybridization (Hickling 1960); genetic manipulation (Pandian and Varadaraj 1988); and sex reversal through sex hormone administration (Yamamato 1951; Clemens and Inslee 1968; Shelton et al. 1978; Guerrero 1982).

Monosex culture by either manually or mechanically selecting males results in half of the potential fish seed being rejected. Popma et al. (1984) developed an efficient system to produce hand-sexed fingerlings but 30% of a farm's acreage had to be devoted to broodfish and fingerling production to support the remaining 70% in food fish production. Fingerlings were reared to 20–30 g for sexing, producing the equivalent of 4,000 kg/ha/y of females, most of which were discarded.

Selective crosses of tilapia using a homogametic male of one species crossed with a homogametic female of another has resulted in all male hybrids (Lovshin 1982). This approach, first reported by Hickling in 1960, became a common method to produce males, but was largely replaced by the mid-1980's. Difficulties in maintaining 2 pure lines of broodfish and keeping them separate, and the space required contributed to the decline. In addition apparent autosomal influences affected sex ratios often resulting in populations that were less than 100% males even if pure lines were maintained.

The use of hormones to alter the sex ratios of fish was first demonstrated in species other than tilapia. Yamamato (1951) concluded that sex hormones, in addition to modification of secondary sex characteristics, also affect the gonads. Androgen induced masculinization and estrogen resulted in feminization. He produced 100% female medaka (*Oryzai latipus*) with an estrogen in 1951 and a nearly all male population with an androgen in 1954. The general technique has successfully altered the sex ratio of rainbow trout *Salmo gairdneri*, goldfish *Carassius auratus*, *Zebra fario* (Yamazaki 1976), grass carps *Ctenopharengodon idella* (Stanley 1976) and tilapia (Clemens and Inslee 1968; Nakamura and Takahashi 1973).

Although commonly referred to as sex reversal, this term requires explanation. Hormone treatment does not alter the genotype of the fish but directs the expression of the phenotype. A treated population of fish may be phenotypically mono-sex but genetically will have remained the same as determined at the moment of fertilization. As a result of hormone treatment, it is possible to have phenotypical male fish which are genetically female or phenotypically female fish that are genetically male. For convenience in the rest of this chapter the altering of the phenotype by administration of sex hormones will be referred to as sex reversal.

Production of all male population through administration of androgen (17-α methyltestosterone) is considered to be the most effective and economically feasible method for obtaining all male tilapia populations (Guerrero and Guerrero 1988). Following the techniques outlined by Popma and Green (1990), less than 15% of a farm's acreage would need to be devoted to broodstock and fingerling production to support the remaining acreage in food fish.

Such efficiency and simplicity in production techniques has resulted in hormone sex reversal becoming the commercial procedure of choice to produce male tilapia fingerlings and has been a significant factor in the rapid growth of the tilapia industry.

MASCULINIZATION

Male tilapia are the desired sex as they grow faster and divert less energy into reproduction. A number of types of chemicals have been used to control sexual development in tilapia. Steroids are a group of lipids with several unique properties affecting growth and development. Steroids are called androgens if they are able to induce male characteristics and estrogens if they induce female characteristics. Androgens have 2 physiological actions: (1) androgenic activity, promoting the development of male sex characteristics; and (2) anabolic activity, stimulating protein biosynthesis. Androgens can be classified into 2 groups: androstane derivatives, having both androgenic and anabolic properties and 19-nor-androstane derivatives, having anabolic

properties but being only weakly androgenic (Camerino and Sciaky 1975). From a sex reversal perspective, and rostane derivatives are of more value because of their potential to direct the sexual development of fish into males. When evaluating an androgen for sex reversal by oral administration of hormone 3 main criteria for selection should be considered: metabolic half-life, androgenic strength and solubility in water.

Testosterone is the principal androgen secreted by the testis and the main androgenic steroid in the plasma of human males (Murad and Haynes 1985). It is often used as the standard to evaluate the androgenic properties of a steroid. It is ineffective when given orally and has a short duration when given by injection due to rapid hepatic metabolism. Synthetic androgens are preferred over natural ones because some can be administered orally and withstand catabolism in the gut. The chemical structure, bonds and attached groups determine the effectiveness (Brueggemeier,1986). Introduction of a 3-ketone function or a 3α-OH group or reduction of the 4,5-double bond enhances androgenic activity. Alkylation of the 17α-position or the 1α-position allows for oral activity.

When natural androgens have been evaluated the results have been variable. Eckstein and Spira (1965) used testosterone as a 1 mg/l bath with blue tilapia (*O. aureus*) with little results. Al-Daham (1970) obtained 89% males using 0.4 mg of testosterone/l. Adrenosterone at 5 mg/l produced an "all male" population where 55% of the fish had no gonadal development (Katz et al. 1976). When adrenosterone was given in the diet at 30 or 50 mg/kg of diet only 74 and 81% males, respectively, were obtained (Guerrero and Guerrero 1997). Nakamura (1981) masculinized *O. mossambicus* by feeding 11-ketotestosterone at 200 mg/kg of diet for 19 d. Haylor and Pascual (1991) prepared a diet for young *O. niloticus* which was 57% ram testis. They examined 27 fish and found 23 males and 4 intersex fish. Phelps et al. (1996) obtained a 65% male population using a diet, half of which was freeze dried bull testes.

A number of synthetic androgens have altered the sex ratio of tilapia when applied as a bath or a feed additive. Clemens and Inslee (1968) produced all male populations of *Oreochromis mossambicus* incorporating 17-α-methyltestosterone into the diet at 10–40 mg/kg. Methyltestosterone (MT) has since become the most commonly used synthetic androgen to alter the sex ratio of fish. It has proven to be effective in a number of different species of tilapia and under a variety of management scenarios (Table 1). Other synthetic androgens have been incorporated into the diet of tilapia for sex reversal are given in Table 2.

None of these androgens can be considered the best for tilapia sex reversal. The range of dose rates and treatment protocols make it difficult to compare compounds. One perspective is to evaluate these androgens based on the local costs to produce a given number of fish. Phelps et al. (1992) discussed how fluoxymesterone was more expensive than methyltestosterone but the effective dose was lower, thus compensating for the cost difference. Selection of an androgen to be incorporated into the feed should be based on local availability, costs, government regulations, and human and environmental safety.

Several androgens have been added to the holding water in an attempt to sex reverse tilapia using a variety of protocols. Al-Daham (1970) treated *O. aureus* fry for 12 d with testosterone at 0.4 mg/l and produced 89% males, but a 6 d treatment was ineffective. Eckstein and Spira (1965) treated 4–5 wk *O. aureus* for 5–6 wk with MT with variable results. Gale et al. (1995) treated *O. niloticus* fry at 10 and 13 days post-fertilization with MT at either 100 or 500 μg/l for 3 h. and were able to significantly skew the sex ratio at 100 μg/l but not at 500. They were able to produce 100% males one trial and 94% in another using mestanolone at 500 μg/l following the same protocol. Torrans et al. (1988) use mestanolone at 0.6 and 1 mg/l to treat *O. aureus* fry for 5 wk and obtained populations that averaged 82% male and 18% intersex fish. Contreras-Sanchez et al. (1997) obtained >90% males exposing *O. niloticus* fry to two 2 h baths of 500 μg/l trenbolone acetate.

Another approach to producing male populations is through the use of non-steroidal compounds that interfere with steroid binding or metabolism. In the sequence of events leading to gonadal differentiation endogenous androgens are aromatised into estrogen. Piferrer et al. (1994) treated a genetically all female population of Chinook salmon (*Oncorhychus tshawytscha*) with an aromatase inhibitor and produced males.

Blocking of estrogen binding sites is another approach to production of males. Tamoxifen, an antiestrogen when given as a feed additive to tilapia at 100 mg/kg of diet produced an all male population (Hines and Watts,1995).

Table 1: Effects of various dosages of 17-α-methyltestosterone (as mg of MT/kg of diet), treatment periods, environments, and percent sex inversion of tilapia (*Oreochromis* spp.); BW = body weight.

Species/Doses	Days	Environments	% Males	References
O. niloticus				
Control	28	45-l glass aquaria	44.5	Okoko (1996)
MT-3.75		Fed 15% BW (4x/d);	80.0	
MT-7.5		Flow through; 29°C	91.7	
MT-15			98.3	
MT-30			99.3	
MT-60			97.0	
MT-120			71.9	
MT-240			50.7	
MT-480			48.3	
MT-600			55.0	
MT-1200			52.0	
Control	28	*Hapas* in ponds;	51.3	Green and Teichert-Coddington (1994)
MT-60		26.1°C; Fed 20% BW (4x/d)	96.8	
Control	28	*Hapas* suspended in tanks;	54.7	Phelps et al. (1992)
MT-60		Fed 20% BW (4x/d)	97.8	
Control	25	95-l steel tank;	50.4	Tayamen and Shelton (1978)
	35	Continuous flow: 23°C	56.9	
	59	Fed 10% BW (4x/d)	54.0	
MT-30	25		99.2	
	35		100.0	
	59		100.0	
MT-60	25		100.0	
	35		100.0	
	59		100.0	
Control	28	115-l Steel tank;	44.0	Shepperd (1984)
MT-30		Flow through; 24°–32°C;	98.0	
MT-60		Fed 15% BW (4x/d)	98.0	
O. aureus				
Control	18	95-l steel tank;	56.0	Guerrero (1975)
MT-15		Continuous flow; 21°C;	84.0	
MT-30		Fed 4% BW/2x/d	98.0	
MT-60			85.0	
Control	22	38-l glass aquarium;	50.4	McGeachin et al. (1987)
MT-60		Continuous flow; 27°–32°C	99.0	
MT-90		Fed 50% BW (4x/d)	98.0	
MT-120			96.0	
O. mossambicus				
Control	19	10-l glass aquarium;	59.5	Nakamura (1975)
MT-50		No flow through; 22°C;	100.0	
MT-1000		fed 1–2/d	61.0	
MT-1000	44		00.0	
Control	19	Aquarium/fed *ad libitum*	54.0	Varadaraj and Pandian (1989)
MT-2			56.0	
MT-5			100.0	
MT-10			100.0	
MT-20			100.0	
MT-30			100.0	
MT-40			100.0	
Red Tilapia (Hybrid *O. mossambicus* x *O. hornorum*)				
Control	28	115-l Steel tank;	39.0	Shepperd (1984)
MT-30		Flow through; 24°–32°C;	94.0	
MT-60		Fed 15% BW (4x/d)	91.0	

Table 2. Other synthetic androgens that have been incorporated into the diet of tilapia for sex reversal.

Androgens	Tilapia species	Dose Rates	Efficiacy	Authors
1-dehydrotestosterone	*O. aureus*	15 mg/kg	69% male	Guerrero (1975)
		30 mg/kg	59% male	
		60 mg/kg	44% male	
ethynyltestosterone	*O. aureus*	15 mg/kg	85% male	Guerrero (1975)
		30 mg/kg	98% male	
		60 mg/kg	100% male	
fluoxymesterone	*O. niloticus*	1 mg/kg	87.3% male	Phelps et al. (1992)
		5 mg/kg	100% male	
		25 mg/kg	100% male	
mestanolone	*O. niloticus*	5 mg/kg	99.5% male	Soto (1992)
		10 mg/kg	97.0% male	
		20 mg/kg	99.0% male	
mibolerone	*O. mossambicus*	1.5 mg/kg	84% male	Guerrero and Guerrero (1993)
		1.75 mg/kg	88.0% male	
		2.0 mg/kg	94.0% male	
19-norethisterone acetate	*O. mossambicus*	1 mg/kg	52% male	Varadaraj (1990)
trenbolone acetate	*O. aureus*	25 mg/kg	98.3% male	Galvez et al. (1996)
		50 mg/kg	99.3% male	
		100 mg/kg	99.0% male	

FEMINIZATION

Female tilapia are not preferred for culture but feminization of genetically male Nile tilapia offers the possibility of all male tilapia through a YY breeding program. Likewise of interest is the feminization of a homogametic male *O. aureus* to produce a functional females for mating with normal male *O. aureus* to produce all male offspring.

Estrogens are those agents which induce feminization. Estrone and 17β-estradiol are 2 natural steroidal estrogens found in the ovary of tilapia (Katz et al. 1971). Synthetic estrogens, such as ethynylestradiol and diethylstibestrol, are more potent than natural estrogens when given orally. This greater activity is due to their stability in the digestive tract and the liver (White et al. 1973).

Sex reversal to female is more difficult than to males. Jensen and Shelton (1979) added the natural estrogens estriol, estrone and 17β-estradiol to the diet of 8–11 mm *O. aureus* in an attempt to produce all-female populations. They were not able to skew the sex ratio of treated fish but noted atypical males at the higher treatment rates. Hopkins (1977) used 17β-estradiol at 220 mg/kg of diet to obtain a population 59% females, 2 % males and 39% “atypical”.

The most commonly used synthetic estrogens for sex reversal are the non-steroidal estrogens, ethynylestradiol (EE) and diethylstibestrol (DES). DES is the more potent and once was used as a growth promotant in livestock until banded by the US Food and Drug Administration in 1979. Both are carcinogens.

The effectiveness of DES and EE to feminize may be dependent on the species of tilapia and the management conditions. Hopkins et al. (1979) fed 100 mg DES/kg diet to *O. aureus* fry for 5 weeks and produced 64% females. Rosenstein and Hulata (1993) obtained 98% and 100% females in 2 sets of *O. aureus* fed DES at 100 mg/kg for 30 d. EE was used at 100 mg/kg with *O. aureus* for 40 d by Melard (1995) to obtain a 94% female population.

Potts and Phelps (1995) fed *O. niloticus* fry for 28 d diets containing up to 400 mg DES/kg or 200 mg EE/kg. They were able to obtain 92% of the population at 400 mg DES with female shaped papilla and 80% having ovaries. EE at 100 mg/kg was not as effective producing a 65% female population. Scott et al. (1989) fed 2 sets of genetically all male *O. niloticus* fry DES at 100 mg/kg and obtained 52% females in one set and 84% in the other. Rosenstein

and Hulata (1994) obtained 100% females feeding DES at 100 mg/kg or EE at 75 mg/kg for 14 d but the results were not consistent among trials.

Bath treatments with estrogens have not successfully feminized tilapia. Rosenstein and Hulata (1993) treated *O. mossambicus* with 17-β-estradiol over a range of concentrations and durations with no effect on the sex ratios.

Toxicity is an issue in estrogen treatments. Eckstein and Spira (1965) reported high mortality of *O. aureus* fry when given stilbestrol diphosphate baths at 400–1000 μg/l.

STERILANTS

Another approach to control tilapia reproduction has been sterilization. Androgens and estrogens have been reported to cause gonadal damage, rendering the fish sterile. Eckstein and Spira (1965) found that stilbestrol diphosphate at 50 μg/l resulted in gonadal destruction in *O. aureus* treated for 2 wk. Adrenosterone at 5 mg/l resulted in a population where 55% of the fish had no gonadal development (Katz et al. 1976). Okoko (1996) found that high doses of MT reduced the gonadosomatic index of "male" *O. niloticus*.

Al-Daham (1970) treated *O. aureus* fingerlings with the chemosterilants metepa and tretamne at 20 ppm and 0.8 ppm, respectively, and was able to limit reproduction. These chemicals did not produce monosex populations but reduced gonadosomatic indices in both males and females. Methallibure controlled tilapia spawning, preventing secondary sexual characteristics and spawning behavior (Dadzie 1975).

FISH SPECIES EVALUATED

Hormonal sex reversal has been demonstrated in a wide range of families of fish including: Andantidae, Atherinidae, Bothidae, Centrarchidae, Cichlidae, Cyclopteridae, Cyprinidae, Cyprinodontiade, Ictaluridae, Percidae, Poecilidae, Polyodontidae, Salmonidae, and Serranidae. It has been particularly effective in cichlids because gonadal differentiation takes place early in the life history. Among the species of tilapia that have been included in studies to chemically alter the sex ratio include those in Table 3.

Tilapia species that have been successfully sex reversed are mouth brooding species where hormone treatment begins within a few days after hatching. Less success has been obtained with a substrate spawner *T. zillii*. Timing and duration of treatment may have been the problem with the lack of success in this species.

MODE OF ACTION OF HORMONE

For sex reversal to be effective it must begin before gonadal differentiation has begun. Sexual differentiation of gonads takes place at some point post-hatch (Yamamato 1951). In newly hatched *O. niloticus* (Alvendia-Casauay and Carino 1988) and *O. mossambicus* (Nakamura and Takahashi 1985) primordial germ cells are found at the dorsal root of developing mesentery in the mesoderm, ventral to the gut and in the endoderm cells of gut. The germ cells eventually migrate to the gonadal region. Paired gonadal analgens are observed 9–10 d post-hatching. The appearance of ovocoel and testocoel, indications of sex differentiation to female and male takes place at 16–20 d posthatching in *O. mossambicus* (Nakamura and Takahashi 1985) and perhaps as late as 30–33 d post-hatching in *O.*

Table 3. Species of tilapia studied for sex reversal

Species	Hormones	Modes of Treatment	Authors
O. mossambicus	MT	feed additive	Clemens and Inslee (1968)
O. niloticus	MT	feed additive	Tayamen and Shelton (1978)
O. aureus	ET	feed additive	Guerrero (1975)
O. honorum	ET	feed additive	Obi and Shelton (1983)
O. spilurus	MT	feed additive	Lone and Ridha (1993)
T. heudeloti	Testosterone propionate	immersion	Hackmann (1971)
O. macrochir	MT	feed additive	Jalabert et al. (1974)
T. zillii	ET	feed additive	Guerrero (1976)

niloticus (Alvendia-Casauay and Carino 1988). According to Nakamura and Takahashi (1985), there is a critical period of sex determination of germ cells in the sexually undifferentiated gonads during which germ cells respond to exogenous as well as endogenous inducers of sex determination. Oogenesis begins before spermatogenesis in *O. mossambicus* (Nakamura and Takahashi 1985) and in *O. niloticus* (Alvendia-Casauay and Carino 1988)

In *O. niloticus* the effects of adrenosterone (androgen) can be divided into 2 stages. At the first stage, development of gonadal germinal tissue is prevented. The second stage is characterized by repopulation of the testicular tissue by gonocytes. Hackmann and Reinboth (1973) concluded that administration of exogenous androgen causes complete or partial degeneration of female gonocytes. Gonads of genetically female fish may not be capable of producing estrogens for the maintenance of oogenesis, and the absence of female hormones brings about testicular development as an autodifferentiation (Hackmann and Reinboth 1973).

Whether steroids are natural inducers of sexual differentiation as proposed by Yamamoto (1969) or that exogenous steroids are pharmacologically altered as suggested by Reinboth (1970) is not clear. Steroidogenesis is not evident in tilapia until differentiation begins (Baroiller et al. 1988). From the numerous studies with tilapia, it is clear that, if exogenous steroids are given before the start of gonadal differentiation and administered past differentiation, then it is possible to alter the sex ratio.

An understanding of the mechanics of exogenous direction of gonadal development is further complicated by the success that has been obtained by short-term immersions in steroid solutions even though the treatment ended well before gonadal differentiation is completed

SEED PRODUCTION SYSTEMS

For successful sex reversal it is critical that the treatment begin with fish of an age where gonadal differentiation has not begun. There are 3 basic approaches to obtaining such a young fish: partial harvest from spawning ponds; complete harvest of fry from ponds or tanks and the egg collection from brooding females. The selection of technique is influenced by factors including the number of fry needed at one time, labor availability, water resources, and facilities availability.

Partial Harvests

Tilapia fry tend to gather along the edge of a pond or tank, particularly in the early morning. By seining along the edge, significant quantities of fish can be collected daily. In relatively small artificial tanks broodfish are commonly stocked at a rate of 0.3–0.7 kg/m^2, depending on the rate of water exchange, with female:male total weight ratios of 2–3:1. Fry are frequently skimmed from the water surface beginning about 10 d after stocking (Figure 1). Brood tanks must be drained and recycled every month or 2 because fry that escape harvest become cannibalistic on recently hatched fry. Monthly fry production in the Philippines is approximately 1,000 fry/kg of brood female (annual production of 1.2 million fry/100 kg of brood females and 30–50 kg of brood males). Guerrero and Guerrero (1985) harvested tanks 4–6 x/d over a 50–72 d reproduction cycle and found 2 peaks of fry abundance, reaching 16.2 fry/m^2/d during the first peak period. To provide an abundance of fry on a consistent basis, the start of reproduction cycles in several tanks should be staggered to avoid such peaks and valleys in fry collection.

The same procedure can be used in earthen ponds stocking up to 2,000–3,000 kg of brooders/ha at a sex ratio 2–3 females/male. Brooders are fed during the spawning period at approximately 1–2% body wt/d and the pond may be fertilized. Some brooders spawn within a few days after stocking and swimup fry can be expected within 10–15 d after brood stocking. With once weekly fry collections Verdegem and McGinty (1989) obtained an average of 153,100 fry/ha/wk (2.2 fry/m^2/d). Over a 116 d period, Little (1989) averaged 2.5 sex reversible size fry/m^2/d from ponds stocked with *O. niloticus* harvested every 5 d, 6 x/d, and 1.5 fry/m^2/d of sex reversible size fry when harvested 3x/d every 5 d.

How long a spawning pond can be kept in production depends on how successful partial harvests are. Ideally all fry are harvested before they reach a larger size. Those that escape capture soon prey on subsequent swim-up fry. Macintosh and De Silva (1984) found that within fry of the same age, cannibalism contributed up to 35% of total fry mortality. Even with careful seining some fry will escape harvest resulting in a progressive decrease in fry harvested due to cannibalism. A spawning pond can be left in production for 8–10 wk before a complete harvest is necessary.

Fry collected by partial harvest from a brood pond are usually of mixed sizes and should be graded

Figure 1. Partial harvesting of tilapia fry by seining the edge of a spawning pond.

before use in sex reversal. Little (1989) found that the number of oversize fry harvested could be kept to a minimum (0.015 fry/m^2/d) if the pond was harvested 6x/d every 5 d.

Partial harvesting of ponds to produce tilapia fry may be acceptable for locations where the production season is year-round and large quantities are not required at any one time. Fry yields from a pond are variable day-to-day therefore several ponds are needed to produce a constant production of fry. The technique is labor intensive but does not require highly skilled labor.

Complete Harvest of Fry From Ponds

Complete harvest of fry can be made in a spawning pond with a catch basin or from a fine mesh net enclosure (*hapa*). Spawning ponds are generally no larger than 2,000 m^2 and are designed to drain completely into a catch basin that is 10 m^2 or larger (at least 1% of pond area). The catch basin should be 30–40 cm deep with a firm bottom, ideally concrete.

The spawning pond is prepared by lining the catch basin with large mesh netting that is about 20% larger than the catch basin. This net is used to remove brooders from the pond at harvest without removing the fry. The pond must be completely dry before restocking or if puddles remain they are poisoned with chlorine or other toxicant to insure no fry remain from the last production cycle to cannibalize fry produced in the subsequent cycle. Tilapia fry can remain alive in small puddles for days if a special effort is not made to eliminate them.

Brooders are stocked at a sex ratio of 1 male:1.5–2 females adding a total weight of fish up to 5,000 kg/ha. Brooders are fed at approximately 1% body weight/d and the pond is not usually fertilized. The fish are allowed to spawn over a 2–4 wk period before the pond is harvested. The timing of the harvest is important to achieve maximum fry yields. Not all females will spawn at the same time but there will be a peak in the spawning activity and a point in time where there is a maximum number of fish of the desired size. If a pond is harvested too soon, part of the reproduction will be eggs or sac fry and are generally lost when the brooders are removed. If the pond is harvested too late, a portion of the fish will have started gonadal differentiation and can not be sex reversed effectively. Green and Teichert-Coddington (1993) developed an equation to time the fry harvest to obtain the maximum number of fry of a sex reversible size. They found that a 195–220 degree-day period was optimum for best production of fry suitable for sex reversal when calculated as follows:

Degree-days to harvest = (Mean daily water temperature – a threshold temperature of 15° C) x number of days to harvest.

Restated from a hatchery perspective the number of days to harvest would range from:

Minimum days to harvest = Minimum degree days/(mean water temp – 15°C)

Maximum days to harvest = Maximum degree days/(mean water temp – 15°C)

For example at 28°C the pond would be harvested between:

Minimum days = 195 degree-days/(28°C–15°C) = 15 days;

Maximum days = 220 degree-days/(28°C–15°C) = 17 days.

Once the appropriate degree days have been reached, fry are harvested by draining the pond into the catch basin early in the day. A screen with a fine mesh and large surface area is placed over the drain to prevent fry from being lost or impinged. Popma and Green (1990) recommend approximately 0.5–0.8 m^2 of screen area to drain a 500 m^2 pond over a 5–10 h period. Brooders are removed from the catch basin by lifting the netting previouslyplaced in the basin. The brooders may be placed directly into another spawning pond or be separated by sex and be held for a few days in a recovery tank.

Fry are captured from the catch basin using fine mesh hand nets (Figure 2). It is important to be organized and efficient during fry collection. Dissolved oxygen concentration in the catch basin often declines rapidly bringing the fry to the surface. Adequate labor should be on hand to catch all the fry and move them into fresh water in a few minutes. Tilapia fry are not as hardy as adults and extra care is needed to insure that healthy fry are harvested. Special care should be taken to prevent excessive turbidity in the catch basin. Fry should not be held in collecting buckets for more that a few minutes before transfer into clean water.

By following such a degree-hour guide, Green and Teichert-Coddington (1993) obtained 1,500–2,500 sex reversible size fry/kg of female brooder stocked with only a minimum of oversized fish. The spawning pond was then prepared again and a new cycle of fry production begun. By making complete harvests and scheduling the timing of the harvest, it is possible to obtain 7.5–10 fry/m^2/d, not counting down time between cycles.

Spawning in Net Enclosures

The use of fine mesh cages or net enclosures (*hapas*) are another alternative for producing fry for sex reversal. *Hapas* have the advantage in that they can be placed in existing bodies of water where other fish species are present and do not require that the pond be drained before the fry can be harvested. The down-time between reproductive cycles is minimum. Complete harvests of spawning *hapas* also allows the collection of eggs or sac fry that may have been lost using techniques discussed earlier.

Spawning *hapas* are typically rectangular in shape, ranging in size from 2 to >500 m^2 and are constructed with 1.6 mm mesh netting (Figure 3). The *hapas* are designed to allow the fish to be crowded to one end for collection. Once crowded together brooders can be removed and females examined for eggs or sac fry and any free swimming fry in the *hapa* can be removed. Brooders are generally stocked at one male: < 2 females at a density of 4–5 fish/m^2 of *hapa* or 0.2–0.6 kg/m^2 of *hapa*.

Figure 2. Fry are captured in the catch basin using a fine mesh hand net.

Figure 3. A fine mesh hapa can be used for tilapia spawning or for holding fry for sex reversal.

Sex reversal is most successful when the initial age and size of fry being treated is controlled. One advantage of *hapas* is that they can conveniently be harvested every 5–10 d to obtain fertilized eggs. By using *hapas*, females can be collected with a minimum of disturbance and each fish can be examined to determine which one is holding eggs. The eggs are rinsed from the mouth and the female returned to the *hapa* to spawn or placed in a conditioning *hapa*. As eggs are found in the mouth, the approximate age can be estimated by their color. Younger eggs are light yellow and older eggs a dark orange or brown. As the eggs are collected those of similar age can be pooled for incubation.

O. mossambicus females hold their eggs and yolk-sac fry tightly in their mouths and rarely release their eggs when disturbed as the *hapa* is crowded together greatly facilitating the collection of eggs. However, *O. niloticus* females not accustomed to frequent handling often release their eggs when disturbed. After a few production cycles, brood females become acclimated to the *hapa* environment and are less likely to release their eggs when crowded during harvest.

Using an incubator system as described by MacIntosh and Little (1995) the sinking eggs of tilapia can be rolled vigorously in a round bottomed incubator with a downward water flow. A high hatch rate can be expected when older eggs are collected and incubated, younger eggs are more difficult to incubate. Fry that are collected right after they swim up and out of the incubator are ideal for sex reversal. They are young and of a uniform size.

Broodstock Replacement in Hapa *Spawning*

Seed production/*hapa* can be improved particularly when the spawning units are harvested frequently and broodstock are replaced each cycle. The advantage of broodstock rotation is that the reproductive cycle of the brood females is more synchronized, permitting a higher percentage of females to spawn during the next cycle. Two or 3 sets of female brooders are maintained, one actively spawning and another 1–2 sets where the females have been separated from the males and are being fed to recover lost energy associated with spawning or from any physical damage. When harvesting every 10 d without broodstock replacement, seed production averaged 106 seed/kg female/d but with female replacement increased seed production to 274 seed/kg/d. (Little et al. 1993). Broodstock replacement can double seed production, but this practice is more labor-intensive and requires additional facilities for broodstock maintenance. A disadvantage of weekly seed collection is that incubation facilities are needed, but short production cycles reduce fouling of nets (if air dried for a couple days between cycles), increase fry production per female brooder, and give uniformly smaller/younger fry. Extended spawning cycles of 21 d for fry production in *hapas* along with a similar period of brood recovery did not improve seed production in *hapas* (Lovshin and Ibrahim 1988).

Where tilapia are spawned in ponds or tanks and harvested every 15 d or more, broodstock replacement each cycle is not necessary but mortalities and females in poor condition should be replaced.

Seed production by any of the above methods is acceptable as long as adequate numbers of proper size fry can be obtained efficiently. Green et al. (1997) provided a summary of seed production that might be expected from various fry production techniques.

Grading

For sex reversal to be effective fish must be of the proper size. Fry collected soon after swimup from an incubator are generally <9 mm and do not have to be graded before being used for sex reversal treatment. Fry that are collected from ponds or *hapas* may be of mixed sizes and should be graded to eliminate fish >14 mm. A grader is a mesh container where fish are added, the small fish are able to swim through the mesh into a receiving *hapa* or tank and larger fish are retained in the grader. Popma and Green (1990) described a grader made of 3.2 mm mesh metal hardware cloth or plastic suitable for separating tilapia fry. They suggested that a grader with a 1 m^2 working area is adequate to grade 50,000 fry. Grader selectivity should be verified to confirm that 85–90% of the 13 mm fish are able to swim through the grader and no more than 5% of the 15 mm fish are able to swim through. If necessary the mesh size of the grader can be reduced by carefully applying paint to the mesh.

SEX REVERSAL OF TILAPIA WITH HORMONE TREATED FEED

Treatment Environment

During sex reversal all fry must receive a daily intake of hormone from the period before gonadal differentiation has begun until it is complete. This requires that the fish be held in a setting where they will receive an adequate quantity of feed containing the hormone. The early investigations into sex reversal using hormone treated feed were conducted in aquaria or troughs receiving clear water (Clemens and Inslee 1968; Guerrero 1975; Tayamen and Shelton 1978). Tanks with flowing water have been successfully to produce commercial quantities of sex reversed fry (Rothbard et al. 1983; Guerrero and Guerrero 1988). Indoor tanks often are not as suitable as outdoor tanks due to greater mortality. Popma (1987) reported average survival of 40% when *O. niloticus* were sex reversed at a density of 3,200 to 4,500/m^3 in indoor tanks having a 5–7%/h exchange rate. Vera Cruz and Mair (1994) obtained >70% survival utilizing outdoor tanks stocked at 5,000/m^3 having a once/d exchange rate. This difference in survival between indoor and outdoor tanks is common on commercial farms as well.

Initial concerns that tilapia must consume no natural food during hormone treatment proved to be unfounded. Buddle (1984) compared the use of indoor tanks with clear water and outdoor tanks and *hapas* in static water ponds as treatment units for tilapia sex reversal. He obtained 96–98% males from those held in *hapas* or treated in indoor or outdoor tanks. Chambers (1984) working with *O. niloticus* obtained 98.5% males and a 95% survival using *hapas* placed in a fertile earthen pond or fertile static water outdoor tanks.

When *hapas* are used to hold fry for sex reversal, they are stocked at densities of 3,000–5,000/m^2 of *hapa* (Popma and Green 1990) or 12 fry/l (MacIntosh and Little 1995). The size of the *hapa* and the number needed should be proportionate to the quantity of fry available on a given day. *Hapas* with a water surface area of 2–5 m^2 and with a water depth of 50–60 cm are convenient for management. The mesh size should be no larger than 1.6 mm but this small mesh will foul during the treatment period. Attention should be given preventing the *hapas* to become fouled to the point where dissolved oxygen becomes low within the *hapa*. To help insure that overall water quality remains high, 100–200 m^2 of pond area should be allowed for 10–15 m^2 of *hapas*.

It has been possible to sex reverse fry stocked free into static or flowing water tanks or earthen ponds. Phelps and Cerezo (1992) stocked *O. niloticus* fry into 20 m^2 outdoor concrete tanks at 150/m^2 and fed a MT treated feed for 28 d and obtained a 98.3% male population which averaged 1.86 g at the end of the treatment period. Stocking fry directly into earthen ponds as also been effective. Phelps et al. (1995) obtained >96% males when *O. niloticus* fry were stocked at 200–260/m^2 into 215 m^2 earthen ponds and fed MT treated feed for 28 d. In a second trial, fish were stocked at only 75/m^2, the percentage of males was 91.3%. Many producers in Colombia successfully sex reverse red tilapia in shallow 15–30 m^2 outdoor tanks, stocking fry at 1000–2000/m^2 and exchanging water at a rate of 4–7x/d. (Popma and Phelps 1998).

Stocking of Fry

Fry are most commonly stocked at densities of 3,000–4,000/m^2 of *hapa*, or flowing water tank. Vera Cruz and Mair (1994) compared stocking densities

of 1,000, 3,000, and 5,000/m^2 of *hapa* using *O. niloticus* and found best sex reversal at 3,000 and 5,000/m^2 but lower survival at 5,000/m^2. High densities help insure an active feeding response needed so all fish are consuming feed. Pandian and Vardaraj (1987) observed that fry can establish a hierarchy in feeding order resulting in small fish not consuming adequate quantities of hormone treated feed for successful sex reversal.

Fry are first graded if necessary, and counted for stocking. An efficient method is to enumerate the fry by visual comparison. As a standard, fry are counted individually into a bucket or pan, adding enough fish that will give a uniform distribution throughout the container. A second bucket or pan of the same color and size is prepared adding water to the same depth and fry are added until the fish density appears the same. Commonly a 15–20 l bucket would be filled with 5–10 cm of water and a standard prepared using 1,000 fry per bucket. When enumerating by visual estimation, care should be given to keeping healthy fish in the standard container and replacing them if they become stressed. If another lot of fish is to be counted that might be of a different size then a new standard should be prepared. It is important to try and avoid having aquatic insects or plant material mixed with the fish being estimated.

Fry can also be enumerated efficiently by weight when a balance capable of weighing 0.1 g is available. A known number of fish are weighed in water and a larger quantity of fish are weighed in water and the number extrapolated. Care should be given to not transfer additional water to the weighing container when the fish are weighed. Sex reversible fry should average 10–30 mg at the start of treatment.

Feed Preparation

A highly palatable feed is needed to obtain an active feed response and effective sex reversal. Commercial fish diets for young fish are suitable. They are generally >40% protein, complete in vitamins and minerals with fish oil added to increase the palatability. Effective diets can be prepared using rice bran or finely ground poultry or hog diet and increasing the percent protein by adding fish meal. The feed ingredients should be reground, mixed and passed through a 0.6 mm mesh screen before use. Vitamins and minerals can be added especially if fry have limited access to natural food, using premixes available for other livestock.

The feed particle size should be the equivalent of a number 00 or 0 crumble of a commercial feed (0.42–0.59 mm) for the first week of feeding. A no. 1 crumble (0.59–0.84 mm) may be given in the second or third week of feeding.

Steroids are not water soluble and are added to the diet by dissolving an appropriate quantity of hormone in alcohol, or fish or vegetable oil to prepare a stock solution. Androgens such as methyltestosterone (MT) dissolve readily in ethanol and a stock solution using 95%–100% pure ethanol can be prepared at a strength of 6 g/l. Ten ml of stock solution added to a carrier and mixed with 1 kg of diet would be adequate to prepare a diet to obtain 60 mg MT/kg of diet.

Lesser strength ethanol or isopropyl alcohol, or vegetable oil may be used as a carrier. However, weaker alcohols will add greater quantities of water to the feed. This additional moisture must be allowed to evaporate off or the feed may become moldy. The prepared feed should not be more than 10% moisture. Excess oil can contribute to rancidity and to hormone loss if oil floats off the feed when fed to fish.

The quantity of carrier is dependent on the type of carrier and the mode of application. When small quantities of feed are prepared, it is convenient to use 200 ml of an alcohol carrier and the appropriate quantity of stock solution per kg of feed. The solution is poured over the feed and thoroughly mixed until all the feed is moist. The hormone can also be applied using a lesser volume of carrier solution by spraying the solution over the feed. Both alcohol and oil have been used as carriers when applied as a spray (McAndrews and Majumdar 1989; Killian and Kohler 1991; Galvez et al. 1995). When small quantities of feed are prepared, the feed is spread into a thin layer, sprayed, mixed and sprayed again. Large quantities of feed can be sprayed in a mixer over a period of time to ensure all the feed is exposed to the solution.

The moist feed is air dried out of direct sunlight or stirred in the mixer until dry then stored under dark, dry conditions. Androgens will breakdown when exposed to sunlight or high temperatures. Both the pure hormone, any stock solution, and treated feed should be stored in the dark at room temperature or less. Varadaraj et al. (1994) compared storage conditions of MT stock solutions and treated feed and the impact on efficacy of sex reversal of *O. mossambicus*. They found that when either the stock solution or feed was exposed to light the efficacy of treatment was significantly reduced. Smith (1996) held a feed containing 60 mg MT/kg in the dark in a

freezer then at various times in a 4°C refrigerator or ambient temperature (28°C ± 1.5) before being fed to *O. niloticus* fry. He obtained populations of >98% male using feed stored under any condition tested including feed held under refrigeration 60 d and an additional 26 d at ambient temperature. When first prepared the feed was analyzed and found to contain 60.4 mg MT/kg and when analyzed after being held in the refrigerator 60 d and an additional 26 d at ambient temperature, the MT concentration was 54.8 mg. When stored under the most harsh conditions the feed which contained 15% fat showed a sight degree of rancidity but that did not appear to effect palatability or the effectiveness of the sex reversal treatment.

Feeding

Young tilapia fry grow rapidly and depending on water temperature, consume 20% or more of their body weight/d at the start of hormone treatment. Such a rapid growth requires the quantity of feed being added should be adjusted daily. This can be done by preparing a feeding chart based on anticipated growth and making weekly corrections of the assumed growth rate. Because of the low average weights involved, it can be difficult to obtain accurate average weights in the field. A practical approach is to extrapolate average weight by measuring length and using a length:weight formula to calculate average weight. A sample of at least 50 fish from each lot to be treated should be measured to the nearest mm. It is best that the fish be densely crowded when collecting the sample. This initial length can be used in the following formula to estimate the average weight:

Weight(g) of 1,000 fry = 0.02 $L(mm)^3$, where L is the mean total length.

Feeding tables can be prepared based on known mean lengths and anticipated increases in length. Anticipated growth is best estimated based on the results from previous sets of fish. A general guide for anticipated growth would be:

Size Range of Fry (mm)	Expected Growth, mm/d
8–12	0.25–0.5
12–17	0.50–0.75
17–25	0.75–1.25

Table 4 is an example of a portion of a feeding table where the length was known on day one at stocking and on day 8 based on a sample measured to the nearest mm. During treatment, the fish should be resampled weekly to determined the mean length and recalculate growth rate. A new growth rate for the upcoming week is estimated based on the growth rate during the last week.

Feeding can be done by hand or by an automatic feeder. The fish should be fed 3 or more times per day for best growth. Bocek et al. (1992) found that effective sex reversal could be obtained when fish

Table 4. Feeding table for 1,000 tilapia fry assuming no mortality; fed at 20% body weight during week 1, and 15% during week 2. Fish are sampled weekly to determine an accurate length.

Week	Day	Daily Growth (mm/d)	Fish Length (mm)	Wt (g) (1,000 fish)	Feed Rate (% body wt/d)	Daily Diet (g/d)
1	1	sample	11.0	26.6	20.0	5.3
1	2	0.3	11.3	28.9	20.0	5.8
1	3	0.3	11.6	31.2	20.0	6.2
1	4	0.3	11.9	33.7	20.0	6.7
1	5	0.3	12.2	36.3	20.0	7.2
1	6	0.3	12.5	39.1	20.0	7.8
1	7	0.3	12.8	41.9	20.0	8.4
2	8	sample	12.9	42.9	15.0	6.4
2	9	0.7	13.6	50.3	15.0	7.5
2	10	0.7	14.3	58.5	15.0	8.8
2	11	0.7	15.0	67.5	15.0	10.1
2	12	0.7	15.7	77.4	15.0	11.6

were fed 2 x/d, 5 d/wk but best growth was obtained when fed daily 4 x/d. When automatic feeders are used, the daily diet should be divided into 4–5 portions so large quantities will be released at each feeding. When small quantities of feed are released uniformly throughout the day, the larger tilapia dominate the area around the feeder and consume most of the feed, resulting in considerable size variation and often poor sex reversal.

Evaluation of Treatment Efficacy

Treatment efficacy should be based on a detailed examination of the gonads of a representative sample of fish. Tilapia can be sexed with reasonable certainty based on the appearance of the genital papilla if they have not been hormone treated. But for hormone treated fish the nature of the gonad does not always correspond with the shape of the papilla. Phelps et al. (1993) followed a MT treatment protocol that gave only a partial sex reversal. They carefully examined the papillae of 270 *O. niloticus* for the opening of an oviduct and based on the appearance of the papilla, 82% were male. Examination of gonads of the fish with "male" papilla revealed that only 60% had gonads that were all testicular tissue, while 29% were intersex, and 11% had normal ovaries. Likewise, 12% of the fish with female papilla had gonads comprised only of testicular tissue.

Sampling error resulting from an inadequate sample size can contribute to a misinterpretation of efficacy. When producing on a commercial scale, a minimum of 300 fish should be collected at the end of the hormone treatment period by crowding the fish together and collecting a random sample. These fish should be grown out to 5 cm or more with a non-hormone treated feed in water free of hormone and preserved in 10% formalin. A representative sample of 100 or more fish should be selected for gonadal examination. The sample must represent the length-frequency distribution. Popma (1987) found for Nile tilapia which were 11–17+ mm at the end of the treatment period, the smaller fish had a greater percentage of females. Hiott (1990) found that when fish were grown to 4–11 cm following MT treatment, females were more common among the larger fish.

For gonadal examination, dissecting equipment is needed along with a microscope slides and a stain. Guerrero and Shelton (1974) describe a gonadal squash technique using an acetocarmine stain. Other effective stains include fast green and hemotoxin. Fish should be preserved a minimum of 10 d in formalin before gonadal examination. The gonadal tissue of fish preserved for less than 10 d remain elastic and often break when being removed. Fish are dissected by making a cut near the anus to below the base of the pectoral fin. The entire gonad, located on the dorsal portion of the peritoneal lining, should be removed carefully beginning ventrally and going forward. For efficient use of supplies, 4–5 sets of gonads are placed on a microscope slide and each given a drop of dye. Another slide is placed on top and the gonads are gently rolled or squashed. When larger fish are examined an obvious ovary with readily apparent eggs may be seen in the body cavity, but on occasions, the gonad may also contain testicular tissue and should be examined microscopically. Thick gonads may need to be sliced longitudinally before they can be examined properly. The entire length of gonad should be examined to see if it contains only one type of gonadal tissue.

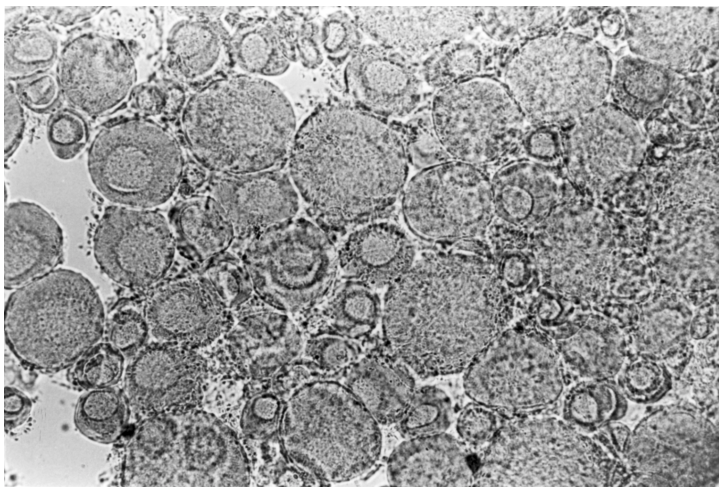
In a gonadal squash of an ovary, eggs of various sizes will be evident throughout the gonad (Figure 4). It should be possible to focus up and down on an egg and see the nucleus as well. Testicular tissue is not as obvious. Lobes of the testis will be apparent but other structures are not as distinct (Figure 5). Connective tissue, oviduct or sperm duct may also appear on the slide.

Gonads may be found that contain both ovarian and testicular tissue (ovotestes). Such intersex fish are found at a low frequency in normal, non-hormone treated tilapia (Clark and Grier 1985; Okoko 1996), most commonly where small portions of ovary are interspersed within the testis. Intersex fish found in hormone treated fish contain a variety of mixtures of ovarian and testicular tissues as to the percentage of the gonad that is of a given tissue type and where such tissues are located (Figure 6).

The reproductive viability of intersex fish is difficult to evaluate. The intersex condition is known only after the fact, when the fish has been sacrificed and the gonad removed. On occasions, bloated hormone treated adults are found with the anterior portion of the gonad putrefied ovary and no oviduct evident. Clark and Grier (1985) reproduced several apparently male *O. aureus* and found that 3 were intersex fish with nonvitellogenic oocytes (25–75 mm) within the testicular tissue.

The minimum acceptable percentage males after sex reversal depends on the culture technique, and acceptable market size. When androgen treatments are effective, the percentage females should be less than 5%. If a small market size is acceptable less than 95% males may be allowable. Lovshin et al. (1990a) found that even in a mixed sex culture

Figure 4. Tilapia ovary as it appears in a gonadal squash with individual eggs having an evident nucleus.



of *O. niloticus*, young 5 g fish stocked at 10,000/ha reached 140 g in 104 d (males = 150 g; females = 126 g) with only a minimum amount of reproduction. The impact of a few females can be more significant if a larger market weight is required. Lovshin et al. (1990b) found that even 2.5% females in a tilapia population depressed growth within 4 mo when no attempt was made to control recruitment. Reproduction was 58% of the weight harvested after 9 mo of culture in ponds when females were 2.5% of the initial stock. Recruitment is most serious after the offspring from the few females begin to spawn, typically 5–7 mo after initial stocking of 20–30 g "sex reversed" fish.

In outdoor ponds, production of fish large enough to yield 5–7 oz fillets requires a 2-phase grow-out or predators being added to control recruitment. Several species of predators including Guapote tigre (*Cichlasoma managuense*), largemouth bass, (*Micropterus salmodies)*, and tucunare (*Cichla ocellaris*) have been used effectively to control tilapia reproduction in "all" male tilapia ponds (Dunseth and Bayne 1978; Green et al. 1997; McGinty 1983; McGinty 1985; Pike 1983). Although tilapia can reproduce in recirculating systems, predators are seldom used to control recruitment. Females can be removed to some extent by grading. Pruginen and Shell (1962) were able to grade *O. niloticus* weigh-

Figure 5. Testicular tissue of tilapia as it appears in a gonadal squash.

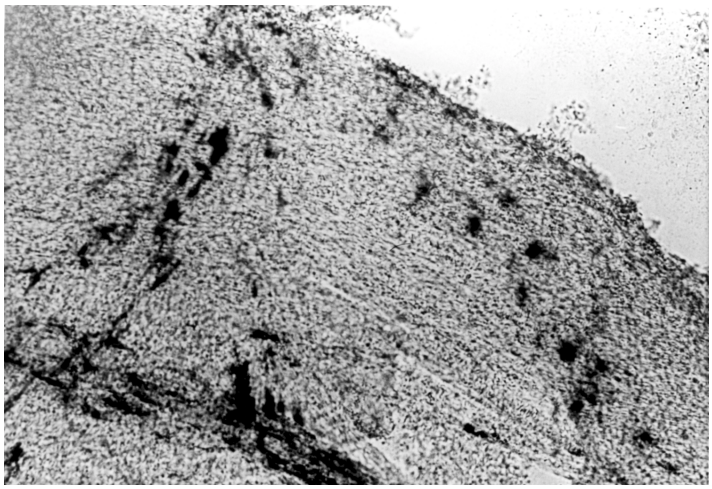
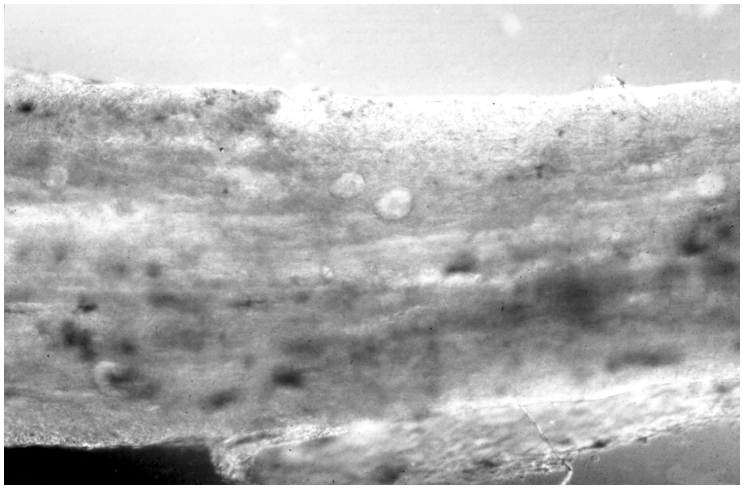


Figure 6. Intersex gonads may have sections of ovarian tissue or isolated eggs interspersed within testicular tissue.



ing 13–17.9 g and separate males with 88+% effectiveness.

FACTORS AFFECTING SEX REVERSAL

Size and Age

The recommendation of Shelton et al.(1978) to begin hormone treatment using 9–11 mm fry is still valid, although larger fish have also been successfully sex reversed. Argue (1992) sex reversed *O. niloticus* beginning with fry less than 19 d old but > 14 mm and obtained 91% males and 4 % intersex. Hiot and Phelps (1993) obtained 87.3% males treating 12–13 mm *O. niloticus* that were <10 d old but only 66.7% males when they treated 12–13 mm fry that were 10 d older. Nakamura and Iwahashi (1982) obtained 98% males when they treated 12.7 mm *O. niloticus*, and 70.2 % males when fry were held 10 additional days and measured 15.9 mm at the start of treatment.

The relationships between growth rate, temperature, gonadal differentiation and successful sex reversal is still not clear but as discussed by Nakamura and Takahashi (1973) hormone treatment must begin before gonadal differentiation begins and continue until it is complete. When gonadal differentiation begins and is completed depends on the production setting. Popma and Green (1990) suggested that after a 25–28 d hormone treatment it may be necessary to grade to remove any fish 13 mm or less which may have not completed gonadal differentiation. Hiot and Phelps (1993) and Argue and Phelps (1996) found that females were more common among the largest of the treated fish.

Fish age and size variation may effect survival. Ellis et al. (1993) compared the use of recently hatched, older fry and a set composed of 50% from each size group. They found after 30 d the smallest fry were more uniform at harvest. The mixed size group not only was more variable at harvest but had 38% lower survival.

The safest approach to obtaining the greatest efficacy of sex reversal and high survival is to begin hormone treatment with the youngest, smallest fry possible, uniform in size, <14 mm.

Treatment Duration

The oral administration of MT treated feed (30 to 60 mg MT/kg feed) to tilapia fry for 21–28 d has yielded populations comprised of no more than 5% females under a variety of protocols (Table 1). The duration of treatment must be adequate to allow all fish to complete gonadal differentiation during the treatment period. Mbarereehe (1992) found that at 18°–22°C, a 40 d treatment period resulted in 95% males but a 20 d treatment gave only 69% males. Bocek et al. (1991) produced 98% males feeding 60 mg MT/kg for 30 d at 21°–23°C. At the end of treatment the fish averaged 14.9 mm. Hiot and Phelps (1993), feeding MT to *O. niloticus* for 28 d at 27°C, found that the effectiveness of hormone sex reversal was correlated to the number of days fry received hormone before reaching 18 mm. Starting with fish

< 11 mm, that grew rapidly during treatment, fish received 14 d of hormone feeding before reaching 18 mm, and were 95.7% males. Starting with 12–13 mm fish under the same growth conditions, fish were larger than 18 mm in 9 d and were only 87.3% males. Owusu-Frimpong and Nijjhar (1981) treated 13.5 mm *O. niloticus* fry with MT for 42 d. These fish had reached only 18–19 mm after 14 d and were successfully sex reversed. Pandian and Varadaraj (1988) treated 10 d old *O. mossambicus* with MT for 11 d obtained 100% males; in another set they treated 13 d old fry for 13 d and obtained 69% males.

Duration of treatment should be related to initial size and growth conditions. As a general rule, fish should receive at least 14 d of hormone treatment before reaching 18 mm. If growth is slower the duration of treatment should be extended until all fish reach this size or a total treatment period of 28 d is exceeded. If growth is too fast, it may be necessary to reduce the quantity or quality of diet to reduce the growth rate.

Environment

Environmental factors such as temperature can impact growth and in turn the treatment duration needed. Temperature alone can also skew sex ratios. Baroiller et al. (1995) skewed the sex ratio of *O. niloticus* to more males by holding fry at 36°C during gonadal differentiation. Desprez and Melard (1998) found similar results with *O. aureus* where the sex ratio was altered to 97.8% male by holding non-hormone treated fry at 34EC. Using *O. mossambica*, Varadaraj et al. (1994) found that the sex ratio of non-treated fry was not altered when reared at 22°, 25°, 27°, 33°, or 38°C but the effectiveness of a 10 mg MT/kg treatment was altered. At 32°C, intersex fish were 35% of the population and at 38°C females were 73% of the population and the remaining 27% were intersex.

Water quality is a consideration when sex reversing tilapia. Most sex reversal is done in freshwater over a range of alkalinities and hardnesses. The literature does not suggest that efficacy is affected by alkalinity or hardness. Several species of tilapia such as *O. mossambicus* and *O. spilurus* can reproduce in brackish or full strength seawater and the fry can be sex reversed under such conditions (Wantanabe et al. 1993; Ridha and Lone 1995). Water quality conditions that will allow good growth and survival of fry are appropriate for sex reversal also. Dissolved oxygen (DO) concentrations should remain above 4 mg/l to insure a strong feeding response. The fry will tolerate lower levels but are stressed and more susceptible to diseases. Fouling of *hapas* and the resultant restricted water exchange can contribute to low DO conditions. Optimum temperature is between 26°–28°C. Temperatures below 24°C significantly reduce growth and may result in some fish not having completed gonadal differentiation during the treatment. Lower temperatures also favor more disease problems.

Dose Rate

The effective dose for sex reversal is dependent on the daily quantity of feed consumed. Varadaraj and Pandian (1989) reported 100% masculization when *O. mossambicus* were fed ad libitum a feed containing 5 mg MT/kg. Where the feeding rate was controlled, Pandian and Varadaja (1987), fed a diet containing 5 mg MT/kg and obtained 60% males and when feeding at 10 % body weight, 80% males at 20%, and 100% males at 30% body weight. They concluded that to obtain a 100% male population a minimum of 1.5 μg MT/g of fish/d needed to be consumed. Rodriguez-Guerrero (1979) feeding 17α-ET to *O. aureus* found that an effective dose corresponded to a mean daily hormone intake over 21 days of 0.46 μg/fish/d. Okoko (1996) fed *O. niloticus* at 15% body weight/d, diets containing 3.75, 7.5, 15, 30, 60, 120, 240, 480, 600, or 1200 mg MT/kg. At 3.75 mg he obtained 80.0% males and 19% intersex, at 7.5 mg the results were 91.7 % males and 8.3% intersex and at 15 mg obtained 98.3% males and 1.75 intersex. He calculated that daily MT intakes of 0.52–2.85 μg/g of fish gave >95% male populations.

Excessive androgen intake can reduce treatment efficacy. Nakamura (1975) obtained 100% males when feeding MT to *O. mossambicus* at 50 mg/kg of diet but at 1000 mg/kg the percent males was 61.4%. Okoko (1996) found that at doses of 240, 480, 600, or 1200 MT/kg of diet at 15% body weight/d resulted in no increase in the percentage of males produced (Table 1).

Exact daily hormone intakes are difficult to accomplish. Knowing the exact weight and number of fry on a given day is difficult and appetite may vary from day to day. In a clear water environment with no natural food available a diet containing 15–30 mg/kg may be effective, while in an outdoor setting with natural food available an optimum dose may be between 30–60 mg/kg of diet given at 15% body weight or more.

Anabolic Considerations

Androgens have both an androgenic and an anabolic effect. The anabolic effect resulting from the use of androgens to sex reverse tilapia is difficult to identify. Several studies that discuss improved growth of sex reversed fish relative to non-treated fish are comparing the growth of near all male populations to that of a mixed sex population after hormone treatment because the presence of females reduces the growth rate due to the slower growth of females or their reproduction (Guerrero 1975; Hanson et al. 1983; Macintosh et al. 1985; McAndrew and Majumdar 1989). Likewise, comparisons of genetically all male tilapia and males obtained by sex reversal, are often complicated by a few females and their reproduction in one or more of the treatments. Hanson et al. (1983) compared growth of a genetically mixed sex population sex reversed to male, a genetically female population sex reversed to male and genetic "males" selected by examination of the genital papilla. Both sets of sex reversed fish (99.4% and 100% male, respectively) grew larger than the hand-sexed fish but the hand sexed "males" contained 6.2% females which may have depressed the mean final weight. Mair et al. (1995) found that genetically male tilapia (99.1% males) and sex reversed "males" (71.6% males) reached similar average weights in 168 d but reproduction represented 10.9 % of the total biomass in ponds with sex reversed males. Tuan et al. (1998) in one trial found no difference in growth of genetically "male" (96.2% male) and sex reversed "male" (99.3% male) populations. In a second trial sex reversed "males" (100% males) grew larger than the genetic "males" but the genetic "male" population was only 82.6% males. Green and Teichert-Coddington (1994) evaluated growth and survival of MT treated and untreated Nile tilapia fry during androgen treatment, nursery and grow-out phases under commercial semi-intensive conditions in earthen ponds. They found no significant difference in growth during any phase of production.

Any improved growth of androgen-treated tilapia is more related to the superior growth of males than the more classical anabolic response related to enhanced protein synthesis and increase inmuscle mass.

Health and Environmental Considerations

Sex reversal of tilapia should consider food safety and environmental issues associated with the use of steroids. It is the obligation of the producer to insure that the public receive the highest quantity fish products possible produced using techniques that have minimal negative effects on the environment. In the US the use of drugs in aquaculture is regulated by the Food and Drug Administration (FDA). As a relatively new industry compared to other forms of livestock husbandry, only a few drugs have been approved by FDA for use in aquaculture. Efforts are now underway to obtain approval for the most commonly used steroid, methyltestosterone. There are numerous scientific studies regarding MT to support the approval process. Other androgens that have been used for sex reversal have a smaller data base and will require considerably more investigation to provide the data needed for the drug approval process.

The short treatment duration and rapid metabolism of MT help insure that tilapia are free of MT before fish reach the consumer. Using growth data by Dambo and Rana (1992), fish reaching 0.5 g at the end of the treatment period consume 29.9–33.2 μg MT during the treatment period. Digested MT is rapidly metabolized and excreted. Curtis et al. (1991) fed tilapia fry for 30 d a feed containing radioactively-labeled MT. Ten d after the 21 d treatment only a trace of MT could be found. The head and viscera were found to contain >90% of the radio-labeled MT, and after 21 days post-treatment <1% remained (Goudie et al. 1986). Johnstone et al. (1983) found >95% of the radio-labeled MT in the viscera and no radioactivity could be found 50 h post-treatment. This rapid metabolism and excretion of MT by a fish treated early in its life history, combined with the extended period needed to produce a marketable size fish results in a safe consumer product.

Detailed studies on the environmental fate of androgens are not available but under certain conditions may produce secondary effects. MT is susceptible to breakdown when exposed to light or high temperatures (AHFS 97 Drug Information, 1997). Both fungi and bacteria can metabolize exogenous steroids. Many different steroid metabolic reactions, including metabolism of MT, are possible in bacteria (Schubert et al. 1972; Jankov 1977), as well as metabolism of steroids to CO_2 and H_2O (Sandor and Mehdi 1979). In an outdoor pond where fry are treated in *hapas* the combination of light, temperature and microbial degradation should result in a rapid break down of MT. In a pilot study without fish, a single 40 mg dose of testosterone was added to one tank of the two tank recirculating system. (Budworth and Senger 1993). Dilution within the recirculating system would result in the initial test-

osterone concentration (17.4 μg/l) being decreased to 10.6 μg/l. It was found that the concentration in water, measured by double-antibody radioimmunoassay, peaked in both tanks about 2 h after application, and then decreased exponentially to near 0 ng/ml testosterone 18 h after application.

In an outdoor tank, Phelps et al. (1992) used small *hapas* spaced approximately 30 cm apart in a static water 20 m^2 tank to hold fish given a MT or fluoxymesterone treated feed or a non-treated feed. The treatments were randomly assigned within the tank and there was no evidence of hormone leaching affecting the sex ratio of non-treated fish. Soto (1992) used a similar physical setup with eighteen 0.12 m^2 *hapas* distributed in a 20 m^2 tank to evaluate the androgenic potential of mestanolone to sex reverse *O. niloticus*. She found no evidence of non-hormone treated fish having a skewed sex ratio even though such fish were surrounded by sets of fish being fed a hormone treated feed. Abucay et al. (1997) found that reusing water that had held tilapia fry during a 25 d MT treatment could alter sex ratios. When a second group of fish were stocked into such water and given a non-hormone treated feed, the sex ratio was skewed. They also found that when "all" female fry were stocked into a cage in an aquarium and MT treated feed added to the bottom of the aquarium where the fish had no access to it, the sex ratio became skewed to males.

In recirculating systems where MT has been given daily, MT may remain in the water column long enough to influence sex ratios. All female gynogenetic, 27–40 d, common carp (*Cyprinus carpio*) were held in a recirculating system (Gomelsky et al. 1994). Four separate experiments were conducted where fish in one tank were fed an androgen treated diet, fish in another tank within the same system were fed an androgen free diet. They found that direct or indirect androgen treatment, either through oral administration (MT fed group) or water exposure (water exposed group), skewed sex ratios.

It is clear that unmetabolized MT and metabolites of MT can accumulate in water of recirculating systems or perhaps static water not exposed to direct sunlight. The degree of accumulation appears to depend on the frequency and dose of MT administered to the target fish. Effects of the excreted metabolites and unmetabolized MT on non-target fish held in the same system could range from elevated serum MT levels to altered sex ratios. However, microbial degradation of androgen in biofilters of recirculating systems appears to occur quickly when androgen is applied in low doses or infrequently. In an outdoor setting the degradation may be more rapid and the effect on non-target organisms less.

CONCLUSIONS

Production of male tilapia through the use of androgens is very effective. It does not require that a portion of the production be discarded as in manual selection, or that 2 separate stocks of fish be maintained as in hybridization. There are several seed production techniques adaptable to most scales of production. The relative ease and predictability of tilapia sex reversal has been a major factor in the rapid growth of the industry.

Although a variety of hormones have been used for sex reversal, methyltestosterone is the most commonly used androgen. Dose rate and treatment durations vary depending on the environment and the experience of the producer. Tilapia fry <14 mm should be treated for at least 14 d before reaching 18 mm and, if growth is slower the duration of treatment should be extended until all fish reach this size or a total treatment period of 28 d is exceeded. A dose rate of 30–60 mg of MT/kg of diet fed at an initial rate of 20% body weight/d should result in successful treatment. The efficacy of treatment should be based on gonadal examinations.

As aquaculture continues to supply an increasing portion of the world's fisheries products, tilapia culture will play a more important role. Sex reversal will remain the industry standard for reproduction control in tilapia.

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