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Sex Reversal of Tilapia in Earthen Ponds

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Aquacultural Production Manual

Sex Reversal of Tilapia in Earthen Ponds

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PREFACE

This Aquacultural Production Manual from the International Center for Aquaculture at Auburn University is oriented towards field application rather than rigorous scientific evaluation of the described techniques. The management practices have been field tested and proven feasible at the described scale of production.

The intended readers are intermediate to upper level technicians with experience in related aquacultural activities. As such, a basic understanding of the principles of aquatic production and of the life history and environmental requirements of tilapia is presumed.

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Sex Reversal of Tilapia in Earthen Ponds

Thomas J. Popma and Bartholomew W. Green[1]

INTRODUCTION

ON COMMERCIAL fish farms female tilapia are usually excluded from growout ponds to prevent overcrowding and stunting resulting from unwanted reproduction. Females can be eliminated manually by visual inspection of the urogenital papilla of juvenile fish, but this technique is labor-intensive. The production of all-male hybrids is also technically feasible, but isolation facilities to maintain the purity of both parental lines are essential, and seed output is often reduced due to partial reproductive incompatibility between two different species. A more recent technique for producing male fingerlings is "sex reversal" or "sex inversion." This can be achieved by offering feed treated with a male hormone to tilapia fry before the primal gonadal cells of females have differentiated into ovarian tissue. The dietary hormones can be suspended once the testes are sufficiently developed to maintain normal levels of endogenous hormone.

Until the early 1980's, sex reversal of tilapia was conducted in clear water to reduce possible interference from natural foods. The water source was generally a well or dechlorinated and oxygenated municipal water. Treatment was usually conducted in shaded containers to retard the development of phytoplankton. The quantity of feed administered required that the containers be cleaned daily and that water quality be maintained by frequent water exchange and aeration. Under these conditions, heavy losses from disease and parasites were common as the technology shifted from experimental to commercial levels. The presumed requirement of clear water did not render sex reversal technically or economically unfeasible for efficiently run operations, but it greatly limited application in small and medium scale fish farms, especially in developing countries.

Since 1984, investigators and producers in several countries, including the United States, Israel, Thailand, Philippines, Jamaica, Ecuador, Panama, and Honduras, have demonstrated that tilapia can be effectively sex reversed on a commercial scale in hapas (fine mesh net enclosures) held in ponds with abundant natural plankton. The fish grew faster, the incidence of disease was reduced, the basal diet did not have to be nutritionally complete, and the infrastructure requirements were reduced. The procedure, as described in this publication, is relatively simple and can be practiced by producers without wells, pumps, or indoor wet lab facilities.

The techniques and results described in this publication are based on experiences by the authors since 1986 at the Escuela Superior Politecnica del Litoral (ESPOL) in Guayaquil, Ecuador, and at the El Carao Aquacultural Experiment Station in Comayagua, Honduras, with Nile tilapia, *Oreochromis (Tilapia) niloticus*. The size of the production units described are the same as those used for field operations. Extrapolation to somewhat smaller or larger units is probably valid, but upscaling would usually be accomplished by increasing the number of production units rather than the size of the unit itself.

This publication is organized to facilitate field application of the hormonal sex reversal technique for production of all-male fingerlings of tilapia. Detailed discussions on the justification and acceptable modifications or alternatives are minimized in the main body of the text. A final section, entitled "Common Questions on the Procedure," is included to assist managers in decisions on the general appropriateness of sex reversal, on site specific modifications, and on overall farm management.

PRODUCTION OF FRY FOR SEX REVERSAL

Concept

The technique to produce fry for subsequent sex reversal is based on the following major considerations. (1) Sex reversal of tilapia must begin before the gonadal tissue of young genetic females has differentiated into ovaries. (2) Depending on water temperature, large numbers of free-swimming fry can first be found in warmwater ponds about 2 to 3 weeks after stocking healthy brood fish, but soon afterwards the number decreases as small fingerlings become cannibalistic on the recently hatched fry. (3) Fry production must be coordinated with the sex reversal operation because the hormone treatment should be begun immediately after fry harvest and the facilities will not be available to receive the following batch of fry for another 25 to 30 days. (4) For efficient farm management, it is generally preferable to sex reverse infrequent batches of large numbers of fry rather than more frequent batches of fewer fry.

Based on the above considerations, the suggested procedure for fry production in ponds involves a 25- to 30-day cycle (including a 2- to 10-day turnaround period) with a single, complete harvest of fry not exceeding 14 mm total length. For most efficient use of labor and facilities, fry production may be accomplished in a single pond. However, additional ponds may be required depending on the number and size of ponds available and the volume and frequency of demand for sex-reversed fingerlings.

Input Requirements

(1) Earthen pond with harvest basin: The results described were obtained from 500- to 2,000-m^2 ponds, but extrapolation to smaller and somewhat larger ponds is presumed valid. An earthen harvest basin should be 30 to 40 cm deep with a surface area of about 10 m^2 or 1% of the pond area, whichever is larger. Concrete harvest basins may be somewhat smaller and also make fry collection more convenient and maintenance less labor intensive. Complete harvest of fry is not recommended in a pond without a harvest basin.

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(2) Water source with salinity less than 10 parts per thousand (ppt).

(3) Nylon netting (13- to 20-mm or ½- to ¾-inch square mesh) with dimensions that exceed those of the harvest basin by 20%.

(4) Fine mesh (1.6-mm or ⅟₁₆-inch) hand-held scoop net.

(5) Fine mesh filter screens for pond draining.

(6) Hapa or cage for temporary holding of brood fish between cycles (3- to 4-m^3 capacity per 100 kg of brood fish).

(7) Brood fish (70 to 100 kg for production of 50,000 target-size fry per cycle), sex ratio of 1.5 to 2 females per male.

(8) Supplemental feed (40-50 kg of feed per 100 kg of brood fish per cycle).

(9) Fish grader, as described in Step 10 of Procedure.

(10) Labor requirements per cycle in a 1,000-m^2 reproduction pond stocked with 100 to 150 kg of brood fish: 15 person-hours for pond preparation, stocking of brood fish, and feeding, plus 15 person-hours, divided among 3 to 4 persons, for pond draining, harvesting and grading of fry (For 500-m^2 reproduction ponds, the labor requirements decrease by 30%; for 2,000-m^2 ponds the labor needs increase by 50%).

Additional details are provided in the Procedure section.

Procedure

The procedure to produce fry for subsequent sex reversal is described in 10 steps:

(1) VERIFY WATER SUPPLY CHARACTERISTICS

Chemical composition is adequate for fish growth and average water temperature in ponds is at least 22°C, but not more than 32°C. The salinity of water must be less than 10 ppt and, ensure the quantity of water is sufficient to drain and refill the pond each cycle.

(2) ELIMINATE ABSOLUTELY ALL FISH IN POND BEFORE FILLING

This can be accomplished by complete drying or by disinfecting puddles with rotenone or swimming pool type granular chlorine (generally calcium hypochlorite) dissolved in water. In either case, apply 10 to 20 g per m^3 of puddled water. (This dosage is based on the assumption that the rotenone is 5% active ingredient and the commercial granular chlorine is approximately 60% active.) The following day verify the complete absence of fish because some small puddles may have been missed. Repeat the treatment if live fish are found.

Another effective technique which is less labor intensive but more costly, is to disinfect with rotenone after filling the pond with just enough water to cover the pond bottom. If average water depth is 10-20 cm, approximately 750 ml or g of commercial rotenone will be needed in a 1,000-m^2 pond. Ponds should be drained to verify the complete absence of fish before restocking. To prevent downstream fish kills after draining, either wait 1 or 2 weeks for natural degradation of the rotenone or apply 2 to 3 g of potassium permanganate ($KMnO_4$) per ml of 5% rotenone for immediate neutralization of the rotenone.

Quicklime (or burnt or builder's lime) has given less consistent results, especially in ponds with organic-rich bottom muds. Approximately 100 kg per 1,000 m^2 surface area of pond bottom should be applied if the more effective fish irradicants are not available.

(3) DRAPE THE LARGE MESH NETTING IN THE HARVEST BASIN, FIGURE 1

Secure at the borders so that few brood fish will swim under the netting.

(4) FILL THE POND WITH WATER SO THAT WATER DEPTH IN MOST OF THE POND IS 50 TO 80 CM

If necessary, filter water to prevent entry of wild fish. In some regions, manuring may be required to control excessive clay turbidity, but fertilization to stimulate plankton growth is not normally recommended. Plankton production in a previously fed pond is usually adequate to maintain nutritional health of the fry.

(5) STOCK ALL BROOD FISH ON SAME DAY, SOON AFTER POND HAS BEEN FILLED

Fry production is more a function of brood biomass than of pond size. To produce 50,000 acceptable size fry for sex reversal, stock 35 to 55 kg of mature females and sufficient males so the sex ratio is 1 male per 1.5 to 2 females. The pond area required to safely and efficiently accommodate this biomass is 500 to 1,000 m^2.

(6) MANAGE POND DURING SPAWNING/PRIMARY NURSERY PERIOD

A supplemental feed is recommended, but do not overfeed. Most commercial fish rations at a daily rate of 1% of fish biomass will maintain brood fish in good spawning condition. A slightly higher daily feed rate (2-3% of fish weight) is recommended only when feeds are of lower quality or the brood fish are undersize. Low dissolved oxygen is seldom a serious problem. Predators, especially birds, may have to be controlled in some regions. Frog and toad egg masses should be removed from the pond because sorting tadpoles from fry at harvest may produce high fry mortality.

(7) DRAIN THE POND 14 TO 23 DAYS AFTER STOCKING BROOD FISH

The primary factor determining the appropriate harvest date is water temperature. A suggested guide is:

Average water temperature	Cycle duration
less than 25°C	20 to 23 days

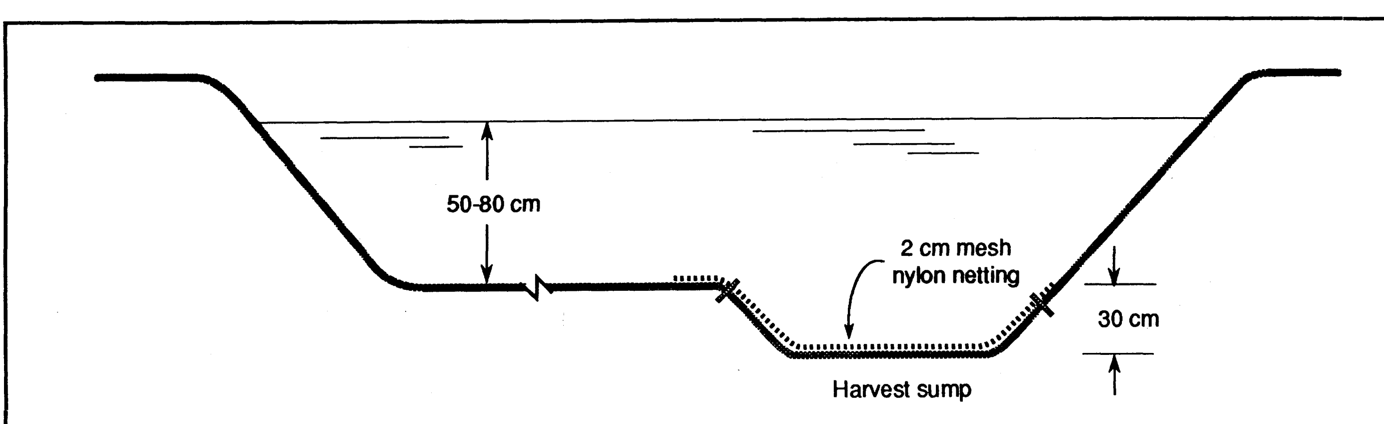


FIG. 1. In fry production ponds the placement of net over harvest sump bottom is critical.

25 to 28°C	17 to 21 days
more than 28°C	14 to 18 days

In planning the harvest of fry, consider the following factors:

The pond should be drained slowly to prevent excessive loss of fry when stranded on the pond bottom.

Fry should be harvested in the early morning before increasing water temperature endangers their survival.

Dissolved oxygen levels should be at least 4 mg/l.

During drainage, the water must be filtered through a fine mesh screen (such as window screen) to reduce fry losses. When the surface area of the filter screen is increased, the velocity of the drain water and the number of fry being impaled on the screen are reduced, figure 2.

As a general "rule of thumb," the following maximum mesh size, minimum area of the filter screen and total drainage time are recommended:

	Pond size	
	500 m^2	2,000 m^2
Maximum mesh size (mm)	1.6	1.6
Area of filter screen (m^2)	0.5 to 0.8	1.0 to 1.5
Drainage time (hours)	5 to 10	10 to 15

(8) AFTER POND HAS DRAINED, LEAVING WATER ONLY IN THE HARVEST BASIN, REMOVE BROOD FISH BY LIFTING COARSE MESH NETTING ON THE BOTTOM OF THE HARVEST BASIN

These same fish may be used as brooders in the following production cycle, which may begin in 3 to 10 days depending on the anticipated availability date for the sex-reversal facilities. Temporary holding for easy recapture and restocking is therefore desirable for farms that anticipate a continuous program of fry production. If a nutritionally balanced diet containing mineral and vitamin premixes and some protein of animal origin is available, the fish may be temporarily kept at low densities in holding cages (about 30 kg/ m^3), and fed a high quality feed at a rate of 2 to 3% daily of body weight.

It may be more productive to keep the sexes separated during this turnaround period, but the results described in this publication were obtained by maintaining all brood fish in a single cage suspended off the bottom of the pond or in concrete tanks. If males and females are held together in concrete tanks or in structures which allow the females to spawn and pick up the released eggs, it is appropriate to examine the females' mouths and remove all eggs before restocking the spawning pond.

If a high quality supplemental feed is not available, brood fish that must be maintained for more than a week between cycles should be released in an enriched pond.

(9) COLLECT FRY FROM HARVEST BASIN SOON AFTER BROOD FISH HAVE BEEN REMOVED

Water exchange in the harvest basin is usually not required as the oxygen demand is greatly reduced once the adult fish have been harvested. On the contrary, the turbidity that often accompanies water exchange may weaken the fry and make collection more difficult.

Most fry in the harvest basin will soon come to the surface if not disturbed. They may then be harvested by skimming with a 40- to 100-cm-wide scoop net made of materials such as 1.6-mm mesh nylon netting or fiber glass window screen.

Fry are delicate at this size, so special care is required to reduce stress from turbidity and handling.

Collect first around the edges of the harvest basin; this is especially appropriate for larger basins that are not concrete lined.

Take special care to keep the scoop nets off the bottom muds when collecting fry.

Transfer the fry quickly to fresh water; never keep them out of water more than about 20 to 30 seconds.

Wash the fry off the scoop nets from the back side of the netting into a holding bucket rather than shaking or pushing them off the net.

Never keep the fry more than 10 minutes in the holding bucket before transferring them into clear, well-oxygenated water.

Fry collection from the basin is a relatively fast operation. Two persons can harvest 50,000 to 100,000 fry in less than an hour.

In well-drained ponds, the collection of fry that were stranded in puddles is not recommended since the labor requirements are high and the number and subsequent survival of these fish are low.

Large numbers of air-breathing insects are often collected along with the fry. When the entire harvest is placed in still water for a few minutes, the two groups often stratify vertically, with the fry remaining deep and the insects near the surface. Most of these insects can then be removed by skimming. Alternatively, an oil:diesel fuel mixture (1:10) can be applied at a rate of 2 gallons per 1,000 m^2 prior to harvest, but this practice is not recommended because of equipment fouling and possible deleterious effects on the fry.

(10) IMMEDIATELY GRADE HARVESTED FRY TO REMOVE THOSE INDIVIDUALS LARGER THAN 14 MM

Graders with the required characteristics are not commercially available but are easily constructed. The following considerations

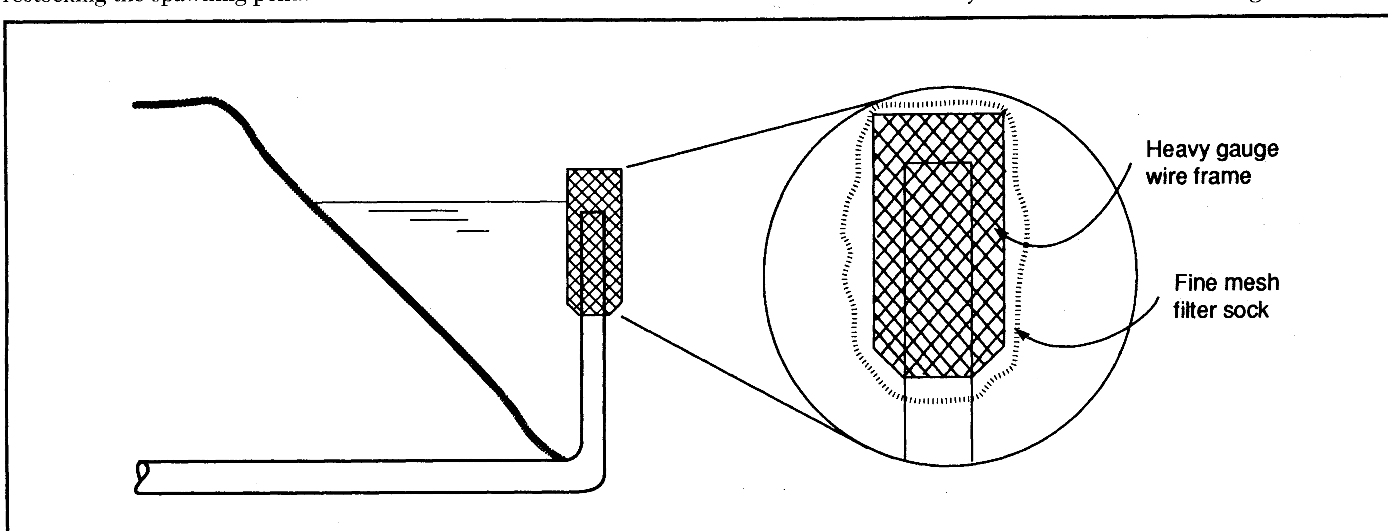


FIG. 2. The effective filter area for standpipe type drains is increased by a rigid oversize wire bonnet that fits over the standpipe; the fine mesh screen material then fits over the bonnet.

are suggested:

An in-pond technique for temporary storage and continuous grading of several batches of fry is a floating grader placed within a fine mesh hapa (1.6-mm or less). The volume of the net enclosure should be at least 20 times greater than the interior volume of the floating grader to stimulate movement of fry from the grader into the net enclosure.

3.2-mm (1/8-inch) metallic screen or "vexar" is acceptable material for grading. "Target size" fry pass through the screen and are acceptable for sex reversal; "oversize" fry are retained in the grader and subsequently rejected, figure 3.

The selectivity of a new grader should be tested because the gauge of the screen material and the coating process influence the size of the openings. The mesh selected should be small enough to retain all the 16-mm fry and most of the 15-mm fry, but large enough for most 13-mm fry to pass. If more than 10 to 15% of the 13-mm fry are retained, a larger mesh screen should be tested. If more than 5 to 10% of the 15-mm fry pass through the grader, a uniform coat of paint may be sprayed on the screen to reduce the size of the mesh.

A grader with 1 m^2 of effective surface area is sufficient to grade at least 50,000 fry. Smaller graders also can be used, but the capacity is proportionally reduced.

The size of a floating grader can be decreased and its efficiency increased by bending the screen in the bottom of the grader to form a corrugated grading surface. The corrugation orients the mesh more perpendicular to the normal swimming position of the fry, figure 4.

The length and width of the grader depend on the degree of corrugation and the required capacity of the grader. To process 100,000 fry over a 30-minute period, the floating grader need be no larger than 90 cm long by 70 cm wide.

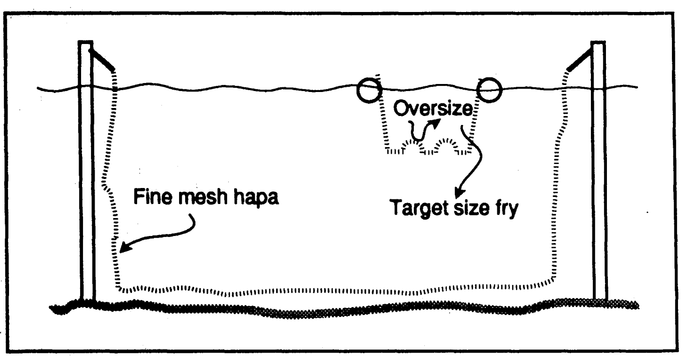


FIG. 3. Grader used to reject over-sized fry.

Fry are transferred either directly from the harvest basin or from a holding tank to the floating grader. After about 15 minutes the grader is raised and lowered several times to excite the remaining fry to escape through the mesh. The objective is to crowd the fry without lifting them completely from the water. The grader is then removed. Fry remaining in the hapa are of an acceptable size for sex reversal. Oversize fry retained in the grader are not acceptable for sex reversal, but some can be reared for brood stock replacement.

Expected Results

Under the management conditions described, figure 5, average results per spawning cycle from 50 kg of female brood fish (plus a male for every 1.5 to 2.0 females) in 500 to 1,000 m^2 of pond space are:

Total number of fry harvested:	60,000 to 80,000
Target size fry (14 mm or less)	
Percent of total:	60 to 100 %
Total number:	40,000 to 60,000
Harvest/handling mortality:	5 to 15 %

The most common causes of failure to achieve expected results from an individual production cycle include the following:

(1) Inappropriate harvest dates. Spawning and egg/larval development is temperature related. See Step 7 of Procedures for general recommendations. If the pond is harvested too late, the percentage of oversize fry and cannibalism are greatly increased. If the pond is drained too early, an unacceptably low percentage of fry will have developed to the free-swimming stage.

(2) Failure to adequately disinfect the pond or prevent entry of wild fish. Abundant fry in the pond at the time brood fish are stocked may result in a 90% reduction in the harvest of target size fry.

(3) Draining mishaps, such as broken filter screen, prolonged drainage time as a result of clogged filter screens, accidental complete drainage of harvest sump before fry collection, etc.

(4) Diseases, parasites, predators, and poor water quality are infrequent causes of depressed harvests under the management scheme suggested.

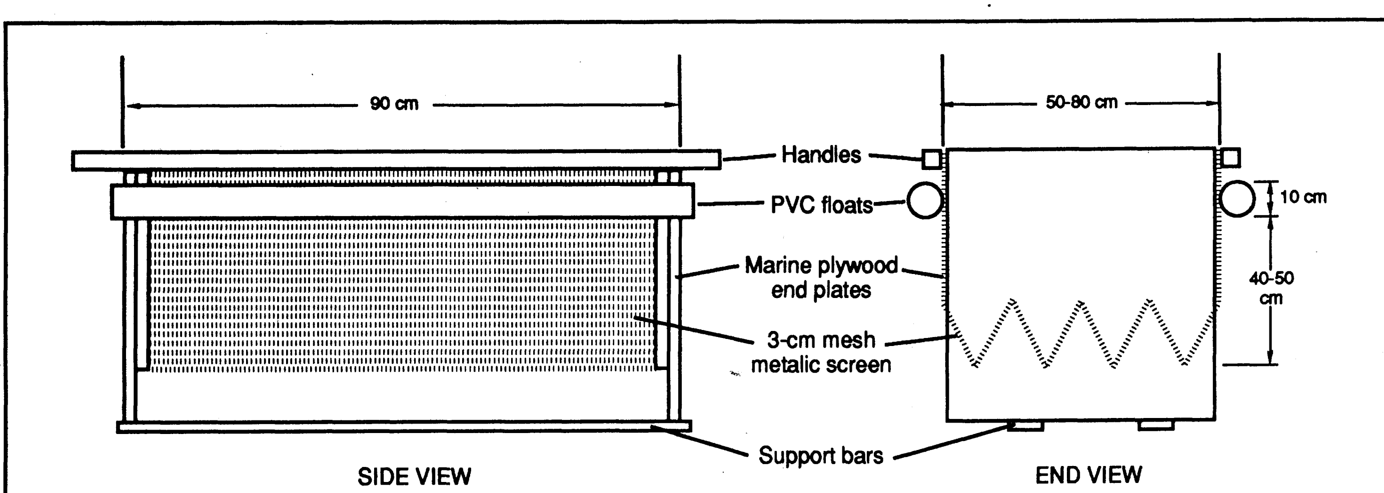
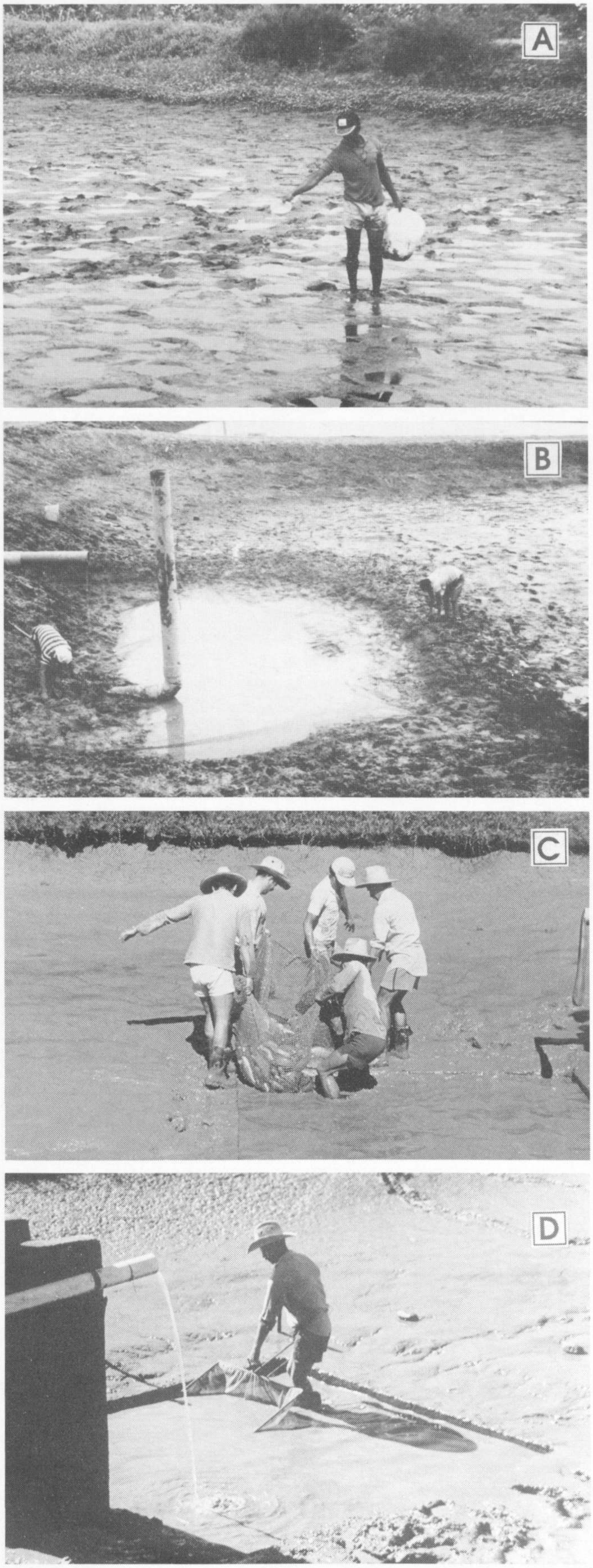


FIG. 4. Floating grader used to separate fry.



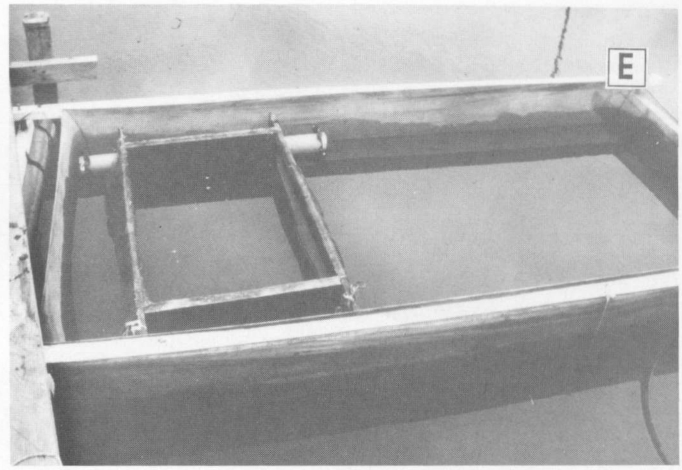


FIG. 5. To prevent cannibalism on recently hatched fry, all fish must be eliminated from reproduction ponds by thorough drying or disinfection (A). A large mesh netting is draped in an earthen or concrete harvest basin (B), and the pond is then filled with water and brood fish are stocked. Then 2-3 weeks later the pond is slowly drained to the harvest basin and the netting is lifted to remove the brood fish (C). Fry are skimmed from the water surface with a scoop net (D). If fry too large for sex reversal are present, the harvest is placed in a 3-mm mesh grader that is floated in a fine mesh hapa to receive the target size fry small enough to swim through the mesh of the grader (E). If 50 kg of female brood fish are stocked in a 500-1,000 m² pond, production of target size fry is approximately 50,000 per cycle.

PREPARATION AND ADMINISTRATION OF HORMONE TREATED FEED

Concept

Once the harvested fry are graded to remove the oversize individuals, they are transferred to hapas where they will be given a hormone-treated feed until the gonadal tissue has differentiated into testes. The hormone treatment can then be suspended because the testicular tissue produces sufficient natural hormone to continue development into functional male fish. The precise moment when the treatment can be suspended is incompletely understood, but at temperatures of 24 to 29°C this normally occurs after 3 to 4 weeks when all fry are at least 14 mm total length.

During hormone treatment, the density of the fry should be high to optimize the use of the hapas and to reduce the amount of natural food available to each fish, thereby increasing the probability that all fry will consume the hormone treated feed. After the treatment is completed no additional dietary hormone is required.

Methyltestosterone, the hormone most often used for sex reversal, is insoluble in water but readily dissolves in 80 to 90% ethyl alcohol. To "fix" the hormone into the feed, an alcohol-hormone solution is mixed into the feed, and the alcohol is subsequently allowed to evaporate.

Input Requirements

(1) Earthen pond that has 100 to 200 m^2 of pond area per batch of 50,000 fry, with minimum water depth of 1 m.

(2) Nylon hapas (1.6 mm or 1/16-inch mesh) that have total surface area of 10 to 15 m^2 per 50,000 fry, with individual hapas 1 to 5 m^2 and depth of 60 to 90 cm; asphalt-treated netting is more resistant to degradation by sunlight and has an expected life of 2 to 3 years with near continuous use.

(3) Hormone impregnated feed is required. (Details on the preparation are provided in Step 1 of Procedure.)

(4) A balance with 2- to 5-g increments and 10-kg capacity.

(5) Labor requirements are 50 to 70 person-hours per batch of

50,000 fry, including feed preparation, fry handling, feeding, and harvest.

Procedure

The sex reversal operation is divided into seven major activities:

(1) PREPARE THE HORMONE IMPREGNATED FEED

Selection of nutritional ingredients

The hormone-treated feed should be of good nutritional quality and highly palatable to ensure ingestion of the required amount of hormone. Crude protein levels of 25 to 45%, at least half of which is of animal origin, and vitamin and mineral supplements are recommended, but 20% protein feeds have been used successfully. A commercial starter ration for fish is ideal, but a finely ground, balanced shrimp or fish feed is acceptable.

A base diet also can be prepared by mixing a finely ground commercial chicken or swine ration starter with fish meal. The chicken or swine starters generally contain mineral and vitamin premixes of adequate quality, and the fish meal increases the protein content and the palatability of the diet.

The dry ingredients should be sieved to remove particles that are too large to be ingested by the fry. A 0.6-mm mesh screen may be most appropriate, but no obvious problems were observed with sieves made of commercial window screen.

Alcohol-hormone solution

A sufficient quantity of the alcohol-hormone solution must be added to the dry ingredients to ensure an even distribution of the hormone. Normally, about 0.5 liter of solution is mixed in 1 kg of diet. However, the small amount of hormone needed (60 mg/kg of diet) can be dissolved in a much smaller volume of alcohol. It is therefore practical to prepare a concentrated "stock" solution of the hormone that will subsequently be diluted to a greater volume before mixing with the dry ingredients. The alcohol in the stock solution should be 90-95% ethyl alcohol, but need not be reagent or USP grade; glycerin may be added at 0.5% by volume to render the alcohol unfit for human consumption. The alcohol into which the stock solution is mixed may be 80-95% ethyl or isopropyl alcohol.

> Stock solution of hormone: Dissolve exactly 6.00 g of methyltestosterone in exactly 1.00 liter of 90-95% ethyl alcohol. (This quantity is sufficient to treat approximately 300,000 fry.)

The stock solution should be stored in a dark bottle. It can be kept at room temperature but preferably under refrigeration. Shelf life is at least 3 months.

List of ingredients per kg of diet

Alcohol-hormone stock solution exactly 10 ml
Ethyl or isopropyl alcohol about 500 ml
Dry ingredients 1,000 g

Procedure for mixing ingredients

(a) Prepare the ground and sieved dry ingredients.
(b) Mix the hormone-alcohol "stock" solution with the alcohol.
(c) Add the above solution slowly and mix with the dry ingredients.
(d) Allow the alcohol to evaporate in an oven at 60°C or at room temperature with no direct sunlight by spreading out the mixture to a maximum thickness of 3 to 5 cm. Mix lightly by hand 2 or 3 times.
(e) If the mixture has been completely dried in an oven, it should be sealed in airtight containers and/or stored in a refrigerator to retard bacterial or fungal contamination. If dried at room temperature, the shelf life of the diet may be extended by sealing in plastic bags when it feels dry to the touch, but before all the odor of alcohol has disappeared—usually 6 to 12 hours. Refrigerate or freeze for a shelf life of at least 2 months. The diet can be kept at least 1 week at room temperature.

Quantity of treated feed needed

Sex reversal usually requires 250 to 400 g of treated feed per 1,000 fry. All the feed needed for the entire treatment is usually prepared before treatment begins.

(2) PREPARE THE POND FOR SEX-REVERSAL HAPAS

Verify pond and water characteristics

Chemical composition of water supply should be similar to that indicated for the fry production pond.

Cooler water temperatures (20 or 23°C) reduce fry growth but do not negatively affect sex reversal.

A harvest sump is not required, but the pond should be drained at least once per year to eliminate escaped and wild fish. (Free-swimming fish weaken or even make holes in the net enclosures while attempting to get at the feed or the fry.)

Fill pond if necessary

The convenience of chemical fertilization or manuring is site dependent. (Increased abundance of natural food will increase fish growth, thus increase feed requirements. However, manuring may be needed to reduce inorganic turbidity, and fertilizer induced plankton blooms may improve the dissolved oxygen dynamics.)

Chemical treatment for control of aquatic invertebrates, as commonly used in primary nursery ponds, is usually not necessary.

(3) SET UP HAPAS

Number and size (See "Input requirements")

Location

Easy access to hapas is important because feed must be given at least twice daily. The hapas should be at least 30 cm above the pond bottom to permit water circulation and prevent turbidity. For these reasons, net enclosures are often tied to wooden walkways or piers that extend a few meters from the shore.

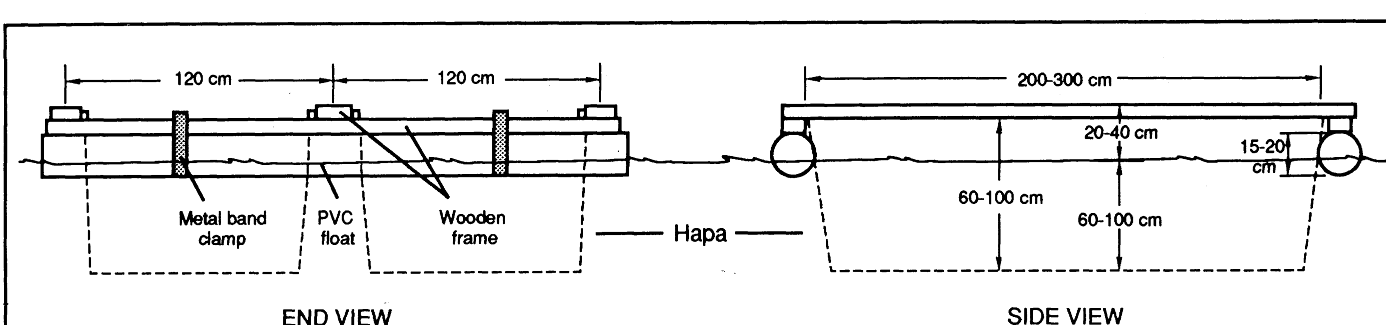


FIG. 6. Where pond water levels fluctuate, hapas can be secured to a floating frame.

Securing the enclosures

The hapas should extend at least 20 cm above the water surface to prevent the fry from escaping. If the water level in the pond is constant, the hapas can be tied to stakes driven into the pond bottom. However, if water level is likely to fluctuate, it may be advisable to secure the hapas to floating frames, thus ensuring a constant distance from water level to top of netting, figure 6.

Covering the enclosures

The hapas generally need not be covered, but in some regions protection from predatory birds may be appropriate. If a covering is required, it should not completely shade the sunlight, which may be an important factor in disease prevention.

Accessory equipment

Floating feeding rings made of 3-cm-diameter flexible tubing should be placed in the net enclosures to reduce the loss of treated feed, especially on windy days. A circular ring with a diameter of 50 to 80 cm is adequate for 10,000 to 20,000 fry in a 3- to 5-m^2 hapa.

Submerged feeding platforms are sometimes placed about 40 to 50 cm below the feeding rings to further retard feed loss, but their importance is incompletely understood. A plastic sheet should be preferred over a rigid platform which causes excessive wear of the netting. Fish have been successfully produced in hapas with no submerged feeding platform.

(4) STOCK THE HAPAS WITH GRADED FRY

Stocking rate

Suggested stocking density in net enclosures is 3,000 to 5,000 graded fry per m^2 of net enclosure. Fry have been successfully sex reversed at lower densities, but the enclosures are inefficiently utilized and the relative abundance of natural food may endanger the treatment if the diet is not highly palatable. Higher densities may also be possible, but competition for feeding space may result in more undersize fish that have not consumed sufficient hormone.

Counting

Before treatment, fry are too small and delicate to enumerate with methods based on estimates of average weight. The following procedure, based on a visual comparison against a known standard, is recommended:

(a) Select two identically-shaped and light-colored containers with vertical sides and flat bottoms (about 30 cm diameter). White 20-liter plastic buckets are convenient.

(b) Add about 5 cm of clear water to each bucket.

(c) Count 1,000 fry into one bucket. (Fry segregate by size so they should be momentarily crowded when selecting a representative sample for counting.)

(d) Place the second bucket next to the "standard." With a shallow, fine mesh scoop net (about 10 or 15 cm diameter), transfer fry to the second bucket until the fry densities in both buckets visually appear to be identical.

(e) Stock the fry in the second bucket into the treatment hapa, and repeat the comparison process.

This method of comparing against a standard is rapid and relatively accurate. In one hour, two people usually process 50,000 to 100,000 graded fry. Error is seldom more than 15%.

(5) FEED THE HORMONE-TREATED RATION

Feeding frequency

Two to 4 times daily during daylight hours, 7 days per week. (No detrimental effect was noted when 1 day per week the entire daily ration was given in a single meal.)

The daily ration need not be divided into meals of exactly equal weight. Normal procedure is to measure the daily ration for each net enclosure and to visually estimate an appropriate fraction of that total for each meal. The daily ration is most accurately measured by weighing, but a calibrated measuring cup is adequate and less time consuming.

Feeding rate

15 to 20% of fish weight daily until fry reach an average length of 15 mm with gradual reduction down to 10% of fish weight daily until the end of treatment.

Calculation of the daily ration

The daily ration per hapa is based on the desired feed rate, the known total number of fry in the hapa and the average weight per fish as calculated from the known or estimated length. The approximate length-weight relationship of fry is:

$W = 0.02 \times L^3$

Where:

W = weight per 1,000 fry in grams, and

L = average total length of fry in mm.

Based on the above relationships and recommendations, the suggested daily rations, according to fish length, can be summarized:

DAILY FEED RATIONS ACCORDING TO FISH TOTAL LENGTH

Average length	Daily ration per 1,000 fry	Average length	Daily ration per 1,000 fry
8 mm	2 g	17 mm	13 g
9	3	18	15
10	4	19	16
11	5	20	17
12	6	21	19
13	7	22	21
14	8	23	24
15	10	24	27
16	11	>24	30

Fry may double their weight in one week. Rations should therefore be adjusted on a daily basis. The ration for the first day of treatment is taken from the above table after measuring a subsample of fry at stocking. Subsequent daily increments in length are estimated from known or assumed growth rates. Common growth rates at temperatures of 25 to 28°C for the stocking densities and feed quality previously described are:

Length of fry	Growth rate
8 to 12 mm	0.2 to 0.3 mm/day
12 to 17	0.3 to 0.6
17 to 25	0.6 to 1.2

These figures should be used only as an initial estimate of expected growth because natural productivity, temperature and feed quality are highly variable. At each specific location, growth rates during treatment should be determined by weekly sampling of fry during the first few cycles. Once growth rate under a given set of conditions is known, a daily feeding chart can be developed to facilitate long term management.

EXAMPLE OF 2-WEEK FEED CHART

Assumptions: initial fry length, 9 mm; growth rate, 0.3 mm per day up to 12 mm length and 0.5 mm per day thereafter. Method of calculation: first determine the expected length of fry for each day according to the above assumptions, then round the length to the nearest mm and find the daily ration from the previous table "Daily Feed Rations According to Fish Total Length."

Day	Fish length	Daily ration	Day	Fish length	Daily ration
	mm	*g/1,000*		*mm*	*g/1,000*
1	9.0	3	8	11.1	5
2	9.3	3	9	11.4	5
3	9.6	4	10	11.7	6
4	9.9	4	11	12.0	6
5	10.2	4	12	12.5	6
6	10.5	4	13	13.0	7
7	10.8	5	14	13.5	8

(6) HARVEST THE SEX-REVERSED FINGERLINGS

Treatment duration

Fry can be effectively sex reversed in 20 days, but occasionally only 95% of the fry develop as phenotypic males. Sex reversal success is more consistent when the treatment duration is 25 to 28 days.

Minimum acceptable size

After a 25- to 28-day treatment, few fry are less than 14 mm. However, if more than 5% are 13 mm or less, those individuals should be culled because as many as 25% of them may be females. The grader that had been used previously to remove oversize fry for sex reversal is adequate for this purpose.

Estimating final numbers

At the end of treatment, the average weight of the treated fish is usually 0.1 to 0.3 g. At this size, it is more convenient and accurate to estimate the final number of surviving fish by dividing the total final weight of fish in the hapa by the average weight of a subsample. A representative subsample (about 100 to 150 g) can be obtained only after tightly crowding all the fish into a corner of the net enclosure. Fish of this size are still delicate. They should be weighed in tared buckets with water to reduce the time out of water.

EXAMPLE OF ESTIMATING FINAL NUMBERS

Bucket with water	=	430 g
Bucket with water plus fry	=	550 g
Counted number of fry in bucket	=	480
Weight of all treated fish	=	4,250 g
Estimated total number of fry $\frac{4{,}250}{(550\text{-}430)/480}$	=	17,000

(7) SECONDARY NURSERY OF SEX-REVERSED FRY

After sex reversal, the fish may be stocked directly for growout to market size, but survival may be highly variable. For greater predictability and more efficient utilization of facilities, a period of secondary nursery in ponds is suggested. All wild fish should have been eliminated from the pond, and a productive plankton community should be established prior to stocking. In poorly prepared ponds, survival during nursery may be as low as 5 to 10%. In properly managed nursery ponds, survival usually varies from 60 to 90%, with an average of about 75%.

An effective nursery technique is to fill and fertilize a dry or disinfected pond with filtered water 7 to 10 days prior to stocking. After initial fertilization with chicken litter at a rate of 1,500 to 2,000 kg/ha or fresh manure from cattle or swine at a rate of 3,000 to 5,000 kg/ha, the nursery ponds generally develop plankton blooms with Secchi disk visibility of 30 to 40 cm in 5 to 7 days. Urea (10-15 kg/ha) can supplement the initial manure application. Fry can be stocked at a rate of 100,000 to 125,000 per hectare. During nursery, the pond should be manured weekly at 1,000 kg/ha. A supplemental feed at 5% of the fish biomass daily improves growth but is not critical for high survival.

If there is a significant possibility that untreated tilapia may enter the nursery pond, a subsample of about 200 treated fish should be maintained in a more controlled environment such as tanks or fine mesh net enclosures. Subsequent sex determination by examination of the gonads will help clarify whether an unacceptably high percentage of females in a nursery pond was due to contamination or incomplete sex reversal. This precaution is also recommended if treated fish are to be sold to other producers who might later complain about the quality of the seed.

Expected Results (Fig. 7)

Hormone Treatment Phase

Survival during treatment: 70 to 90 %

Average size after a 25- to 28-day treatment

Weight: 0.1 to 0.3 g

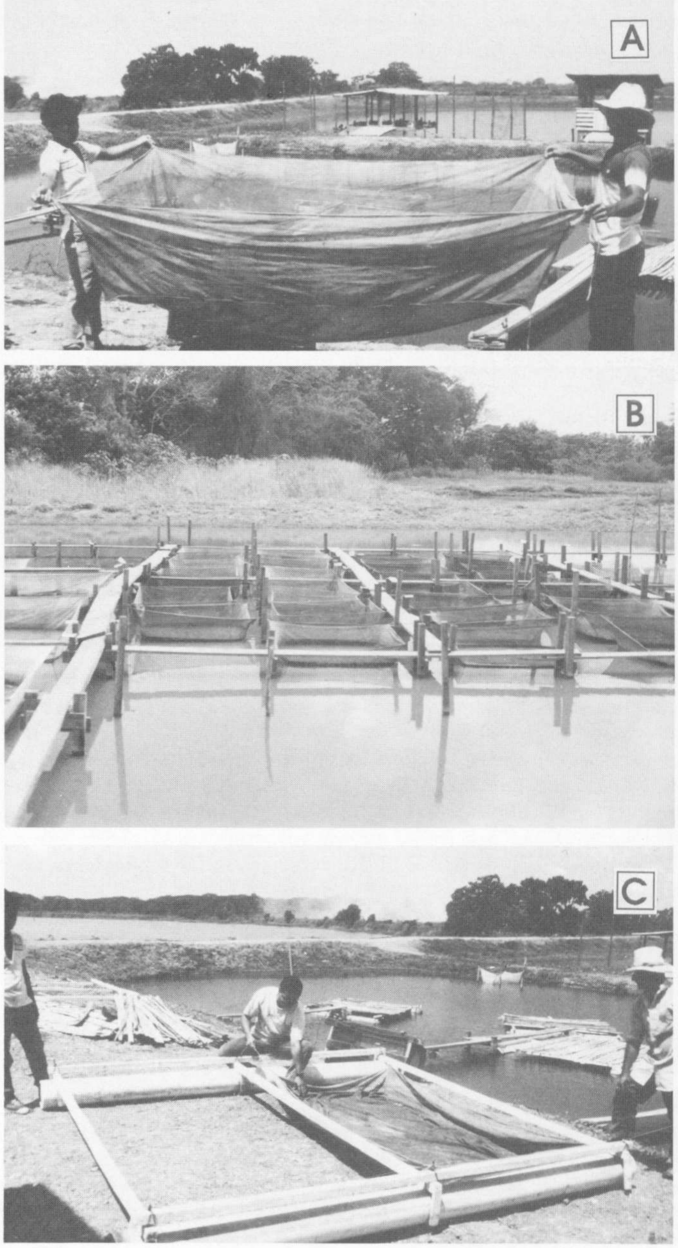
Length: 18 to 25 mm, with at least 95% greater than 14 mm.

Percentage phenotypic males: 97 to 100%

Secondary nursery phase

Survival during nursery: 60 to 90 %

Average size after 40 to 60 days: 15 to 25 g



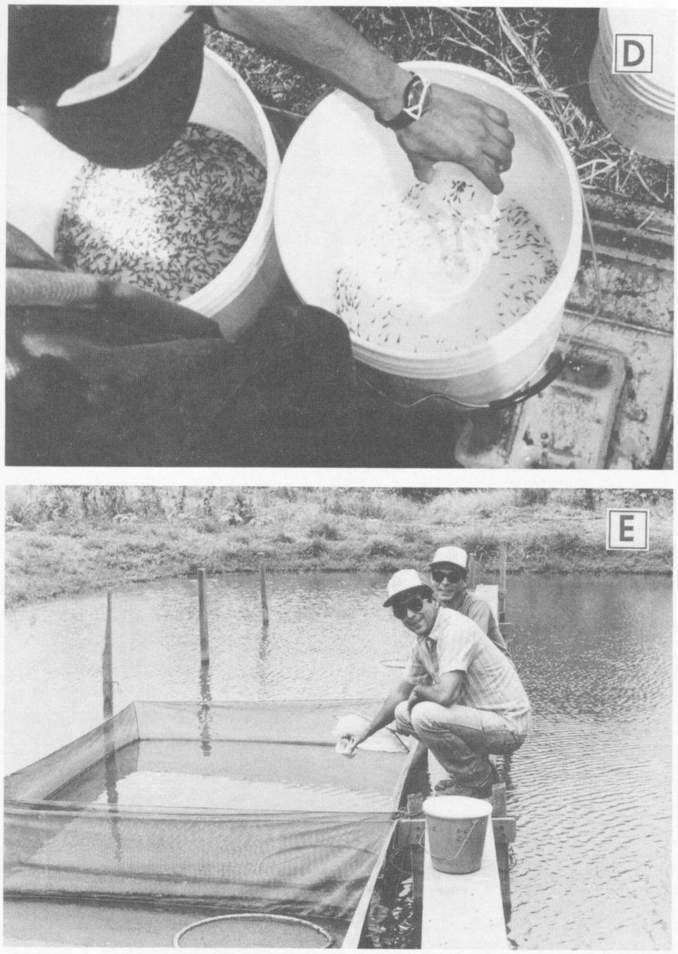


FIG. 7. Pictorial summary of sex reversal procedure. Tilapia fry can be sex-reversed in fine mesh hapas (A) that are tied to fixed stakes (B) or to floating frames (C). Fry less than 15 mm in length are counted into the hapas by visially comparing against a standard of known number of fish (D). They are given a hormone-treated feed 3 or 4 times daily for 4 weeks (E). After daily feeding for 4 weeks, 97-100% of the fish are phenotypic males.

COMMON QUESTIONS ON THE PROCEDURE

The previous description was organized to facilitate field application of the technique. Therefore, detailed discussion on the justification and acceptable modifications or alternatives to specific activities was minimized. This section is included to assist managers in decisions on the general appropriateness of sex reversal, on site specific modifications and on overall farm management.

How is the Effectiveness of the Sex-reversal Treatment Determined?

The phenotypic sex of tilapia can be determined by visual examination of the genital papilla or by sacrificing the fish to examine the gonads. Sex identification by external examination is possible because females have two independent orifices on the genital papilla for release of eggs and urine, while in males the ducts carrying sperm and urine join internally and exit from a single urogenital orifice, figure 8. There are two principal disadvantages with this technique: (1) Even with experience, visual examination is not consistently reliable until the fish have grown to a weight of about 20 g, and (2) during sex reversal, the masculinization of the genital papilla may occur prior to the masculinization of the gonad. Consequently, individual fish that ingest a subeffective dose of hormone may externally appear to be males but may develop internally as females with ovaries. Nevertheless, for practical purposes this technique may be satisfactory, especially on farms where treated fish are reared in nursery ponds to advanced juvenile or early adult stages before stocking in a growout pond.

When sex-reversed fry are stocked into growout ponds or sold at an early age, it is often important to evaluate the sex-reversal treatment earlier and more reliably. In that case, examination of the gonads of a subsample of treated fish is the suggested method. The gonad is examined microscopically, and oocytes are readily observed if the gonad is an ovary. The relatively large, round oocytes become even more obvious if the gonad is stained before examination, figure 9.

A SUMMARY DESCRIPTION OF THE TECHNIQUE

(a) After sex reversal, rear a subsample of fish in a controlled environment to a minimum size of 4 to 5 cm. (Initially rear them to 8 to 10 cm until the crew gains experience.)

(b) Preserve the fish in a 7 to 10% formalin solution for at least 2 weeks. (The formalin toughens the tissue and makes removal of the gonad much easier.)

(c) Open the fish, extract the thin gonad which lies along the dorsal (top) side of the abdominal cavity, and place it on a glass slide.

(d) Put a drop of aceto-carmine stain on the gonad, and lightly "squash" it with a glass cover slip. (The stain can be purchased in prepared form or made by adding 0.5 g of carmine to 100 ml of 45%

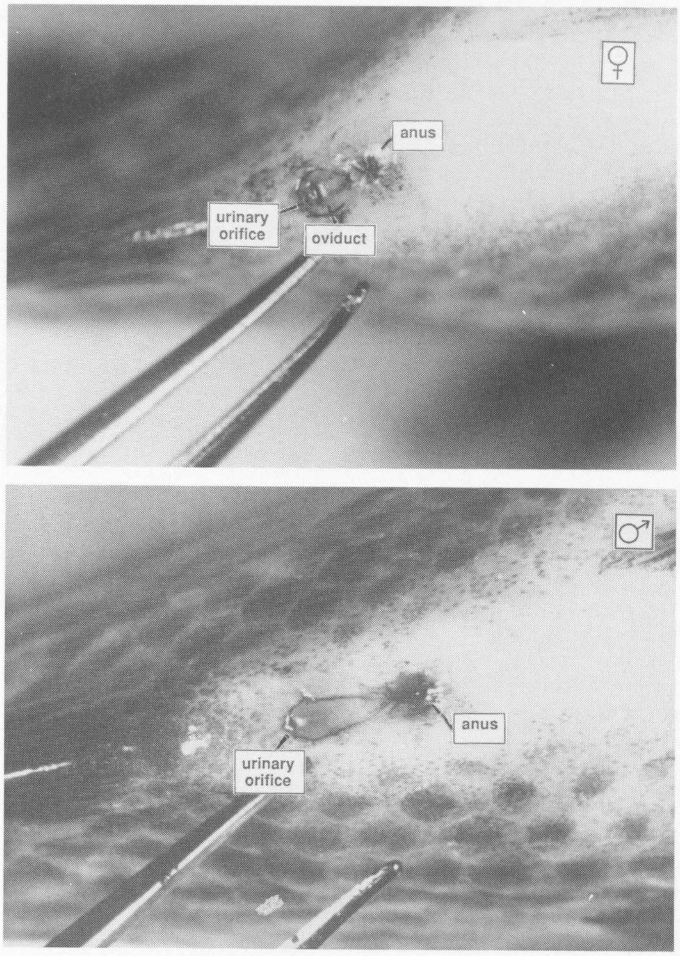


FIG. 8. The phenotypic sex of tilapia can be determined by visual examination of the genital papilla once they have reached a minimum weight of 15-20 g. The urinary duct and oviduct exit independently from the genital papilla of a female (left), while in males (right), the ducts carrying seminal fluid and urine unite internally and exit via a single urogenital pore.

acetic acid, boiling a couple of minutes, and filtering the cooled solution to remove coarse particles.)

(e) After a couple of minutes, examine the gonad at 50X magnification. The fish is a presumptive male if densely packed oocytes are not found. Occasionally, an ovo-testes will be observed with scattered oocytes; few, if any, of these fish will be reproductively functional females.

The reliability of the estimate of percent males in a batch of treated fish is a function of the number of fish in the sample. The 95% confidence interval (95% assurance that the true average is within the range indicated) becomes tighter as the sample size increases from 20 to 1,000 fish. If average percent males in a sample is 95%, the effect of sample size on the confidence interval is:

sample size (N)	95% confidence interval
20	75.2 - 99.9 %
50	84.9 - 99.1 %
100	88.7 - 98.4 %
200	91.0 - 97.6 %
500	92.3 - 96.7 %
1,000	93.5 - 96.3 %

The effect of sample size (N) on the 95% confidence interval of varying percent males in a sample is:

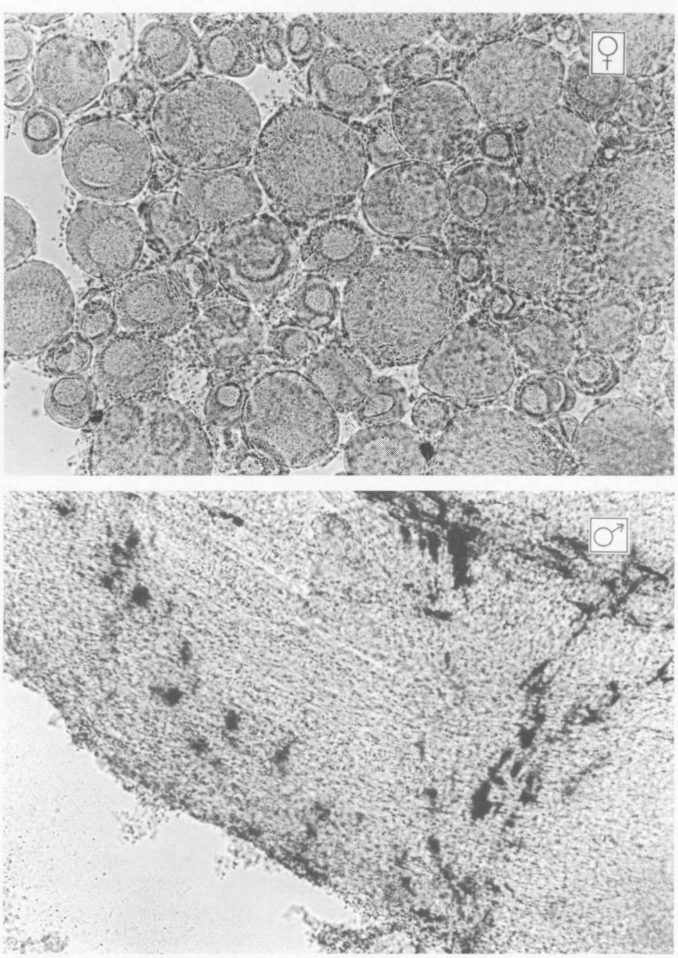


FIG. 9. Oocytes at various stages of development are apparent in the ovaries of 4- to 5-cm females (above). In phenotypic males, oocytes are absent from the testes, which appear granular in texture (below).

Average percent males	95% confidence interval		
	N = 100	N = 200	N = 500
96	90.1-98.9	92.3-98.3	94.0-97.5
97	91.5-99.4	93.6-98.9	95.2-98.2
98	93.0-99.8	95.0-99.4	96.4-98.9
99	94.6-99.9	96.4-99.9	97.7-99.7
100	96.4-100	98.2-100	99.3-100

What Percentage of Females can be Tolerated in Growout Ponds?

Sex reversal, as described in this publication, is generally not 100% effective. Most fry ingest more hormone than required to achieve sex reversal, but feeding behavior, competition, injury, disease, and numerous other environmental or genetic factors may prevent some fish from consuming sufficient quantities of the hormone-treated feed. The percentage of phenotypic females after treatment is often less than 1%, but on occasion may be as high as 5%. Some of these females may be sterile, but others will have normal reproductive function. In warmer climates, with average daily water temperatures of at least 25°C (77°F), they may spawn in 3 months. Limited reproduction late in the growout cycle has little negative effect on growth, but the danger of stunting increases primarily as a function of the number of females and the duration of the growout cycle. Results are variable, depending on management practices and environmental conditions, but the following limits are given as a general "rule-of-thumb " for warmer climates:

Time from sex reversal to harvest of food fish	Maximum safe percentage of phenotypic females
3 months	7 - 10 %
5 months	3 - 5 %
7 months	1 - 2 %
10 months	0%

Polyculture with an effective predator mitigates the negative impact of a few spawning females but may unduly complicate the logistics of pond management.

What Hormones Can Be Used?

Synthetic testosterone-related compounds are developed as anabolic (primarily for muscle development) and/or masculinizing agents. Some must be injected intramuscularly while others may be taken orally. Methyltestosterone and ethynyltestosterone can be taken orally, and are about equally potent as an anabolic and as a masculinizing agent. Because of the general availability and effectiveness of methyltestosterone, little research has been conducted on sex reversal of tilapia with related hormones. However, preliminary work has demonstrated their feasibility. The most likely candidates for investigation are testosterone compounds that can be taken orally, are strong masculinizing agents, and are only slightly soluble in water.

Are there less expensive alternatives to alcohol available?

The 500 ml of ethyl alcohol per kg of diet, as suggested in this brochure, may cost as much as the hormone itself. Unfortunately, little research has been performed on suitable substitutes. Some relevant points are:

(1) It is probable but unproven that lesser quantities of ethyl alcohol would not reduce the effectiveness of the treatment.

(2) In many countries, potable ethyl alcohol is unavailable or is made more expensive as a result of special taxes. Ethyl alcohol denatured with glycerine (0.5% v/v) can be safely used in the preparation of a sex-reversal diet.

(3) Isopropyl alcohol, as mentioned in the text, is a proven substitute for ethyl alcohol.

(4) Methyltestosterone is soluble in some fish oils. An additional

benefit from spraying a hormone-oil mixture on the feed could be increased palatability of the diet. The increased risks, however, are the danger of rancidity from improperly stored feeds and the possible loss of hormone if the oil floats off the feed in the water.

Can other methods be used for fry production and sex reversal?

As previously described, large numbers of uniform-age fry can be produced by draining a reproduction pond down to a harvest basin about 3 weeks after stocking brood fish. However, other techniques may be more appropriate under certain conditions. Suppose water is scarce, or a good harvest basin is not available, or a large number of fry are not needed on a single harvest day. In those cases, the reproduction pond can be partially harvested on several occasions, using a fine mesh seine with the lead line held just off the pond bottom.

Partial harvesting should begin no later than 3 weeks after stocking brood fish. Depending on the frequency and intensity of the partial harvesting, the pond may remain productive for 2 to 3 months. Eventually, fry production is reduced by the cannibalistic juveniles that escape capture.

The principal disadvantages of partial harvesting are: (1) it is more labor intensive than the complete harvest technique as a result of additional sorting of fry by size and removal of unwanted by-catch; (2) the increased handling stress produces greater harvest mortality; and (3) as the biomass increases in the pond, slightly reduced growth will increase the average age of fry and the risk that the gonads of more genetic females have already differentiated into ovaries. (The maximum acceptable length of "target size" fry obtained by extended partial harvesting has not been determined.)

An alternate method of fry production is: Brood fish are stocked at a density of 3 to 7 per m^2 of hapa with a sex ratio of 1 to 5 females per male; fry (and possibly fertilized eggs) are collected every 5 to 10 days. With the more frequent harvest schedule, the percentage of free-swimming fry is greatly reduced, and would therefore be practiced only if incubation facilities are available. If hatchery equipment is available, the brood density is set at the lower end of the range and the proportion of males is increased to improve the probability that most eggs will be fertilized. A temporary resting or recovery period for brood fish between cycles apparently improves fry production in hapas. Promising large scale production techniques are currently under development at the Asian Institute of Technology in Bangkok, Thailand.

Fry production in hapas has several advantages and disadvantages over the open pond technique:

(1) Undrainable ponds can be used.

(2) Water is conserved because fry production ponds are not drained as frequently.

(3) The pond may be used simultaneously for other purposes, such as nursery or growout.

(4) Brood fish are used efficiently. (Harvest mortality of fry is lower, and fertilized eggs taken from the females can be incubated in hatcheries.)

(5) A relatively deep harvest basin is not required.

(6) Fry production in hapas is more labor-intensive because fry must be harvested almost weekly, instead of every 3 to 4 weeks as in ponds.

(7) Spawning hapas and incubation facilities are relatively expensive. The use of spawning hapas more than triples the total area of hapas required for the fry production / sex reversal operation.

(8) Brood fish are more susceptible to predation and theft.

What other containers can be used to sex reverse fry?

Tilapia fry can be sex reversed effectively in any container that maintains environmental conditions appropriate for growth. Aquaria, stainless steel troughs, plastic pools, and concrete tanks up to 30 m^3 have been used, the latter on a commercial scale in Israel. The problems are:

(1) The amount of hormone-treated feed administered requires that the containers be cleaned daily and that water quality be maintained by frequent water exchange and/or aeration.

(2) Disease and parasites are more problematic under such conditions, especially when the container is shaded. Mortalities of 50 to 80% are not unusual.

(3) Labor and start-up costs are generally higher.

Is sex reversal affected by the frequency that hormone-treated feed is offered?

Feeding frequency during sex reversal is a subject of great practical importance currently under investigation. In yet unpublished studies, tilapia were effectively sex reversed when the hormone-treated feed was offered only 6 days per week and when the daily ration was divided into only two meals. However, further studies are suggested before these practices are recommended for commercial operations.

Can Sex-Reversed Tilapia Be Used as Brood Fish When They Mature?

A few genetic females may consume so little hormone during sex reversal treatment that they develop into normal, functional females. Another small fraction of genetic females could be sterile individuals with ovo-testes as a result of insufficient amounts of hormone. Megadoses of hormone also could cause sterility in a few fish, but the majority of the sex-reversed fry develop into reproductively functional males. About half of these fish were genetic males from the moment of fertilization, and their offspring would have a normal sex ratio of 1 female:1 male. The remaining sex-reversed fish can function reproductively as males, but they remain genetic females, and when crossed with normal females, all or most (depending on the species) of their offspring would be normal females. This is the opposite of what is wanted for monosex growout of tilapia. Therefore, although the majority of the treated fish are not sterile, a producer would take special care to eliminate all sex-reversed tilapia as potential brood fish.

Do Sex-Reversed Females Grow More Slowly Than Genetic Males?

When sex-reversed fish reach market size, there is no obvious bimodal distribution (two size groups) as is the case in mixed-sex culture. Many factors related to the phenotypic and genotypic sex of the fish determine its growth rate. The relative importance of each factor is incompletely understood, but the net effect is that, for all practical purposes, genetic females that have been sex reversed to phenotypic males grow the same as normal males.

We noted that when sex-reversed tilapia are harvested after growout, a low percentage of the fish (less than 1%) have distended abdomens. The urogenital papilla are phenotypically male, but the gonads may be filled with a watery liquid containing few or many eggs. These rare individuals are probably sterile "intersex" fish with ovo-testes. The liquid accumulation may be related to the lack of a continuous oviduct leading to the exterior. The fish appear to grow normally and have no other sign of abnormality.

How much does the hormone cost increase fingerling cost ?

Seed production requires many inputs, with the cost of individual inputs varying from country to country. It is, therefore, impossible to give a simple answer to this question, but, in general, the cost of the hormone is a minor fraction of the total cost of producing monosex seed of tilapia. In the United States, the cost of the hormone in 1989 was $26 per 10 g, which is sufficient to produce nearly a half million sex-reversed seed. In many developing countries, it may be more difficult to obtain the veterinary grade hormone, and the grade approved for human medicine may cost three times that value.

In Ecuador and Honduras, the total cost for fry production and sex reversal was equivalent to $5-8 (U.S.) per 1,000 fry in 1987. The cost of the hormone treated feed was about 10% of that total, and the cost of the hormone itself was about 10 to 20% of the cost of the feed (that is, 1 to 2% of the total cost of producing sex-reversed fry). In most countries, the approximate range of costs for the major ingredients needed to sex reverse 1,000 fry is:

Ingredients	U.S. dollar
Basic feed	0.30 - 0.80
Alcohol	0.20 - 0.30
Hormone	0.05 - 0.25

Hormone costs would be reduced if a lesser quantity were required. Several investigators have shown on experimental scale in clear water that tilapia can be successfully sex reversed when fed at only 10% of body weight daily with a ration containing only 20 or 30 mg of methyltestosterone per kg of feed—that is, only about one-fifth as much hormone as recommended in this publication. However, we would not recommend these lower levels until they have been field tested on commercial scale in water containing abundant plankton. This precaution appears even more appropriate when considering that the cost of the hormone at suggested levels is only 1 or 2% of the total cost for sex reversal.

What Effect Do Hormones Have on Humans Who Eat Hormone-Treated Fish?

The justification for the use of methyltestosterone to sex reverse fish intended for human consumption is based on considerations of the total quantity of hormone consumed by the fish and its rate of elimination from the fish after the sex-reversal treatment is suspended. The total quantity of hormone consumed by the fry during sex reversal is small in comparison with normal therapeutic doses for humans. The minimum recommended daily dose of testosterone for androgen-deficient human males is more than 100 times greater than the total amount consumed by a tilapia fry during sex reversal. In reality, most of that small amount is metabolized and eliminated before the fish grows to a marketable size as the liver converts it to more water soluble compounds which are excreted in the bile and urine. When methyltestosterone is orally administered to fry during sex reversal, 90% of the hormone is excreted within 24 hours, and just 3 weeks after the hormone diet is withdrawn less than 1% of the hormone remains in the fish. During growth to a marketable size, the juvenile and adult fish continue to excrete the remaining hormone. At the time of harvest, the quantity of dietary testosterone remaining in the sex-reversed fish is insignificant in comparison with the amount produced naturally by a normal adult male tilapia. One study demonstrated that the plasma testosterone level of sexually active, untreated males in the presence of females was actually higher than levels found in same-age, sex-reversed fish in monosex culture. Considering all these factors, it is doubtful that the low residual levels of the dietary testosterone found in fish months after sex reversal would be a health hazard for consumers. The use of methyltestosterone for sex reversal of food fish has not yet been approved by the U.S. Food and Drug Administration.

Can all species of tilapia be sex reversed?

The procedure described in this publication is based primarily on results obtained with *Oreochromis (Tilapia) niloticus*, but sex reversal with orally administered methyltestosterone at the same dosage levels has been demonstrated with other mouth-brooding tilapias, including *O. aureus, O. mossambicus, O. hornorum* and the "red" tilapia which is generally a mixed strain species. It is possible that the minimum acceptable initial size of fry should be slightly smaller for the mossambica and hornorum groups.

Hormonal sex reversal has not yet been successful for the substrate spawning tilapias, such as *Tilapia rendalli* and *T. zillii*.

Can You Sex Reverse From Male to Female?

After sex reversal with androgens was demonstrated to be feasible, scientists began considering the use of estrogens to produce phenotypic female tilapia from genetic males. The fattening of an all-female population has little appeal to practical aquaculturists because females grow more slowly and several could spawn with a single accidentally introduced male. However, for some species of tilapia there existed the possibility of crossing a sex-reversed homogametic "female" with a normal male of the same species to produce 100% male offspring. This would not be possible with *Oreochromis (Tilapia) niloticus* or *O. mossambicus* but would theoretically be feasible with O. aureus. With other species of tilapia sex reversal with estrogens may make it possible to produce "supermales" which produce only male offspring. Unfortunately, sex reversal to female with estrogens was much more difficult than sex reversal to male with androgens. Even with antiandrogens, such as methalibure, few sex-reversed phenotypic females are produced. Additional research is needed to clarify the value of estrogens in the production of all male seed.

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