Production Of All-Male *Tilapia Aurea* By Sex-Reversed Broodstock

By Douglas Tave

The major goal for virtually all tilapia breeding projects has been to try and keep the fish celibate during grow-out. To that end, most genetic work with tilapia has been designed to prevent reproduction by producing all-male populations.

All-female populations can be produced, but females grow significantly slower than males. Additionally, if the breeding program is not totally successful, the presence of a few males in the grow-out ponds would lead to toga parties and orgies, and a significant portion, if not the major portion, of biomass at harvest would be composed of the results of unprotected sex.

Several breeding programs have been developed to produce all-male populations for grow-out. The most common approach has been to use interspecific hybridization (see my November/December, 1987 column). A second approach has been to use supermales (see my March/April, 1990 column). A third approach has been to use sex-reversed broodfish.

Although direct sex reversal by feeding fry androgen-treated feed is probably the most common technique used to produce all-male populations of tilapia, I don't consider this to be a breeding program. No breeding or directed mating is involved in this approach. It is an aspect of hatchery management.

The direct sex reversal of tilapia fry is quite simple and relatively inexpensive. The only drawback is the fact that the fish have been treated with hormones. In today's volatile consumer market this could be a marketing problem if a bored actress decides she needs a crusade. Remember alar-treated apples? The recent release of a genetically engineered tomato has brought other nuts out of the closet. Unfortunately, you cannot discuss the safety of genetically-engineered food or hormone-treated fry on an intellectual basis with these folks; to them, this is a type of religion, and there is no need to discuss beliefs.

One way of circumventing the problem of selling hormone treated-fish, even though the hormone is essentially gone within three weeks of its withdrawal, which is almost 18 months before the fish are sold, is to use hormones to produce sex-reversed fish and to spawn them to produce an all-male population.

I'm sure some fool out there will object to this procedure, since we're tampering with the sex of the fish, and we then force these poor critters into what is essentially a genetically homosexual relationship. Oy!

The ability to produce an all-male population by using sex-reversed broodfish is totally dependent on the sex-determining system of the species. Most aquacultured species have the XY sex-determining system (see my March/April, 1989 column), and you cannot produce all-male populations with species that have this sex-determining system by using sex-reversed broodfish. You can produce 75% male populations, but that's it. If you want, you can use sex-reversed broodstock to produce all-female populations, and this is being done with salmonids.

The only species in which you can produce all-male populations by spawning sex-reversed broodfish are those that possess the WZ sex-determining system, and precious few aquacultured food species are known to possess this system: two species of tilapia and the Japanese eel.

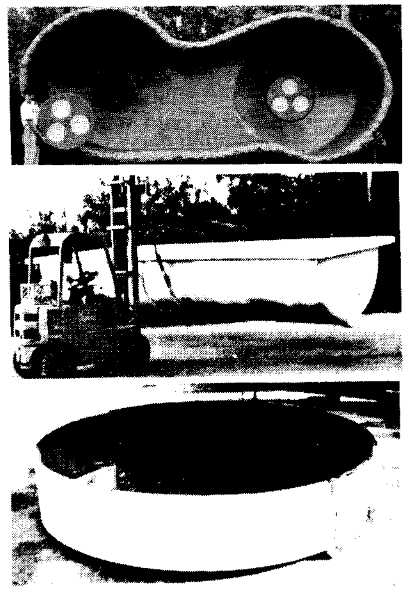
The WZ sex-determining system is essentially the opposite of the XY sex-determining system. In the WZ sex-determining system, males are ZZ and females are WZ. Males are said to be homogametic, because they can produce gametes with only one kind of sex chromosome (Z); females are said to be heterogametic, because they can produce gametes with two types of sex chromosomes (Z and W).

Tilapia are rather unique in that there are two known systems of sex determination: *Tilapia nilotica* and *T. mossambica* have the XY sex-determining system, while *T. aurea* and *T. hornorum* have the WZ sex-determining system. This means you can use sex-reversed broodfish to produce all-male populations with *T. aurea* and *T. hornorum* but you can't do it with *T. nilotica* and *T. mossambica*. You could use sex-reversed broodfish to produce all-female *T. nilotica* and *T. mossambica* populations, but I can't imagine why you'd want to do that.

This is frustrating, because we can't use this breeding program with *T. nilotica*; on the other hand, we can use it with *T. aurea*, so we should be somewhat grateful. Just think, if all tilapia had the XY sex-determining system, not only would we be unable to produce an all-male population by spawning sex-reversed broodfish, we would also be unable to produce an all-male population by creating interspecific hybrids.

A 2-and-1/2-year breeding program was conducted at Nir David Fish Breeding Farm, D.N. Gilboa, Israel to produce cold-tolerant all-male tilapia by using sex-reversed broodfish. The aquaculturists at Nir David decided to use sex-reversed fish to produce an all-male population for two reasons: first, this approach would enable them to produce an all-male population without the use of a direct hormone treatment; second, this approach would produce a more cold-tolerant all-male population of tilapia.

Many tilapia fish farms in Israel grow all-male *T. nilotica* ♀ x *T. aurea* ♂ hybrids. *T. aurea* is the most



ld tolerant species of tilapia, and hybrids have a cold-tolerance that is intermediate between the two parental species. Therefore, an all-male population of *T. aurea* would be more cold tolerant than an all-male hybrid population. Cold tolerance is an important phenotype in regions with cold winter temperatures.

The strain of *T. aurea* used in this breeding project was one that had been maintained at Nir David since 1980. Aquaculturists at Nir David had hybridized males from this strain with female *T. nilotica* for 13 years to produce all-male hybrids. This suggested that the line was stable and that the sex-determining system was stable.

One of the biggest problems in tilapia breeding is the fact that sex determination is not as simple as we'd like it to be. There appears to be one or more autosomal sex-influencing or sex-modifying genes. Although there is little doubt that such a gene(s) exists, no foolproof theory has been proposed. An autosomal sex-determining gene was discovered in common carp (see my January/February, 1994 column), so it is only a matter of time before one is discovered in tilapia.

The autosomal sex-modifying gene(s) in tilapia is one of the reasons why few breeding programs have been able to produce 100% male populations. The other reason is contaminated broodstock. If species are not pure, they won't breed as expected because they will have four sex chromosomes—W, X, Y, and Z. I've heard some entrepreneurs brag that their stocks of tilapia are 7/8 or 15/16 pure, and claim that this means their stocks can be considered to be a pure species for breeding purposes. Someone in Army intelligence might buy this nonsense, but that's all it is. The species is either pure or it ain't. Period.

The strain of *T. aurea* at Nir David was uncontaminated and bred true in crossbreeding programs for 13 years, so it was a good candidate for a sex reversal breeding program.

Two females and two males were stocked in a 4 x 2 x 0.5 m plastic-lined pond. After the females spawned, a random sample of 200 fry was transferred to a 1-m^3 tank for sex reversal.

Fry were fed a ration that contained 60 mg of 17 α ethynyl estradiol/kg feed. They were fed thrice daily at 20% body weight per day for 30 days.

After the sex reversal treatment, the fry were cultured in tanks at a density of 100 fry/m^3 for 40 days, at which time they averaged 10 g. At the end of that period, half the fish were sacrificed to determine the effectiveness of the sex reversal protocol, and the other 100 fish were stocked in an earthen pond for grow-out. Gonadal examination revealed that 82% of the hormone-treated fish were females.

When the fish reached 40 g, they were sexed and all males were culled. The females were transferred to a spawning pond.

It is impossible to differentiate normal WZ females from sex-reversed ZZ "females." The sex-reversed females are fish that are males genotypically, but females phenotypically.

The only way these two types of females can be identified is by a progeny test. Eight females were stocked in individual 4 x 2 x 0.5 m plastic-lined ponds along with two males. Six of the females spawned. After spawning was detected, the males were removed and the females were allowed to incubate their offspring for 90 days.

Random samples of 80-100 fry were taken from each family and sexed. In this type of progeny test, the sex ratio of the offspring will tell you whether the female is a normal WZ female or is a sex-reversed ZZ "female." A normal female will produce offspring with a 1:1 sex ratio (half sons and half daughters). A sex-reversed female will produce nothing but sons.

Two of the families were 100% males, which meant that their mothers were sex-reversed ZZ "females." The other females produced daughters, so they were culled.

The two sex-reversed ZZ "females" were stocked in individual spawning ponds, and two males were stocked in each pond. Because the progeny test had revealed that both females were ZZ "females," these matings (ZZ "♀" x ZZ♂) were done to produce 100% ZZ (male) offspring.

Half of the fry produced by these matings were sex-reversed as described earlier, while the remaining fry were grown and sexed to verify that these matings produced nothing but males.

Eighty-five percent of the ZZ hormone-treated fish were sex-reversed and were ZZ "females." The second-generation sex-reversed fish were grown until mature, and 185 sex-reversed ZZ "females" were stocked with 30 normal ZZ males in a 20 x 4 x 0.8 m pond. All offspring produced by these fish should have been males.

Five samples of between 80 and 100 fry were taken from the pond over a 2-month period. Of the 480 fish examined, 477 were males (99.4%). There are two possible explanations for the presence of the three females. First, they could have been produced by an autosomal sex-modifying gene. Secondly, they could have been contaminants transferred to the pond by birds, since the pond was not covered.

While the breeding program did not create sex-reversed broodfish that produced a 100% male population, it did create sex-reversed broodfish that produced a 99.4% male population. These results are very encouraging, especially since the grow-out pond was uncovered. If this type of breeding program is conducted with pure, stable strains of *T. aurea*, it can be used to produce all-male fish for grow-out, and no fish that are grown for market will be treated with hormones. As a bonus, this breeding program will produce an all-male population that is more cold-tolerant than one that is produced by interspecific hybridization.