# OBSERVATION ON AQUARIUM SPAWNING OF ESTROGEN-TREATED AND UNTREATED TILAPIA

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*Abstract:* Genotypic male *Tilapia aurea*, previously treated for sex reversal to a female state, and their sex-reversed female ($F^1$) parent were stocked into aquaria with untreated females at various ratios. The reproduction of the 2 types of females with normal males was compared. None of the treated fish spawned; most were later found to have ovotestes. The $F^1$ sex-reversed female spawned repeatedly after she was paired with a male in a larger tank, indicating that the presence of normal females under confined aquarium conditions may have a repressive effect upon estrogen sex-reversed females.

Proc. Ann. Conf. S.E. Assoc. Fish & Wildl. Agencies 34:81-87

The tilapia (family Cichlidae) are widely cultured, but excessive reproduction in ponds is a problem in culturing them for human consumption (Bardach et al. 1972). Various methods of monosexing have been used to control reproduction. The use of hormones to reverse the phenotypic sex is one means of producing single-sex populations (Guerrero 1975).

Potential use of functionally sex-reversed fish as brood stock, to be spawned with normal (untreated) fish of the opposite phenotypic sex, was discussed by Shelton et al. (1978). Depending on the homogametic sex, monosex progeny should be produced by such a spawning program (Jensen and Shelton 1979). *Tilapia aurea* males are considered to be homogametic (ZZ) for sex determination. Thus, if a normal male (ZZ) is spawned with an estrogen sex-reversed female (genotypic male = ZZ), only male progeny should be produced. Progeny testing of hormone-treated fish had substantiated that the genotype is not altered by the transformation of the phenotypic sex (Hopkins 1977).

Successful alteration of secondary sex characteristics and the gonads of an individual is dependent on the efficacy and dosage of the hormone, method of administration, and time and duration of treatment in relation to the period of gonadal differentiation for the particular fish species (Yamamoto 1969). The most effective treatment for estrogen sex-reversing male *Tilapia aurea* appears to be oral administration of a combination of 17 $\alpha$ -ethynylestradiol and methallibure (Hopkins et al. 1979). Methallibure is a chemical hypophysectomizing agent that has enhanced the effect of estrogen treatment on this species.

Several problems exist in the use of estrogen-treated *T. aurea* as broodstock. Selecting broodstock from a hormone-treated population can result in pairing individuals of the same gonadal sex, since the dimorphic urogenital papillae alone may be altered by the exogenous hormones (Hopkins 1977). Also, the number of pairings of estrogen-treated fish that have resulted in spawns has been low in comparison to spawns from untreated fish under the same conditions (Liu 1977). However, spawning behavior of functionally sex-reversed fish has not been examined. The primary objective of this study was to compare interaction, behavior and spawning success of normal and estrogen-treated females stocked together and separately, with a normal male.

Financial support for a portion of this study was provided by a grant from the Office of Water Research and Technology of the U.S. Department of Interior through the Water

Resources Research Institute of Auburn University under Matching Grant Agreement No. 14-34-001-8055 (Project B-075-Ala).

The Cooperative Fishery Research Unit is jointly supported by the Alabama Department of Conservation and Natural Resources, Auburn University Agricultural Experiment Station, and the U.S. Fish and Wildlife Service.

## METHODS

Hormone treated and untreated female *T. aurea* were observed in aquaria when paired with untreated males. Each aquarium was 60 x 60 x 35 cm and held about 100 liters of pond water. Only the front panel was glass so that fish were not affected by activity in adjacent aquaria. To reduce losses caused by aggression, welded wire with 5 x 7 cm openings was positioned inside about 15 cm from the front of the aquaria, extending above the water line from side to side. The wire provided a place of partial refuge for the females and visually delineated a territory for the male. Fish were fed daily ad libitum with pellets containing 40 percent protein. Water flow into each aquarium was about 2 liters/min. Aquaria were stocked in mid-June, when daily water temperature averaged 22° C. Neither the photoperiod nor the water temperature were controlled.

One normal (untreated) male and 3 phenotypic females of approximately equal size (16-18 cm) were each marked with a Floy® anchor tag and placed in each aquarium. Either 0, 1, 2, or 3 hormone-treated fish (genotypic males), presumably females, were stocked with 3, 2, 1, or 0 normal, untreated (genotypic) females, respectively. Each treatment was replicated 2 or 3 times. Each fish was examined upon introduction into the aquaria. Untreated females had normal-appearing papillae with an obvious transverse genital slit. Papillae were normal-appearing in 46 percent of the estrogen-treated fish (genotypic males), slightly atypical in 24 percent, and moderately to highly aberrant in 30 percent. No treated females with moderately to highly aberrant papillae were used in this experiment.

Two females ($F^1$) had been estrogen treated and previously identified by progeny testing to be functionally sex reversed (genotypic males). Both had produced 100 percent male offspring when spawned with normal males. One of these 2 sex-reversed females ($F^1$) died early in the study. The other female was stocked in aquarium 6 (Table 1) with 2 normal females and 1 normal male. The remaining treated fish used were offspring ($F^2$) of this surviving progeny-tested female, and had been estrogen treated for reversal of their potential male phenotype.

Surgical removal of the premaxillae (Lee 1979) from each normal male reduced agonistically induced mortality of the females (Rothbard 1979). Females that died during the study were replaced with females from the appropriate progeny group. Two days after spawning occurred, eggs were removed from the mouths of incubating females and artificially incubated on a shaker table, following the recommendations of Lee (1979).

## RESULTS

Since none of the presumably sex-reversed $F^2$ females had previously spawned, the experiment was a progeny test for these fish. Thus, the treated $F^2$ group could contain some individuals incompletely affected by the estrogen treatment (i.e., have a female-like papilla but retain functional testes or perhaps ovotestes).

Little prespawning aggressive behavior was observed in the first 2 weeks of the experiment when the water temperature was about 22°C. Spawning coloration and aggression intensified during the 3rd week when the water temperature increased to about 25°C. The typical spawning coloration of a *T. aurea* male includes a bright red margin on the fringes of the dorsal and caudal fins, a bluish coloration on the operculi, but (in the absence of other males) no apparent vertical barring of the body. After 2 more weeks, the water

Table 1. Spawning and mortality in aquaria stocked with untreated and estrogen-treated *Tilapia aurea* females at various ratios.

| Female ratio[1] | | SPAWNS | | | MORTALITY | | |
|---|---|---|---|---|---|---|---|
| (treated:untreated) and aquarium # | Duration (weeks) | Total[3] | Weekly mean per aquarium | Weekly mean per untreated female | Untreated females | Treated fish | No. of treated fish yielding milt |
| 0:3 | | | | | | | |
| 9 | 7 | 4 | 0.57 | 0.19 | 2 | -- | -- |
| 10 | 8 | 8 | 1.0 | 0.33 | 0 | -- | -- |
| 1:2 | | | | | | | |
| 1 | 7 | 6 | 0.86 | 0.43 | 1 | 0 | 0 |
| 2[2] | 4 | 3 | 0.75 | 0.38 | 0 | 0 | 1 |
| 6 | 4 | 4 | 1.00 | 0.5 | 0 | 0 | 0 |
| 2:1 | | | | | | | |
| 3 | 5 | 0 | 0 | 0 | 0 | 1 | 0 |
| 4 | 4 | 1 | 0.25 | 0.25 | 0 | 1[4] | 1 |
| 3:0 | | | | | | | |
| 5 | 6 | 0 | 0 | -- | -- | 1 | 2 |
| 7 | 5 | 0 | 0 | -- | -- | 0 | 3 |
| 8 | 4 | 0 | 0 | -- | -- | 1 | 3 |

[1]/ Each aquarium contained 1 untreated male.

[2]/ Aquarium 6 contained the $F^1$progeny-tested female and 2 untreated females.

[3]/ Spawns only by untreated females.

[4]/ Female; others had female papillae but with testes.

temperature rose to 27-28°C; the male in each aquarium soon became assertive and aggressively chased the females throughout the aquarium. Later, only the central area behind the partial barrier was actively defended by the male. However, the male often chased a female that was in front of the barrier and occasionally allowed females to wander behind the barrier.

Female-female interactions usually resulted in a recognizable hierarchy within 48 hours after stocking. The confrontation between 2 normal females usually involved frontal displays with operculi slightly flared, extension of the dorsal, pelvic, and pectoral fins, and prolonged and repeated open-mouth contact. Body coloration was subdued. The dominant female usually occupied the lower level of the aquarium in front of the wire barrier, and pursued the other females only for short distances. Subdominant females tended to occupy positions near the water surface, assuming a posture with the body angled upward. The dominance order was reinforced by periodic charges that usually include a feigned nip on the anterior portion of the body of a subdominant female. Occasionally, the aggressive female made contact with the other fish. In each aquarium, 1 female was least dominant of the 3 and was charged or nipped by the other 2 females. The second female in the hierarchy was charged only by the dominant female. Subdominant females displayed a variety of color patterns that usually include vertical barring on the sides. The dominant females was generally lighter in color.

A complication in these interactions was caused by some of the estrogen-treated fish that were apparently incompletely sex reversed. Although they were considered females on the basis of the appearance of the papillae, their behavior after stocking was often a more accurate indicator of their primary sex. Incompletely sex-reversed fish were rarely aggressive enough to displace the male, but some frequently showed characteristically male-like behavior and spawning coloration which interfered with the establishment of a more ordered female hierarchy. Other incompletely sex-reversed fish did not show male behavior or coloration, and did not participate in the ordered female hierarchy previously described. Most treated fish participated in the hierarchy establishment and eventually assumed subdominant female roles. Several females died as a result of aggression from the normal male and/or treated fish. The treated fish were more capable of inflicting physical damage, since their premaxillae were intact. A treated fish showing this behavior was removed and examined for milt. If the fish was a male it was replaced with another treated female. The 6 treated fish first stocked into aquaria 7 and 8 (Table 1) were all replaced. One of the 3 fish originally stocked in aquarium 5 was replaced, and the replacement fish was also exchanged when it produced milt.

During courtship the male displayed a continuously erect dorsal fin and the genital papilla became increasing extruded. The pursuit of a female by the courting male was more aggressive than the chasing observed between females. Nipping and nudging were directed toward the female's vent. Spawning occurred more frequently with the dominant female than with subdominant females.

Prespawning reproductive behavior of the female included several attempts to either pick (imaginary) eggs up into her mouth or "clean out" a nest site on the bottom of the aquarium behind the wire barrier. The male's agonistic behavior would change rapidly to courtship behavior during this period as the brooder pair would next simulate egg deposition and fertilization which was described by Rothbard (1979). After the termination of feigned spawning behavior, the female would begin egg deposition which was followed by fertilization.

Spawning by untreated (normal) females occurred in every aquarium except one (Table 1), although not every normal female in each aquarium spawned. In contrast, none of the treated fish spawned. The mean number of spawns/untreated female was 0.44/week for the female stocking ratio (treated: untreated) of 1:2 and 0.26/week for those stocked at 0:3. Lee (1979) observed a mean of about 0.23 spawns/week with 3 normal females and a single male in these same aquaria. The mean number of spawns/week was significantly higher in aquaria with a 0:3 and 1:2 female ratio ( $\alpha$ = .05) than in those with other ratios. The mean number of spawns/untreated female/week was significantly higher ( $\alpha$ = .05) in aquaria with a 1:2 (treated/untreated) female ratio (but not in those with a 0:3 female ratio) than in aquaria with a 2:1 female ratio.

No spawning by treated fish had occurred in the aquaria by the end of July, including the $F^1$ female that had spawned successfully during the previous year; consequently, all fish were transferred outdoors to 4,200 liter circular plastic pools. Each treated fish was individually stocked with an untreated male (premaxillae removed). Those untreated females that had not spawned in aquaria, each spawned at least once in the plastic pools. Pool spawns by untreated females are not included in the tabular data. Three spawns within a 6-week period occurred in the pool containing the progeny-tested ($F^1$) sex-reversed female (Table 2). All 3 spawns were fertilized by the same male and resulted in large numbers of viable and presumably 100 percent male offspring. One hundred fish sampled from the first spawn were all males. No spawning in the plastic pools containing other treated fish ($F^2$) was observed by 1 October, when the experiment was terminated. Gonadal examination of treated fish ($F^2$) showed that only 2 fish had ovaries of normal appearance. The remaining 14 fish had gonads that were apparent ovotestes.

## DISCUSSION

Progeny testing of estrogen-treated *T. aurea* has been previously accomplished and functionally sex-reversed females were identified, but from pairings of 1 male with a single test female under less confined conditions than those provided by the aquaria in the present study (Liu 1977). Hormone-treated females may be less aggressive than untreated females, and therefore, may not be as competitive in spawning. The $F^1$ sex-reversed female that had previously been proven fertile spawned repeatedly when removed from confinement and the presence of 2 competing females. Each of these 2 untreated females spawned twice during the period of confinement. Also, normal (untreated) females that did not spawn in the aquaria spawned when moved to the larger plastic pools. It appears that the stress of additional crowding and the presence of competing females adversely affects individual spawning success.

Estrogen-treated fish of the $F^2$ generation that had been incompletely sex reversed directed their aggression toward the normal females or other treated fish, disrupting normal spawning interaction. Fish having ovotestes were usually co-dominant with untreated females, but were incapable of culminating any response to male courtship because ovulated oocytes would be unable to pass through testicular elements.

The number of spawns/untreated female was somewhat higher when 1 treated fish was stocked with 2 untreated females than when compared with other female ratios. However, the presence of 2 treated fish in an aquarium significantly reduced the spawning activity of the untreated female. Under these circumstances there may have been too much agonistic behavior from incompletely sex-reversed females. The low spawning success in progeny tests of estrogen-treated *T. aurea* has been previously reported (Liu 1977, Hopkins 1979). The presence of ovotestes in these estrogen-treated *T. aurea* not displaying obvious male characteristics indicates that the aberrant gonadal morphology associated with incomplete sex reversal may partially explain the low spawning rates.

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Table 2. Spawning frequency of an estrogen sex-reversed female *Tilapia aurea*.

| Site of spawn | No. of spawns | Period of time (wk) | Male offspring (%) | No. offspring sampled | Area per brooder ($m^2$) |
|---|---|---|---|---|---|
| 4,200-liter plastic pool (1978)[1] | 1 | 8 | 100 | 40 | 3.65 |
| 100-liter aquaria (1979) | 0 | 6 | -- | 0 | 0.09 |
| 4,200-liter plastic pool (1979) | 3 | 6 | 100 | 100 | 3.65 |

[1]/Hopkins 1979.

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