Aqua Tips

Feminization of Commercial Swordtails, *Xiphophorus helleri,* by Dietary Administration of 17ß-Estradiol

Clyde S. Tamaru, Lei S. Yamasaki, Kathleen McGovern-Hopkins, Spencer Malecha, and Douglas Vincent

University of Hawaii at Manoa

This article was written as part of the work for the project, "Improving Outputs in the Commercial-Scale Production of Swordtails in Hawaii, Year 2," which was funded by the Center for Tropical and Subtropical Aquaculture (CTSA) under a grant from the U.S. Department of Agriculture Cooperative State Research, Education, and Extension Service.

One of the major constraints in the commercial production of common swordtails is sex ratios that are highly skewed towards the female sex. Because the common male phenotype (Fig. 1) is expected to make up at least a third to a half of a single order, determining a means to produce swordtail populations where the sex ratios are more evenly distributed would be highly desirable. However, understanding what the underlying mechanism(s) that are responsible for these variant sex ratios remains elusive, because the factors that control sex determination in swordtails still remain uncertain.

Photograph by Mike Yamamoto.

Figure 1. Photograph of common swordtails: Male (upper) and female (lower).

It may seem paradoxical that one would want to investigate feminizing swordtail populations, given that increasing the male phenotype appears to be the desired outcome. A variety of swordtails with elongated fin rays, known as the lyretail, however, is valued at 2-3 times that of the common variety. In this variety, the upper and lower rays of the caudal fin are elongated, giving rise to the term "lyretail," after the Greek musical instrument. The elongated fin is the

physical expression of a genetic change in the fish, which also impacts the ability of the male lyretail swordtail to mate, due to an over-development of the gonopodium. Because of the inability of male lyretails to copulate with other swordtails, lyretails are traditionally produced by mating a female lyretail with a common male, resulting in 50% of the progeny being lyretail. This observation may be explained using the classical model of Mendelian inheritance of dominant (i.e., lyretail trait) and recessive (i.e., common swordtail) alleles segregating randomly in a swordtail population (Norton, 1991). This was confirmed with the production of the homozygous genotype for the lyretail trait using artificial insemination and progeny testing (Tamaru et al., 2003). As expected, the offspring of a cross with a homozygous lyretail individual results in progeny that are phenotypically 100% lyretails. In this particular scenario, possession of homozygous lyretail females would be the desired situation, because production of all lyretails could be accomplished by simply mating lyretail females with common swordtail males.

Direct feminization in fish is achievable by exposing sexually undifferentiated individuals to natural or synthetic estrogens. It has also been reported that estrogen therapy can result in elimination of the gonadal ducts, reduction of the size of the gonad and reduction in the number of germ cells ultimately resulting in sterility. Confirmation that the feminization procedure does not have any adverse side effects on common swordtails also forms the basis for this report.

Material and Methods

Feminization Trial

The natural oestrogen, E_2, was obtained from Sigma-Aldrich, St. Louis, Missouri, USA, and was administered orally to the fry in five treatment groups (i.e., Control, T1, T2, T3 and T4), representing dosages of 0, 100, 200, 300 and 400 mg hormone/kg feed, respectively. The experiment was conducted in a randomized block design with each treatment block being triplicated. The fry in the control group were fed the same diet without any hormone. The feminization experiment was conducted in 14 38-L aquaria that were each equipped with a continuous source of aeration, undergravel filters, municipal tap water and conducted as static systems.

Each tank was stocked with 30 three day-old fry with an approximate body weight of 11 mg wet weight per individual. The fry were fed twice a day with the prepared diets for a period of 30 days at 3% of the body weight. After the 30 day period, the fish were fed a hormone-free diet and reared for an additional five months to insure that the fish attained sexual maturity, at which time their sex could be accurately determined.

Reproductive Performance

Reproductive performance was assessed using 27.9 cm diameter plastic containers (n = 40), each equipped with two 8.3 cm diameter screen windows. All of the containers were fitted with a Styrofoam collar and floated in a 11 m^3 concrete raceway filled with municipal tap water. The raceway was provided with a continuous source of aeration and water exchange at approximately 10% per week. The surviving five month old phenotypic female swordtails (n = 20) from one replicate of the highest dosage tested (400 mg/kg feed) were placed into separate breeder cages. Female swordtails that were not exposed to estrogen therapy were stocked into the remaining tanks. The average body length of this group was 49.7 ± 5.3 mm. A single male swordtail was

placed with each female and allowed to mate naturally. The animals were fed twice a day with TetraMin Tropical floating flakes consisting of 48% crude protein, 8% crude fat, and 2% crude fiber. Each of the plastic containers was checked for the presence of fry twice a day. The brooders remained in their cages until fry were produced, which was achieved in approximately two months. Production of only one clutch of fry was used to determine reproductive competency, and these fish were then returned into their holding containers and allowed to grow out for an additional four months. After the elapsed time, the resulting sex ratios from each container were obtained by using secondary sex characteristics (e.g., sword and gonopodium for males) to differentiate between the sexes.

Results and Discussion

The mean percentages of females resulting from the various treatments are summarized in Figure 2. The mean percentage of females in the control group was 74.5 ± 6.4% and would significantly ($P<0.001$) increase to 97.0 ± 5.2% at the highest dosage of E_2 (e.g., 400 mg/kg feed). No apparent differences were detected in percent survival, growth, or gonadal somatic indices of fish exposed to the various treatments. Histological examination of the gonads did not reveal the presence of "intersexes" in treated individuals. The results from previous and current investigations clearly indicate that the period when the female sex can be influenced most in swordtails is during the first month after birth using an oral administration of E_2 with either a high dose, short duration or low dose, longer duration exposure strategy (Lim et al., 1992).

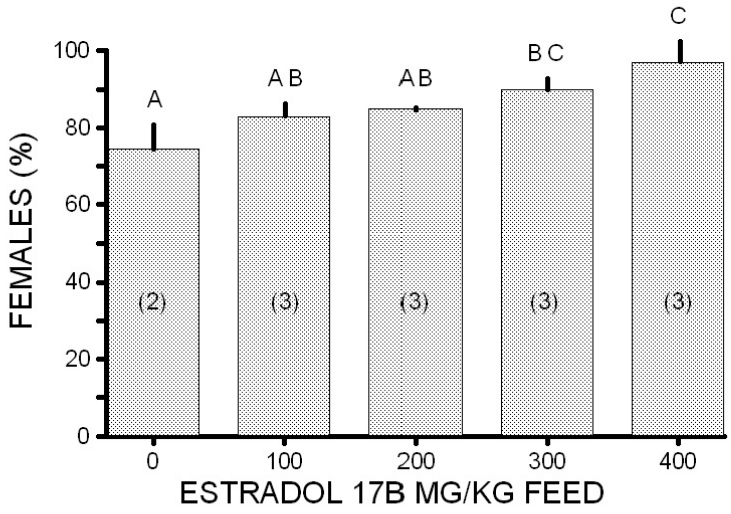

Figure 2. Summary of the average percentage of females obtained in response to the various dosages of E_2 that were investigated. Numbers in parenthesis represent the number of replicates. Bars ± S.D. that do not share a letter are significantly ($P<0.05$) different from each other.

Only one female out of 20 female individuals that were not exposed to E_2 treatment did not produce any fry during progeny testing. All (n = 20) of the females who had received the 400 mg/kg feed dosage of E_2 were observed to have produced fry. All of the treated individuals were phenotypically female and clearly fertile. All 20 females produced fry, indicating that the feminizing therapy did not have any negative impacts on reproduction.

Most intriguing, however, are the resulting sex ratios from the progeny testing that was achieved after the growout period. A summary of the observed sex ratios of four varieties of swordtails being produced at Windward Community College Aquaculture Complex and the resulting sex ratio from the sum of the progeny of the 20 separate crosses is presented in Table 1.

Table 1. Summary of sex ratio of swordtails at Windward Community College Aquaculture Complex.

Variety	Females	Males	Total	Sex Ratio (F:M)	C^2	P Value
Red	116	50	166	2.3	13.7	<0.001
Pineapple	167	77	244	2.2	17.1	<0.001
Half Black	115	25	140	4.6	40.4	<0.001
Red Wag	103	37	140	2.8	16.5	<0.001
Red Wag Feminized	126	154	280	0.8	2.8	0.09

Consistent with the challenge reported previously is the female dominated sex ratio observed for the various swordtail varieties (range: 2 – 5 females to males). The female to male ratio, however, of the resulting crosses of individuals that had undergone the feminizing treatment did not statistically differ from a 1:1 female to male ratio. As mentioned previously, this was one of the desired outcomes of the research being conducted. The data is consistent with a polygenic control mechanism for determination of sex in commercial swordtails. In this scenario, the functional male phenotype is expressed when the sum of the male genes in an individual exceeds a certain "threshold". One possible explanation is that genotypic males that have been feminized when mated with another untreated male increase the likelihood of progeny that possess the required number of male genes to become phenotypically male. Results obtained in the laboratory are currently being validated at pilot-scale at the Windward Community College Aquaculture Complex in order to assess the utility of the feminizing treatment on a commercial-scale.

References

Lim, B.H., Phang, V.P.E., and P.K. Reddy. 1992. The effects of short-term treatment of 17a-methyltestosterone and 17ß-estradiol on growth and sex ratio in the red variety of swordtail, *Xiphophorus helleri*. Journal of Aquaculture in the Tropics 7: 267-274.

Norton, J. 1991. Fancy live-bearers, Part Two. Freshwater and Marine Aquarium 14(7):48-51.

Tamaru, C.S., K. McGover-Hopkins, G. Takeshita, and M. Yamamoto. 2003. Creating the homozygous genotype for lyretail swordtails. Tropical Fish Hobbyist, #566, Volume LI(9):66-70.