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Selective Breeding of Farmed Fish

R.O. Smitherman, G. Wohlfarth, W.L. Shelton,
R.A. Dunham, G. Hulata, S. Rothbard

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FINAL REPORT

on

SELECTIVE BREEDING OF FARMED FISH

BARD-US 164-79

April 1980 to November 1983

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SELECTIVE BREEDING OF FARMED FISH

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I. DESCRIPTION OF RESEARCH RESULTS

ISRAEL

SELECTIVE BREEDING OF TILAPIAS

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ABSTRACT

This investigation consisted of two studies in the genetic improvement of tilapias, estimating realized heritability of growth rate and attempting to produce brood stock consistently yielding all male hybrid progenies. In order to practice mass selection in Oreochromis niloticus, a special technique was developed for producing broods consisting of equal aged individuals. This is required because spawning of different tilapia females is not synchronous. Selection was carried out at two ages of fish, juveniles and adults. The progenies of the selected fish were compared to two controls, progenies of random samples and progenies obtained by re-spawning the founder population. Two cycles of selection were carried out and the response to selection tested in two growth tests. No response was obtained and the estimate of realized heritability of growth in the O. niloticus population tested is zero. This result is discussed in terms of genetic variation and inbreeding and the likelihood of obtaining a selection response by first constructing an appropriate base population.

A breeding scheme was constructed aimed at producing brood stock in two species of tilapia (O. niloticus and O. aureus) whose interspecific hybrid progenies are all male. This consists of progeny testing pairs consisting of a female of one species and a male of the other. The criteria for selecting

parents was the sex ratio of their hybrid progeny, all male or segregating into 1 female: 3 males, in the two reciprocal crosses. The first step of the investigation has been partially carried out, resulting in the isolation of a number of proven individuals. The second and third steps, consisting of intra-specific reproduction of the proven individuals and testing these progenies in interspecific crosses could not be accomplished within the time limit of this project.

INTRODUCTION

Breeding methods based on quantitative genetics have as yet contributed little to the improvement of fish or other organisms grown in aquaculture systems. Some domestication must have taken place in stocks of the major fish species established in commercial aquaculture systems, mainly common carp, rainbow trout and channel catfish. Genetic studies have been carried out in rainbow trout and channel catfish (Gjedrem, 1983; Bondari, 1983; Dunham and Smitherman, 1983; Smitherman et al, 1983), but the only large scale application of genetic improvement to commercial culture has been carried out with the common carp in Israel (Wohlfarth et al, 1965; Moav and Wohlfarth, 1973), Hungary (Bakos, 1979) and the U.S.S.R. (Kirpichnikov, 1981). This consisted of progeny testing different strains for their crossbred performance and selecting the better strains for commercial brood stock. Mass selection of the larger individuals was found to be ineffective in the common carp (Moav and Wohlfarth, 1976).

The group of fishes collectively termed tilapias belong to three genera and a large number of species. Their potential importance for aquaculture is enormous in the warmer areas of the world, mainly between the 20°C winter isotherms. This is due to their many favorable attributes like general hardiness; resistance to diseases, low oxygen tension and crowding; high yield potential; ability to grow on a wide range of natural and artificial foods;

ability to withstand a wide range of salinities and their excellent quality as a table fish. Nevertheless the total annual production of tilapias in the world is low (FAO, 1980) and little has been done to improve any of their commercially important traits. This is at least partially due to their one major drawback, early sexual maturity, and uncontrolled spawning in production ponds. The resulting over-crowding leads to inhibition of growth and a major proportion of the yield consisting of small unmarketable fish. An apparent breakthrough potentially solving this problem was made by Hickling (1960) who showed that hybrids between tilapia species may consist of all male broods. As a result the main research effort in tilapia genetics and breeding has been concentrated on the production and testing of different tilapia hybrids (Fishelson, 1962; Chen, 1969; Jalabert et al, 1975; Wohlfarth et al, 1983). Nevertheless all male tilapia hybrid production has never been sustained during the transition from research to large-scale production, though predominantly male hybrids broods are in commercial use (Mires, 1977). The effect of mass selection for growth in tilapia and the realized heritability of this trait were investigated in three studies (Thien, 1974; Tave and Smitherman, 1980; Bondari et al, 1983) and gave different results in different species. This research project consisted of a study in the genetic improvement of tilapias by two not mutually exclusive strategies:

1. Developing a method of producing proven brood stock in two different tilapia species, whose interspecific hybrids consist of all male broods.
2. Mass selection for growth and late sexual maturity in Oreochromis niloticus during two consecutive generations, in order to estimate selection response and realized heritability.

MASS SELECTION FOR GROWTH RATE AND LATE MATURITY IN OREOCHROMIS NILOTICUS,
ESTIMATION OF SELECTION RESPONSE AND REALIZED HERITABILITY

Three previous studies have been concerned with selection response and estimating heritability of growth in tilapia. An asymmetrical response to bidirectional selection for body weight was shown in O. mossambicus during a single cycle of selection by Thien (1971, after Kirpichnikov, 1981). Selection of the larger individuals showed a very small selection response (realized heritability = 0.009 and 0.083 for females and males, respectively) whereas the estimates of realized heritability for down selection were much higher (0.237, 0.137 for females and males, respectively). These results should be considered with some reserve, since the control consisted of a selected medium group and not a random sample. All the fish in this study, the parent generation on which selection was practiced as well as their progeny groups, were grown in aquaria and their mean weights were less than 20 g. The heritability of growth in O. niloticus was estimated from comparisons between half-sib groups (Tave and Smitherman, 1980). Length and weight of all groups were measured at 45 and 90 days. The only heritability estimate differing significantly from zero was the dam heritability (h^2_D) for length at 45 days. A bidirectional selection experiment for growth was carried out in O. aureus (Bondari et al., 1983), the fish being grown in tanks and cages. A response was found for both up and down selection, which was differentially asymmetrical in the two directions and in two testing environments. Realized heritability estimates computed from the up selection were 0.24 and 0.23.

In the study here described, mass selection was carried out on O. niloticus (Ghana stock) by picking out the largest individuals at two ages, pre-maturation and post-maturation. Two controls were used, a respawn of the original parents and a random sample taken immediately before selection was carried out. In addition, it was also attempted to select for late maturity by sequentially

culling individuals showing signs of sexual maturity, e.g., swollen sexual papillae and mating colors. Selection for weight was carried out on two consecutive generations (in 1981 and 1982) and the progenies of these selected fish, together with the control progenies were tested in 1982 and 1983. Selection for late maturity was found not to be feasible.

METHODS

The species chosen, O. niloticus, is reported to be one of the faster growing tilapia species (Wohlfarth and Hulata, 1983; Pullin, 1983) and is also widely used for interspecific hybridization.

Spawning methods. Groups of tilapias do not spawn synchronously (as common carp do) but pair-wise, preceded by nest digging, choice of mate and courtship. As a result, when a number of mature tilapias of both sexes are stocked into spawning ponds, their fry consist of groups of individuals of different ages. Such mixed age populations are clearly not suitable for selection for growth, since the larger individuals are likely to be the progenies of the earlier spawns, rather than the faster growers. Selection of these larger individuals may result in selection for earlier maturity, an undesirable trait in aquaculture, and is unlikely to be instrumental in increasing growth.

Equal aged fry populations, on which to practice selection, were produced by the following techniques: Spawning was carried out in plastic containers of 700 l, each stocked with a number of a females (6-8) and two males. Brooding females were identified and the eggs of each were incubated in a separate hatching vessel (Hulata et al, 1983). By this means the exact date of spawning and hatching, i.e., the age of each fry group was determined. Pooling of fry from different individual spawns was restricted to spawns of the same day or two consecutive days. The female and male fish identified as the parents of these spawns were marked in order to enable their respawning in subsequent years as one of the controls of the selection experiment.

Method of selection. The fry groups were transferred to earthen ponds for nursing, two or three days after hatching was completed, each group being stocked into a different pond. After about 10 weeks, these nursery ponds were drained and all the fish removed. The selection procedure practiced in each group, as described below, was aimed at obtaining four groups of fish: a random sample, a sample selected for late maturity, and two samples containing the large fish, one selected before and one after onset of sexual maturity. The selection procedure was as follows:

1. All fry in each pond were sexed and samples (random and selected) taken separately from females and males. This is required since in tilapias males are larger than females.
2. A random sample was taken from each group, transferred to another pond and there grown till the end of the season. Then random samples were taken from each pond and pooled to serve as "random sample control group". From the remaining fish the larger individuals were selected. The different selected samples were pooled to serve as parents for the "post-maturation selection group".
3. From the remaining sample the smaller individuals (about half the number of fingerlings) were culled. The remaining larger fish were randomly divided into two samples.
4. One of these selected samples was stocked into a different pond, with the aim of selecting for late maturity by sequentially culling the earlier maturing individuals.
5. From the other sample the largest fish were selected. The selected samples from different spawns were stocked jointly into one pond. In the following year they were used as parents for the "Pre-maturation selection group".

This procedure, (Figure 1 and Tables 1-3) was used to produce the two selection lines and one of the controls for the first cycle of selection. The second control was produced by respawning the original parents. For the second cycle of selection samples of the two selection lines were again spawned in containers in the hatchery and the nursing and selection procedure repeated on the same general lines - see Tables 4-5. The two control lines for the second cycle of selection were produced by separately respawning the original parents and the random sample.

Methods of growth testing. The growth tests included four groups of fish, two selection lines and two control lines. These fish, on which no further selection was practiced were mass spawned in earthen ponds. Samples of fry were seined from these spawning ponds, counted and transferred to nursery ponds, with the aim of nursing them to at least 10 g previous to stocking into the growth tests. Growth testing was carried out in separate ponds, i.e., samples of each of the four test groups were stocked separately into a set of replicated ponds in both 1982 (first cycle) and 1983 (second cycle). Growth testing was carried out in separate ponds rather than by the potentially more efficient method of communal testing, since the methodology of communal testing has not been sufficiently investigated in tilapias. It requires differentially marking fingerlings of the different groups, a method of correcting for the correlation between initial weights and weight gains and an estimate of the correlation between performance in communal and separate ponds. This methodology has been sufficiently investigated in the common carp (Moav et al. 1960; Wohlfarth and Moav, 1972; Moav and Wohlfarth, 1974) to enable communal testing, but not in tilapias.

In the first growth test (1982), in which the effect of one generation of selection was evaluated, each group was stocked into three replications. The second growth test (1983) in which the cumulative effect of two consecutive

generations of selection was evaluated, the number of replications was four. In both of these years a number of interspecific tilapia hybrids were tested simultaneously and in the same management. The results of these tests are presented since they enable a comparison between purebred O. niloticus and crossbreeds between this and other tilapia species.

The management of these growth tests consisted of polyculture and intensive manuring. All the ponds were stocked with common carp, silver carp, grass carp and freshwater prawns (Macrobrachium rosenbergii) as well as tilapias. The major nutrient input consisted of dry poultry manure applied six days per week at amounts increasing with increasing biomass of fish from 50 to 175-200 kg dry matter of manure per ha per day (Wohlfarth, 1978). In addition, relatively small amounts of 25% protein feed pellets were also applied daily.

RESULTS

Preliminary Observations. During 1980 several preliminary observations were carried out, aimed at identifying the earliest age at which sexual maturity becomes evident from external inspection. Three full sib progenies of O. niloticus and one of O. hornorum, spawned in the hatchery were each stocked into a separate earthen pond of 400 m² about a week after hatching. These ponds were drained when the fingerlings were between 55 and 85 days old and all the fish examined externally. Samples were also dissected in order to examine gonad development. The details of the observations are noted below:

Species	No. of fish		Age of fish	% males
	stocked	harvested	(days)	
<u>O. niloticus</u>	228	169	55	51
<u>O. niloticus</u>	172	163	75	68
<u>O. niloticus</u>	253	174	85	74
<u>O. hornorum</u>	272	235	65	52

In O. niloticus no breeding color or changes in the genital papillae were noted at either 55 or 75 days. Among the 85-day-old fish a few males were found with reddish colored fins and somewhat swollen papillae. Dissection showed that in 55-day-old fish the gonads were in the form of two tiny transparent threads, at 75 days sperm sacks were apparent in the testes and oocytes in an early vitellogenic phase in the ovaries. At 85 days motile sperm was found in the developed males and several females had oocytes in the vitellogenic phase. The 65-day-old O. hornorum were in a similar stage to the 75-day-old O. niloticus. Phenotypic variation in size was small, probably due to the low stocking rates and there was a preponderance of males among the larger fish. It was concluded that for weight selection the fish should be stocked at higher densities and selection carried out separately on females and males. Pre-maturation weight selection should be carried out at about two months, while selection for late maturity should be started at three to four months.

First cycle of selection. One-year-old individuals of O. niloticus of both sexes were introduced into spawning tanks in March, 1981. During April and May 16, full sib fry groups were spawned and hatched. These were pooled into several groups consisting of two or three individual spawns, which were stocked into seven earthen ponds of 400 m² each for nursing (Table 1). The number of fry per group varied between 930 to 1860. The parents of these spawns, 15 females and 11

males were kept in the hatchery to serve as the "original parent control groups". The first cycle of selection was carried out on these fingerlings, separately in each pond, as described under Methods, between 9 to 12 weeks after stocking. The sex ratio of the fry groups deviated considerably from the expected 1:1 ratio, proportions of males varying between 57% to 89% in different spawns. These unexpected deviations cannot be a result of differential mortality of females since survival was high in the nursing ponds (mean of 79%) and there is no apparent association between proportion of males and survival. As described under Methods, pre-maturation selection (Table 2) was carried out after two samples that had been taken, and not on the total populations in each pond. The selection intensities were computed accordingly, e.g., in males the total number of individuals was 5,113 but selection was carried out on 3,054 fingerlings, since the two samples taken previous to selection comprised 2,059 fingerlings. Since the total number of fingerlings selected was 181, the selection intensity was 5.9%. That of the females was a little lower. Selection distance, computed as the mean difference between weight of selected fish and that of the total population, was 8.9 g and 7.4 g for males and females, respectively. In both sexes the selected fish were ca 20% larger than the population mean. These fish comprised the "pre-maturation selection group".

At the end of the season, the seven ponds stocked with random samples of the original populations were drained and all of the fish removed and sexed. A random sample was taken from each sex of each group to serve as a pooled "random control group". From the remainder the largest individuals were selected, and the selected fish from the seven different ponds pooled to serve as the "post-maturation selection group". Details of this selection are shown in Table 3. The selection intensity for males was 4%. Selection in females was less intense, due to the small number of females on which selection was practiced (366 compared to 958 males) and the need for reasonable number of individuals to

avoid excessive inbreeding and random drift effects. As a result, selection intensity in females was 16%. The selection distance was 89 and 46 g for males and females, respectively, the selected fish of both sexes being ca 20% larger than the population mean.

Selection for late sexual maturity. Three of the ponds intended for selection for late maturity were drained when the fish were four-months-old. It was then found that all the fish showed external signs of sexual maturity, i.e., swollen papillae in both sexes and breeding coloration in all the males. It was concluded that variation in age at sexual maturity is small and that in small populations the likelihood of isolating deviants is low. The problems of producing large tilapia populations consisting of equal aged individuals has been explained above. Thence this line of investigation which did not appear in our original research proposal, was abandoned.

Second cycle of selection. Unexpected difficulties were encountered in obtaining spawning in the hatchery in 1982. The first spawns were either infertile or the number of fry was too small. Suitable samples of fry were obtained between May and July. These were progenies of nine spawns, some of which were pooled, resulting in six fry populations. The three fry populations spawned by fish selected as juveniles consisted of one, two or three individual spawns, respectively (Table 4A). The three fry populations spawned by fish selected as adults were all single spawns (Table 5A). Each fry population was stocked into a separate pond, about a week after spawning.

The technique of selection in this cycle was simplified, compared to the first cycle, since samples for selecting for late maturity were not taken.

Details of the juvenile selection are shown in Table 4. Selection was carried out between 53 to 60 days after the fry were stocked into the ponds, when their age was about 9-10 weeks. Survival of fry was higher for the two groups stocked earlier in the season (76%) than the group stocked in July (57%) -

see Table 4B. Prior to selection the fry of each group were sexed, in order to carry out selection separately in females and males. The number of males exceeded that of females in each group, ca 62% of the fish being males. Selection was carried out by visual inspection, ca 10% of the larger individuals being selected and the rest discarded. The mean weight of the fish at selection was 56 g, that of the selected fish being 8 g and 11 g higher than the sample mean for females and males respectively, see Table 4C. The mean weight of the males was a little higher than that of females in two of the ponds, whereas in the third (H-8) they were equal. The three samples of selected fish were pooled and grown together till the end of the season, when the mean weight of females and males reached 137 and 174 g, respectively.

Details of adult selection are shown in Table 5. Selection was carried out, again separately for females and males, when the fish were between 11 to 22 weeks of age. In one of three ponds (B-2) total mortality of tilapias has occurred, and not a single fish was found. This mortality occurred soon after stocking, when the fish were still very small, since dead fish were never seen floating in this pond. Survival in the other two ponds was lower than expected (see Table 5A). The number of males again exceeded that of females considerably (Table 5B). As in juvenile selection, 10% of the larger fish of each sex were selected and the rest discarded. The mean weight of fish at selection varied between the two ponds as a result of the difference in date of stocking. The mean weight of the selected fish was 25 g and 27 g higher than the sample mean for females and males, respectively (Table 5C). These selection differences are about three times as large as in juvenile selection, probably due to the higher mean weight of fish at selection. The mean weight of males also considerably exceeded that of the females.

Testing selection response. These tests involved the progenies of four groups of fish, two selected groups and two control groups:

1. Progenies of fish selected as juveniles.
2. Progenies of fish selected as adults.
3. Progenies of randomly sampled fish, taken at the time of adult selection.
4. Progenies of the original parents.

Two growth tests were carried out, estimating the response to one generation of selection (1982) and the cumulative effect of two consecutive generations of selection (1983).

First growth test. The four groups of fish were stocked, each group into a separate spawning pond, on April 29, 1982. Spawning took place in May, fry being found in these ponds between the end of May to the beginning of June. Counted samples of fry were transferred from the spawning ponds to nursery ponds on June 14. The fry were nursed for a month, during which period three of the groups reached a mean weight of between 11.1 to 13.5 g. In the fourth group (progenies of fish selected as juveniles) however, mean weight of the nursed fry was almost 25 g. On July 14-15 the nursed fry were stocked into 12 ponds of 400 m². Each test group was stocked into three ponds (replications) at a rate of 300 fry per pond (7,500 per ha). In addition all the ponds were stocked with 60 fry of a hybrid between O. mossambicus x O. aureus (which is distinguishable from O. niloticus by the absence of a striation on its caudal fin), 122 common carp, 40 silver carp and 15 grass carp. Total fish stocking was 537 per pond, (13,425 per ha). The ponds were also stocked with juvenile prawns (Macrobrachium rosenbergii) at rates varying between 400 to 800 per pond (10,000-20,000 per ha). The test was terminated between November 7-11, when the ponds were drained and all the fish removed, sorted, counted and weighed. The results are summarized in Table 6.

1. Mean weight gain of the four groups of tilapias varied between 144 g to 159 g per fish during the growth period of 117 days, equivalent to a

mean daily growth of between 1.23 to 1.37 g per fish. In a parallel test involving tilapia hybrids (in which O. niloticus acted as female parent) mean daily growth was considerably faster between 1.78 to 2.02 per fish.

2. The mean differences in weight gain among the four tested groups were relatively small. The highest weight gain was shown by progenies of fish selected as adults and the lowest by progenies of fish selected as juveniles. The two control groups showed intermediate weight gains.
3. Survival during the experimental period varied considerably between the groups, between 50% in progenies of fish selected as adults to 92% in progenies of fish selected as juveniles. In two control groups, survival was intermediate.
4. The proportional number of males was lower than the expected 50%, with large differences between the groups. In the progenies of fish selected as juveniles, only 27% of the fish were males whereas 51% of the progeny of original fish were males.
5. The weight of males was higher than that of females at the end of the test, as expected. However the mean weight difference between the sexes differed between the test groups, ranging from 87 g in progenies of random samples to 30 g in progenies of fish selected as juveniles.
6. There appears to be a negative association between survival and weight gain. Progenies of fish selected as adults showed the largest mean weight gain and the lowest survival, while the opposite was found in progenies of fish selected as juveniles.

These results are inconclusive and do not show any genetic response to selection. The relatively low weight gain of the progenies of fish selected as juveniles is presumably associated with the unexpectedly low proportion of males in that group. We do not have any reasonable explanation for this phenomenon

and cannot assume that it is a result of the selection carried out in their parents.

In a further test carried out in the same management and the same block of ponds, three different interspecific tilapia hybrids were compared. The females parent of these hybrids was O. niloticus and the male either O. aureus, O. hornorum or itself a hybrid between O. hornorum x O. aureus. Here we are not directly interested in the differences between tilapia hybrids but rather in a general comparison between tilapia hybrids in which O. niloticus acted as one parent to purebred O. niloticus. These hybrids consisted of male fish only. The results (Table 7) show that survival of hybrids was higher than the mean of the O. niloticus groups (96% compared to 72%). The mean growth increment of hybrids (225 g) was larger than the mean of purebred O. niloticus (154 g) or of their males (187 g).

Second Growth Test. The technique used was very similar to that of the first test. The brood fish were stocked into spawning ponds on April 21 and fry first observed on June 5. Counted samples of fry were transferred to nursery ponds on June 12 and nursed for about a month. At the end of the nursing period mean weight of fry of three of the test groups was between 10 and 13 g, while that of the fourth group was 6.5 g. On July 10 these nursed fry were stocked into 16 experimental ponds, each group being stocked into four ponds at a rate of 360 fish per pond (9000 per ha). The ponds were also stocked with 120 common carp fingerlings, 40 silver carp and 12 grass carp per pond and juvenile prawns (Macrobrachium rosenbergii) at stocking rates varying between 400 to 1,600 prawns per pond. Total stocking rate was equivalent to 12,300 fish plus 10,000 to 40,000 prawns per ha. The pond management, manuring and feeding, was similar to that practiced in 1982. The test was terminated between October 30 to November 3 and its results (Table 8) are summarized below:

1. The mean weight gains of the different groups were a little higher than in the previous year, ranging between 159 g and 183 g during the growth period of 110 days. This is equivalent to a mean daily growth of 1.44 to 1.66 g per fish.
2. The mean differences in weight gain between the tested groups were relatively small and not significant. The largest weight gain was shown by progenies of fish selected as adults and the lowest by the random control.
3. Survival was much higher than in the previous test, treatment means varying between 78% to 93%.
4. Final mean weight differences between the sexes were larger than in the previous test, between 82 g and 108 g, but variation between the groups was smaller.
5. The proportion of males was slightly higher than the expected 50% in three of the groups, but in the fourth (random sample control), only 40% of the fish were males. This is unlikely to be a result of differential mortality of females since survival was high (93%).
6. The lower mean weight gain of the random sample control group compared to the other three groups is most reasonably explained by the lower proportion of males in that group. Neither the male nor the female weight gains of the random sample control differed markedly from those of the other three groups.
7. The similarity in growth between the two selected group and the two controls indicate a lack of genetic response in growth to mass selection of the larger individuals.

As in the previous year, a comparative growth test of tilapia hybrids was carried out simultaneously and in the same block of ponds as the selection response test described above. Two tilapias hybrids were tested, O. niloticus x

O. aureus and O. niloticus x O. hornorum. Both of these hybrids consisted mainly of male fish. The results of this test (Table 9) show that the survival of the hybrids was a little higher than that of O. niloticus groups (98% compared to 87%). Their mean growth increment (243 g) was higher than the mean of the O. niloticus groups (173 g) or that of the O. niloticus males (219 g).

CONCLUSIONS

No real differences were found between the controls and the progenies of fish after one or two generations of selection, for weight. Selection response and realized heritability were zero. Presumably this lack of response is due to a low genetic variation for growth in the O. niloticus population on which selection was practiced, as a result of inbreeding. This hypothesis is supported by the observed slow growth of the O. niloticus groups comprising the selection experiment, compared to that of interspecific hybrids in which inbreeding could not have occurred. Tave and Smitherman (1980) concluded that the very low heritability for growth in their study was a result of inbreeding in the Auburn population of O. niloticus due to the restricted number of founder individuals. We believe that the lack of selection response in our study was due to the same cause. According to this logic the selection response in O. aureus (Bondari et al, 1983) is due to a larger genetic variation in the Tifton stock of O. aureus than in the O. niloticus stocks of Auburn and Dor. The number of founder individuals of that stock must have been restricted as a result of two transfers, first from Israel to Auburn and some generations later from Auburn to Tifton. Thence, it is not clear why inbreeding in that stock should be less pronounced than in the two O. niloticus stocks. Methods of increasing genetic variability by constructing an appropriate base population, and thereby increasing the likelihood of attaining a selection response are discussed below.

PROGENY TESTING TILAPIA BROOD STOCKS TO PRODUCE MONOSEX HYBRID POPULATIONS

Producing adequate number of marketable tilapias is primarily hampered by uncontrolled reproduction in grow-out ponds and by the slow growth of females. Several techniques have been evaluated for producing all male populations, by which both these problems would be solved. These include (Wohlfarth and Hulata, 1983):

1. Manual separation of males by visual inspection of secondary sexual characteristics.
2. Producing all male hybrid broods by interspecific crosses.
3. Sex reversal by feeding hormone treated feeds to undifferentiated fry.
4. Use of sex reversed brood fish of known genotype to produce monosex progenies.

Manual separation of sexes and discarding the females is wasteful, labor intensive and some mistakes in sexing always occur. Interspecific crosses do not consistently produce all male hybrid brood on a commercial scale. An example is the hybrid between female Oreochromis niloticus x male O. aureus, which is grown commercially in Israel. Stocks of these two species from different commercial hatcheries in Israel produce hybrid fry in which the proportion of males varies from below 60% to above 90%, but never 100% males. Inconsistent results have been postulated to be caused by "contamination" of the stocks or segregation of alleles due to natural polymorphism in sex-determining factors.

All male populations have been consistently produced with other interspecific crosses using species which are exotic to Israel, i.e., female O. niloticus x male O. hornorum and female O. mossambicus x male O. hornorum. These two hybrids were found to be inferior to O. niloticus x O. aureus, the standard hybrid in Israel, in commercially important traits such as growth rate, winter survival and skin coloration (Wohlfarth et al, 1983). Our approach was

attempting to establish proven brood stocks yielding all male populations of O. niloticus x O. aureus.

METHODS

The method chosen for this investigation is based on the observation that some, but not all, single pair spawns of the cross between female O. niloticus x males O. aureus yield all male hybrid progenies (Pruginin et al, 1975). A progeny testing program involving single pair spawns of this cross and selection of parent fish yielding all male hybrid broods could supply proven brood stock. However, the number of fish which is feasible to isolate by this technique is far smaller (different order of size) than the amount of proven brood stock required for commercial production. In order to reproduce the proven individuals, fish of both sexes are required in each of the two species, but the above progeny testing technique yields only females of O. niloticus and males of O. aureus. This problem could be solved by isolating proven females of O. aureus and males of O. niloticus, by an additional progeny testing program. Intraspecific spawns of proven individuals, selected from these two component progeny testing programs, could then provide commercial brood stock in sufficient numbers.

In order to select individual pairs from the progeny testing of O. aureus female x O. niloticus male pairs, an adequate selection criterion is required. This was obtained from the available knowledge in interspecific crosses in tilapias. In four of the crosses, potentially yielding all male broods, the reciprocal cross yielded hybrid broods whose sex segregation was 1 female : 3 males (Wohlfarth and Hulata, 1983).

The method for producing proven brood stock of tilapias, here investigated thus requires three generations of testing (see Figure 2):

1. A progeny testing program involving a series of single pair reciprocal crosses between O. niloticus and O. aureus. In crosses between female O.

niloticus x male O. aureus those parents are chosen whose hybrid progenies are all male. In the reciprocal cross the selection criterion is a 1 female: 3 male segregation in the hybrid progeny.

2. Reproduction of the selected fish by multiple intraspecific spawning

between chosen individuals, in each species.

3. Testing the procedure by multiple spawns between female O. niloticus x male O. aureus obtained in the second generation and examining whether these hybrid broods are all male.

The first generation of this program consisted of a large series of single pair reciprocal crosses. To achieve this we established spawning families consisting of 5-8 females of one species and one male of the other species. These families were stocked in 700 l plastic containers, equipped with internal gravel filter and thermostatically controlled heating elements. Through daily inspection brooding females were identified, and after 2-3 days they were gently caught in a fine mesh net and the eggs or larvae collected into a bowl and transferred for further incubation (Rothbard and Hulata, 1980). Each brood, identified by the serial number given to its parents upon first spawning, was reared separately in a small container and later in a cage suspended in large indoor concrete vessels to a size enabling sexing. This was either by external inspection of secondary sexual characteristics, or, more often, by direct inspection of dissected gonads under the microscope.

RESULTS

About 40 females and 10 males of O. niloticus of the Ghana strain, and 25 females and 15 males of O. aureus were used during the project. These fish were collected from various commercial tilapia hatcheries in Israel. During the last year, 12 females of O. niloticus from Egypt and 3 males of O. aureus from another Israeli source were also used.

Males were rotated among female groups, or replaced by new ones during the study. Males and females were discarded when results indicated that they did not meet selection criteria. Not all of the fish participated in spawning. Some males and females resided in the system without reproduction for long periods. A wide variation in spawning frequency of females stocked together in the same container was noted (Hulata et al, in press). A sharp decrease in spawning activity occurred subsequent to moving the project from Dor to the new facility at Bet Dagan (summer 1981).

More than 80 single pair hybrid spawns were obtained from crosses involving O. niloticus females and O. aureus males. About a third of these were lost due to infertility, technical problems during artificial incubation and later mortalities. Most problems occurred in the early phase of the project. Some occurred during the latest phase due to the absence of one investigator (G. H.) and the resignation of our trained hatchery technician.

Results of the surviving progenies, whose sexing has been completed by this time, are presented in Table 10. Females of O. niloticus originated from two commercial stocks: Ein Hamifratz (Marked with an asterisk in the table) and Gan Shmuel hatcheries. Males of O. aureus used in the early phase were from the Hazorea stock. When it was found that male nos. 372 and 176 did not produce all-male hybrid progenies, we rechecked that stock and found visual signs of contamination which were confirmed by electrophoretic analysis. All males were from the Ein Hamifratz stock. O. niloticus females (No. 16, 19, 21, 22, 23, 25) spawned with Hazorea males were lost in a fire in the hatchery and could not be retested.

As a result of this progeny testing we now possess 9 O. niloticus females and 4 O. aureus males which are considered "proven" as defined in the research plan. Our results confirm previous observation that in tilapia hybrids obtained from commercial mass spawns, the proportion of males is higher when the parent

fish of both sexes are from Ein Hamifratz stock than from Gan Shmuel. A few O. niloticus females from the Gan Shmuel stock were rejected, but none from the Ein Hamifratz stock.

One male (No. 14 of the Ein Hamifratz stock) was rejected since its progeny with an already "proven" O. niloticus female (No. 102) did not consist of males only. This exemplifies the possibility of progeny testing individuals by crossing with a previously proven mate.

About 50 single-pair hybrid spawns were obtained from the reciprocal crosses, involving O. aureus females x O. niloticus males. All parents used were derived from the Ein Hamifratz stock. About a third of these progenies did not survive to sexing, and others suffered from heavy mortalities. In fourteen progenies sexing is not yet completed due to their small size.

In these crosses, the selection criteria is not the absence of females but their frequency, the required proportion being 1 female:3 males. This statistical event is clearly sensitive to mortalities, as occurred in this study. All spawns sexed so far are listed in Table 11, including replicate spawns of the same parental pairs. In some cases these replicate spawns showed different sex segregations, e.g. the progeny of female O. aureus No. 3 x male O. niloticus No. 66, which segregated into 1:3 and 1:2 in replicated spawns. Four females (No. 3, 6, 10 and 315 showed approximately the required segregation in some of their spawns. These results are not considered reliable enough to define these females as "proven". For similar reasons the three males participating in these spawns did not give conclusive results.

Proceeding to the second phase of the breeding program was not possible by the end of the project. We hope to confirm some of the above mentioned candidates, after sexing of progenies is completed, so that this study can be continued.

CONCLUSIONS

We were not able to conclude this study due to unexpected technical difficulties. Therefore we are not in a position to prove or disprove whether the proposed method for producing proven brood stocks in tilapias, consistently yielding all male hybrids, is correct and applicable to commercial aquaculture.

In a recent study Majumder and McAndrew (1983) hybridized female O. niloticus x male O. aureus taken from natural stocks. The proportion of males varied between 65% to 86% in seven different crosses, but none produced an all male brood, as in previous studies. This is taken as proof for the existence of natural polymorphism of sex determining factors. Since it is not possible to solve this problem by periodically renewing stocks from natural sources without testing their crossbred performance, the only solution is by a breeding program.

The progeny testing scheme attempted in this investigation was discussed with colleagues on numerous occasions. In spite of some hesitations, no one suggested an alternative scheme, with better chances of success. We, therefore, regret that we could not complete the investigation within the given period, and intend to continue working on this model.

DISCUSSION

The conclusions generated from the two components of this investigation, noted separately at the end of each of the main chapters do not require repetition. Therefore this "discussion" takes the form of some short conclusive remarks.

The investigation was not intended to yield results of immediate practical application to aquaculture. Its aim was a study of two alternative but not mutually exclusive methods of genetic improvement in tilapias. A technique was developed for mass selection in tilapias, which could probably be applied to other non-synchronously spawning fishes. We also developed a model of selection by progeny testing for sex ratio in hybrid progenies, which may be suited to

producing monosex broods in other fish species. The result enable placing different strategies for selective breeding of tilapias in some order of priority.

The lack of response of our tilapia population to selection for faster
growth does not necessarily mean that tilapia populations in general cannot be improved by mass selection. The likelihood of attaining a selection response could probably be increased by constructing a population with a larger genetic variation, by crossing individuals from two or more distinct populations. Selection would then be carried out on the F_2 or F_3 generation of this populations.

Nevertheless it appears to us that the potential benefit to aquaculture of mass selection in tilapias may be less than in other species. This is due to the need of first constructing a base population as well as the cumbersome technique required for producing populations of equal aged individuals, on which to practice selection. Crossbreeding, within or between species, which is not beset by these problems may be a more efficient method of selective breeding.

We still believe in the progeny testing technique, which we developed for the production of brood stock, consistently yielding all male hybrid broods. We were not able to prove this, due to unforeseen technical problems involved. Since no alternative method has yet been proposed, we intend to continue with this scheme.

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Table 1. First selection cycle: Spawnings and pre-maturation growth phase (1981).

Date of transfer to pond	No. of pooled spawns	Pond No.	No. of fry in pooled group		% males	Date of draining for selection
			at stocking	at draining		
April 15	2	A-9	1249	1066	79	June 29
April 23	2	A-4	930	770	68	July 2
April 26	2	A-3	1013	702	67	June 29
April 29	2	A-6	1267	841	76	July 24
May 3	2	A-11	1130	883	89	July 14
May 3	3	A-2	1485	1188	81	July 15
May 8	3	A-8	1860	1576	57	July 20

Table 2. First selection cycle: Pre-maturation selection procedure (1961)

Pond No.	No. of fish	Av. Weight (g)	Random Sample		Late Maturation		Selected-fastest early growth		Selection Distance (g)	Selection Intensity (%)
			No. fish	Av. Wt(g)	No. fish	Av. Wt(g)	No. of fish	Av. Wt (g)		
A. Males										
A-9	837	36.3	165	37.2	165	39.2	21	45.2	8.9	4.1
A-4	525	42.2	105	42.0	105	45.0	13	51.5	9.3	4.1
A-3	467	36.8	93	36.6	93	40.1	14	41.4	4.6	5.0
A-6	638	57.1	128	59.2	128	65.0	19	75.8	18.7	5.0
A-11	787	42.2	157	42.5	158	45.8	39	49.7	7.5	8.3
A-2	968	42.1	195	41.0	195	46.7	48	50.8	8.7	8.3
A-8	891	47.0	186	47.7	186	50.6	27	53.7	6.7	5.2
Total	5113	43.4	1029	43.8	1030	47.6	181	52.3	8.9	5.9
B. Females										
A-9	229	30.5	45	31.5	45	35.7	5	42.0	11.5	3.6
A-4	245	37.2	48	37.5	49	41.6	6	45.0	7.8	4.1
A-3	235	34.3	47	33.8	47	38.7	5	44.0	9.7	3.5
A-6	203	45.9	40	46.8	40	51.0	6	60.0	14.1	4.9
A-11	96	34.8	19	37.4	19	37.9	5	38.0	3.2	8.6
A-2	220	35.6	43	37.9	43	40.0	11	39.1	3.5	8.2
A-8	685	37.2	138	38.8	138	39.7	21	43.8	6.6	5.1
Total	1913	36.7	380	37.8	381	40.5	59	44.1	7.4	5.1

Table 3. First selection cycle: Selection at adult size (1981)

Pond No.	Total No. of fish	Av. weight (g)	Random sample		Selected-biggest		adult size Av. Wt (g)	Selection Distance (g)	Selection Intensity (%)
			No. of fish	Av. Wt. (g)	No. of fish	Av. Wt (g)			
A. Males									
8-1	164	351	15	349	5	480	129		3.4
9-9	98	324	15	316	5	376	52		6.0
8-6	78	330	12	345	4	385	55		6.1
A-8	117	302	15	293	5	428	126		4.9
A-3	143	347	15	344	5	412	65		3.9
A-9	178	314	15	327	5	406	92		3.1
A-2	180	299	16	292	5	390	91		3.1
Total	958	323	103	323	34	412	89		4.0
B. Females									
8-1	35	185	10	192	8	208	23		32.0
8-9	42	159	7	177	8	186	27		22.2
8-6	54	210	15	204	8	251	41		20.5
A-8	32	153	10	152	5	192	39		22.7
A-3	17	156	5	150	2	200	44		16.7
A-9	43	159	10	166	8	201	42		24.2
A-2	143	153	15	148	8	228	75		6.3
Total	366	166	72	172	47	212	46		16.0

Table 4. Second selection cycle: Juvenile selection (1982)

A. Dates, no. of spawns and no. of fry per spawn

Pond	Date of		No. of spawns	No. of fry		Survival (%)
	stocking	selection		stocked	selection	
H-8	19.5	21.7	1	1478	1130	76.5
H-16	2.6	6.8	2	893		
				753		
				1646	1256	76.4
H-18	15.7	6.9	3	750		
				850		
				823		
				2423	1391	57.4

B. No. of fry stocked and selected

Pond	Female			Males			Female plus Males		
	total	selected	intensity	total	selected	intensity	total	selected	intensity
			(%)			(%)			(%)
H-8	430	42	9.8	700	62	8.9	1130	104	9.2
H-16	486	46	9.8	788	80	10.2	1256	126	10.0
H-18	525	54	10.3	866	88	10.2	1391	142	10.8
Total	1423	142	10.0	2354	230	9.8	3777	372	9.8

C. Weight (g) of fish at selection

Pond	Female			Males			Mean		
	total	selected	selection	total	selected	selection	total	selected	selection
	sample	sample	distance	sample	sample	distance	sample	sample	distance
H-8	53.2	62.9	9.7	53.3	64.5	11.2	53.3	63.7	10.4
H-16	73.4	78.5	5.1	80.5	90.6	10.1	77.0	84.6	7.6
H-18	37.1	46.7	9.6	40.1	53.0	12.9	38.6	49.9	11.3
Mean	54.6	62.7	8.1	58.0	69.4	11.4	56.3	66.1	9.8

Table 5. Second selection cycle: Selection of adults (1982)

A. Dates, no. of spawns and no. of fry per prawn

Pond	date of		No. of spawns	No. of fry		Survival (%)
	stocking	selection		stocked	selection	
H-2	10.5	11.10	1	2326	1501	64.5
B-2	15.7	12.10	1	1002	0	0
A-17	2.8	12.10	1	1835	848	46.2

B. No. of fry stocked and selected

Pond	Females			Males			Females plus Males		
	total	selected	intensity (%)	total	selected	intensity (%)	total	selected	intensity (%)
H-2	450	45	10.0	1051	107	10.2	1501	152	10.1
A-17	392	40	10.2	456	46	10.1	848	86	10.1
Total	842	85	10.1	1507	153	10.1	2349	238	10.1

C. Weight (g) of fish at selection

Pond	Females			Males			Female plus Males		
	total	selected	selection	total	selected	selection	total	selected	selection
	sample	sample	distance	sample	sample	distance	sample	sample	distance
H-2	147.3	182.0	34.7	188.9	234.0	45.1	168.1	208.0	39.9
A-17	38.0	53.5	15.5	58.0	67.6	9.6	48.0	60.0	12.6
Mean	92.7	117.8	25.1	123.5	150.8	27.3	108.1	134.3	26.2

Table 6. Testing the response to one generation of mass selection for growth in *Sarotherodon niloticus* (1992).

Progeny of	Pond	No. of fish at the end of test	%	Mean weight (g)				Weight gain (g)					
				males stocked		at end							
				Females	Males	Females	Males	Females	Males				
				Total survival (%)				Mean	Mean				
Original	15	73	106	179	59.6	10.4	148.2	180.6	167.4	137.8	170.2	157.0	
Parents	22	100	120	220	73.3	45.4	11.1	141.3	204.5	170.0	130.2	193.4	158.9
	32	90	91	181	60.3	49.7	11.9	142.6	204.0	173.1	130.7	192.1	161.2
Mean		87	106	193	64.4	51.4	11.1	144.0	196.3	170.1	132.9	185.2	159.0
Random	25	134	101	235	78.3	42.9	12.3	131.2	232.4	174.7	118.9	220.1	162.4
Sample	29	158	104	262	87.3	39.7	12.9	129.6	220.1	165.5	116.7	207.2	152.6
	28	150	91	241	80.3	37.7	12.4	128.9	197.5	154.8	116.5	185.1	142.4
Mean		147	99	246	81.9	40.1	12.5	129.9	216.6	165.0	117.3	204.1	152.5
Selected	18	102	72	174	58.6	41.3	13.7	153.6	211.5	177.6	139.9	197.8	163.9
Adults	23	60	36	96	32.0	37.5	13.5	162.3	190.6	172.9	148.8	177.1	159.4
	30	117	63	180	60.0	35.0	13.4	146.3	222.0	173.1	132.9	208.6	159.7
Mean		93	57	150	50.0	37.9	13.5	154.0	208.0	174.5	140.5	194.5	161.0
Selected	19	198	65	263	87.6	24.7	23.5	162.4	197.3	171.1	138.9	173.8	147.6
Juveniles	17	201	84	285	95.0	29.4	26.0	149.2	179.2	158.0	123.2	153.2	132.0
	20	204	78	282	94.0	27.6	24.0	169.6	193.6	176.2	145.6	169.6	152.2
Mean		201	76	277	92.2	27.2	24.5	160.4	190.0	168.4	135.9	165.5	143.9

Table 7. Performance of three interspecific tilapia hybrids, 1982.

Tilapia	Pond	(%)	Mean Weight		Weight	Gain
hybrid	No.	Survival	Initial	Final	Total	Daily
S.n	12	99	14.1	204	190	1.62
x	21	100	18.1	234	216	1.84
S.a	27	91	16.7	239	222	1.89
Mean		97	16.3	226	209	1.78
S.n	11	101	24.6	221	196	1.67
x	16	95	27.2	266	239	2.04
S.h	26	94	18.3	270	252	2.15
Mean		97	23.4	252	229	1.95
S.n	13	96	22.2	260	238	2.03
x	14	96	23.0	259	236	2.02
S.(hxa)	24	89	21.8	258	236	2.02
Mean		94	22.3	259	237	2.02
Grand Mean		96	20.7	246	225	1.92

S.n = *Sarotherodon niloticus*, S.a = *S. aureus*, S.h = *S. hornorum*,

S. (hxa) = Hybrid *S. hornorum* x *S. aureus*.

Table 8. Testing the response to two generations of mass selection in *S. niloticus*

Progeny of	Pond No.	No. of fish at the end of test				males %	Mean weight (g)		Weight gain (g)			
		Total survival (%)		stocked	et end		Weight gain (g)					
		Females	Males				Females	Males				
Original Parents	21	154	181	335	93.0	54.0	16.1	146.8	270.2	213.5	130.7	254.1
	24	169	170	339	94.2	50.1	10.0	125.0	215.4	170.3	115.0	205.4
	29	185	159	344	95.5	46.2	17.0	146.5	249.3	193.6	129.5	232.3
	31	148	169	317	88.0	53.3	11.1	127.4	241.4	184.6	116.3	230.3
	Mean	164	170	334	92.7	50.9	13.5	136.4	244.1	190.5	122.9	230.6
Random Sample	22	211	127	338	93.9	37.5	11.8	142.3	228.4	174.6	130.5	216.6
	23	217	113	330	91.6	34.2	12.7	148.5	232.9	177.5	135.8	220.2
	33	189	150	339	94.2	44.2	13.4	127.2	211.4	164.4	113.8	198.0
	32	195	141	336	93.3	42.0	12.1	139.2	214.9	170.9	127.1	202.8
	Mean	203	133	336	93.2	39.6	12.5	139.3	221.9	171.8	126.8	209.4
Selected Adults	25	131	179	310	86.1	57.7	9.0	143.4	224.6	201.8	134.4	215.6
	19	125	166	291	80.8	57.0	9.0	145.1	248.6	204.1	136.1	239.6
	28	148	163	311	86.4	52.4	10.4	117.5	229.4	176.1	107.1	219.0
	30	147	173	320	88.8	54.1	9.5	129.4	239.2	188.7	119.9	229.7
	Mean	138	170	308	85.5	55.2	9.5	133.9	235.5	192.6	124.4	226.0
Selected Juveniles	27	81	117	198	55.0	59.1	6.6	119.1	190.4	161.2	112.5	183.8
	26	135	175	310	86.1	56.5	6.1	126.1	220.8	179.5	120.0	214.7
	34	127	141	268	74.4	52.6	6.3	128.7	226.2	180.0	122.4	219.9
	20	132	220	352	97.7	62.5	7.0	128.9	225.9	169.4	121.9	218.9
	Mean	119	163	282	78.3	57.8	6.5	125.7	215.8	177.5	119.2	209.3

Table 9: Performance of two interspecific tilapia hybrids, 1983.

Tilapia hybrid	Pond No.	Survival (%)	Mean Weight(g)		Weight gain(g)	
			Initial	Final	Total	Daily
S.n	16	99	13.7	271	257	2.34
x	12	101	13.7	280	266	2.42
S.a	17	94	14.1	250	236	2.15
	11	104	13.8	215	201	1.83
	Mean	100	13.8	254	240	2.18
S.n	13	96	22.6	277	254	2.31
x	14	97	19.9	236	216	1.96
S.h.	15	95	21.1	276	255	2.32
	18	98	21.8	282	260	2.36
	Mean	97	21.4	268	247	2.24
Grand Mean		98	17.6	261	243	2.21

S.n = *Sarotherodon niloticus*, S.a = *S. aureus*, S.h = *S. hornorum*

Table 10. Sex of single pair progenies of O. niloticus x O. aureus
(m=males only, m+f=males and females in progeny. Number
of replicates in parentheses. * = Ein Hamifratz stock

<u>O. niloticus</u> females	<u>O. aureus</u> males						
	No. 372	No. 176	No. 55	No. 19	No. 14	No. 11	No. 5
No. 16	m+f(4)	m+f(2)					
No. 19	m+f(3)	m+f(3)					
No. 21		m+f(2)					
No. 22	m+f	m+f(2)					
No. 23*	m	m+f					
No. 25*		m+f					
No. 28*						m	
No. 30*			m				
No. 33*			m				
No. 41*			m				
No. 42				m			
No. 48	m+f						
No. 49	m+f	m+f					
No. 57				m			
No. 68*							m(2)
No. 75					m+f		
No. 102				m	m+f(2)		
No. 161						m	
No. 162						m	

Table 11. Sex segregations (females : males) obtained in single pair
progenies of S.aureus x S. niloticus. (Progenies marked
with an asterisk suffered from high mortalities

S. aureus females	S. niloticus males			
	No. 33	No. 37	No. 66	No. 7
No. 2			1 : 7*	
No. 3		1 : 2*	1 : 3*	
			1 : 2*	
No. 4			1 : 1	
			1 : 20*	
No. 5			3 : 2	
No. 6		1 : 3*	5 : 3	
			3 : 2	
No. 10		2 : 5		
		1 : 2*		
No. 315				1 : 4

Figure 1. Procedure of selection performed in each pond, on males and females separately.

Figure 2. Procedure for progeny-testing *Oreochromis niloticus* brood stocks, producing all-male interspecific hybrid broods.

UNITED STATES

SELECTIVE BREEDING OF TILAPIA AND CATFISH

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ABSTRACT

Mass selection improved body weight of Tilapia aurea but not that of T. nilotica. Realized heritability and increase in body weight for T. aurea and T. nilotica were 0.24, 16%; and -0.10, -7%, respectively. The different responses from the two species might have been related to different breeding histories. Androgen-induced sex-reversal, progeny testing, and subsequent breeding programs for production of monosex female populations of T. nilotica were successful. Estrogen-induced sex-reversal, progeny testing, and subsequent breeding programs for production of monosex male populations of T. aurea and T. hornorum were not successful because of difficulties in effectively sex-reversing genetic males to functional phenotypic females. Hormone treatment by the administration of methyltestosterone was a most promising program for producing all-male populations and this technique elicited an anabolic response in treated individuals. Isozyme variability allowed identification of T. aurea, hornorum, mossambica, and nilotica. This variability was used to determine parentage and genomic changes in red tilapia. The level of heterozygosity at loci encoding enzymes was greatest in Nile strain of T. nilotica, intermediate in Ghana strain, and least in Ivory Coast strain. Species differences in the quantity of genetic variation detected through electrophoretic assays may have been related to the contrasting selection responses of T. aurea and T. nilotica.

Mass selection improved body weight in three strains of channel catfish. The realized h^2 and increase in body weight ranged from 0.24-0.50 and 12-18% in the three strains. Crossbreeding also improved body weight in channel catfish but the tested strains had different combining abilities; 50% of the crosses resulted in overdominant growth by the F_1 . In field tests the best select line, crossbreed, and interspecific hybrid (channel catfish x blue catfish) had similar growth rates. Select lines of channel catfish had significant changes from the randomly bred control populations in isozyme allele frequency. Analysis indicated these changes resulted from both selection and random genetic drift. The selection program for increased body weight in channel catfish culminated in the first release of genetically improved fish to U.S. fish farmers.

INTRODUCTION

The husbandry of an aquatic organism cannot reach its potential until fish culturists have control of the biology of that organism. Genetic control is one aspect of that biology. Until now, modern genetic theory has contributed little to the improvement of farmed fish. Consequently, aquaculture is generally based on non-improved breeding stocks. Research problems requiring elucidation involve the basic genetic mechanisms of important farm-reared fishes of Israel and the United States - tilapias and catfishes, and manipulation of these mechanisms to increase production efficiency in aquaculture.

Specific objectives of the research were (1) to test the feasibility of production of monosex male tilapia fry through (a) direct hormone sex reversal, (b) breeding of hormone sex-reversed brood fish, and (c) interspecific hybridization; (2) to compare crossbreeding and hybridization with mass selection of tilapias and catfishes for improvement of growth; and (3) to apply electrophoresis for (a) stock identification, (b) maintenance of stock purity, and (c) tracing parentage.

TILAPIA

BIDIRECTIONAL MASS SELECTION FOR GROWTH IN TILAPIA NILOTICA

Relatively few selection experiments have been done for improving fish growth rates. Ch'ang (1971) reported realized heritabilities for selection in the positive direction of 0.10 and 0.16 for adult male and female T. mossambica grown in aquaria. Moav and Wohlfarth (1976) demonstrated that common carp, Cyprinus carpio, had a realized heritability of zero for mass selection in the positive direction. Kincaid et al. (1977) obtained a realized heritability in 147-day-old rainbow trout, Salmo gairdneri, of 0.06 for individual selection and 0.26 for family selection. Dunham and Smitherman (1983), using mass selection, found realized heritabilities ranging from 0.24 to 0.50 with standard errors of 0.06 to 0.13 in 3 strains of market-size channel catfish. Bondari (1983), using a combination of crossbreeding, family selection and mass selection, found a heritability for high body weight in channel catfish of 0.10. Bondari et al. (1983) reported a heritability of 0.23 ± 0.05 when selection for high body weight was applied on 2-year-old T. aurea.

The present study deals with T. nilotica which has not been tested for response to selection for rapid growth. However, Tave and Smitherman (1980) estimated, by sib analysis, that the heritability for rapid growth in 45- and 90-day-old T. nilotica of the same strain was insignificant. Few, if any, comparisons between predicted and realized heritabilities of fish have been made.

The present study was done on early growth of fish for several reasons. If fish were selected prior to maturity, then variation created by the effect of maturation-related hormones on the growth of the fish would be minimized (Kirpichnikov, 1981). Further, prematuration selection would obviate the labor of separating sexes prior to selection. Furthermore, if fish could be selected

early for growth, then valuable space and time could be saved by not maintaining fish which would be culled at a future date.

The objectives of the present study were three fold: to obtain a realized heritability for rapid early growth in T. nilotica (Ivory Coast) by measuring the response to selection for this trait in the same strain; to compare this to Tave and Smitherman's (1980) predicted response to selection for the same trait; and to test for response to indirect selection for late growth, by growing to market size, progeny of fish mass selected for early growth.

METHODS

History of brood stock. T. nilotica from Auburn University stock (Ivory Coast strain) were used. The Auburn stock was founded in 1974 when 100-200 fingerlings produced by 5-10 pairs of brooders were obtained from The Centro de Pesquisas Ictiologicas, Pentacoste, Ceara, Brazil. The Brazil station had previously founded their stock with 50-100 fish obtained from the Station de Recherches Piscicoles, Bouake, Ivory Coast in 1971 (Tave and Smitherman 1980).

In 1981, a base population (F_1 generation) was created from which to select fish. In 1982, the response to selection was obtained from progeny (F_2 generation) derived from the select and control parents. In 1983 the F_2 progeny of select and control parents were grown to market size.

F_1 generation. The primary goal of the 1981 production phase was to generate a large number of fry of the same age to stock in a common environment to grow for a maximum of 60 days. Wohlfarth et al. (1981) demonstrated that T. nilotica were sexually immature prior to 60 days of age in a climate similar to that at Auburn, Alabama, U.S.A. About 10,000 fry were needed as a base population from which the parents of the F_2 generation could be selected in adequate numbers and at a relatively high selection intensity. The selection was to be done on a population of fish at a stocking rate a commercial farmer might use for fingerling production.

One 0.04-ha earthen pond, that had been fertilized and limed (Boyd 1979), was stocked with 10,000 fry (250,000 fish/ha) 8-9 mm in length. An initial size differential in carp fry at stocking becomes magnified with time (Moav and Wohlfarth 1974; Wohlfarth and Moav 1970; Wohlfarth and Moav 1972). Thus, it was deemed essential that the tilapia fry produced for this study be the same age and size at stocking order to minimize environmental variation. Preliminary observations indicated that newly hatched fry grew 1 mm a day for the first 4-5 days in hatching jars without feed and at a water temperature of 29°C. Thus, fry which were within 1 mm in length were considered within one day of age.

Fry and fingerlings were fed ground catfish feed (32% crude protein) throughout the growing period. Feed allowances were derived empirically by sampling the fish population every two weeks, and adjusted every other week using projected weight gain. For the first two weeks of the growing period, fry were fed at 15% of their body weight. Thereafter, fish were fed at 5% of their body weight.

Fingerlings were harvested 11 September, 1981, 58 days after hatching. One day prior to harvest, a random sample of 76 fish was taken from the pond to determine, with the use of gonadal squash-smear analysis (Guerrero and Shelton 1974), if there was a size differential due to sex. An estimated 500 females from each select group were needed to produce adequate numbers of F₂ fry for determining response to selection. Thus, about 1000 of both the largest and the smallest fingerlings were selected for high line (H) and low line (L) broodstock.

On 15 October, all fish were transferred to an overwintering facility. This facility included cylindrical tanks (1.1 m³) containing reservoir water that was maintained at 20°C, aerated with compressed air, and exchanged weekly. Fish were fed daily with trout feed (36% crude protein) at 1% of their body weight. On 30 April, the fish were separated by sex and transferred to outdoor plastic

pools and concrete tanks for further growth. Sex ratios in each select group were calculated for comparison with results of the squash-smear analysis.

F₂ generation. The goal of F₂ fry production was to produce large numbers of fry simultaneously within the H, L and control (C) groups. Fry in the C group were produced by a repeat spawning of the F₁ parents. Brood fish from each group were stocked into circular plastic pools (7.3 m²; 0.7 m deep) and rectangular concrete tanks (20 m²; 0.75 m deep).

Twelve 0.02-ha earthen ponds that had been limed and fertilized (Boyd 1979), were used for growing the F₂ generation. Each pond was stocked with one grass carp (Ctenopharyngodon idellus), weighing about 1 kg, to control aquatic weeds.

Ponds were randomly assigned to H, L and C treatments. Each pond was stocked with 5,000 fry (250,000 fry/ha). There were 5 replicates of the H and C treatments, and only 2 replicates of the L treatments due to insufficient numbers of fry.

Feed allowances were derived empirically by sampling the fish population every two weeks, and adjusting every other week using projected weight gain. Feed allowances for all ponds were based on the mean weight of the fastest growing group (treatment) of fish at each sampling. The fish in all the ponds were fed the same quantity of ground catfish feed (32% crude protein), beginning at 15% of body weight and decreasing to 4% of body weight.

All fish were harvested 59 days after hatching. Total and mean weights of fish were determined for each pond.

On 7 June, 1983, fish of the C and H lines were communally stocked with male, sex-reversed T. nilotica in two 0.04 ha ponds (El Gamal 1984). Each pond was stocked with 300 fish; 100 fish from each group were used. The sex-reversed fish were added to increase the stocking density of the ponds to a commercial level. Pectoral fins, right and left for C and H, respectively, were clipped to distinguish the groups. The mean stocking weights (g) of the C and H fish were

59 and 55, respectively. The fish were offered catfish feed (32% protein) daily ad-libitum. The fish were sampled on 15 July and harvested on 24 September, after 109 days.

RESULTS

F₁ generation. The mean weights (g) of the fish sampled at 21, 35, 42 and 58 days of age were 0.4, 2.2, 3.9 and 9.2, respectively.

A total of 8707 fingerlings were harvested, a survival rate of 87%. The total weight was 2005 kg/ha with a mean fish weight of 9.2 g. The largest 15% and the smallest 16% of the population were retained as H and L brood stock. The mean weights of the H and L fish were 14.4 and 6.7 g, respectively. The selection differentials for the H and L fish were 5.2 and 2.5 g, respectively. The gonadal squash-smear analysis of 76 fingerlings randomly sampled one day prior to harvest, indicated no difference ($P < 0.01$) between the mean lengths of males and females.

Fish within each select group were separated by sex on 30 April when they were 7 months old. There was a significant difference in sex ratios of the groups ($P < 0.01$). The H group was comprised of 643 males and 471 females (1.4 males:1 female) while the L group was comprised of 399 males and 591 females (1 male:1.5 females).

F₂ generation. About 50,000 fry each were harvested from H and C groups, but only about 15,000 fry were harvested from the L group. Apparently, the lower production in the L group was due to a lower frequency of spawning, probably because of the relatively small mean size of the brooders (43 g).

Mean weights (g) adjusted for survival of the H, L and C groups of fish were 7.1, 6.7 and 7.6, respectively (Table 1). The difference between the C and H population means was insignificant ($0.10 > P > 0.05$), but the difference between the C and L means was significant ($P < 0.01$). The realized heritabilities for selection in the positive and negative directions were -0.10 ± 0.02 and $0.36 \pm$

0.08, respectively. Data from one control pond were excluded from analysis, because a very high survival rate (6,658) indicated that the stocking rate for this pond was high relative to the mean rate (4,883) for all the ponds.

The mean weights of the C and H lines at the time of sampling at harvest are in Table 2.

Analysis of variance showed no difference ($P < 0.05$) between the mean weights of C and H fish.

DISCUSSION

Sex ratios in select groups. Gonadal squash-smear analysis data, on fish sampled at 56 days of age (1981), indicated that males and females were of the same mean size. However, these indications proved to be incorrect when the fish were separated by sex at 7 months of age (1982). The male:female ratio in the high line (58% male) was the opposite of the male:female ratio in the low line (60% female).

Response to selection. A realized heritability of -0.10 ± 0.02 for rapid early growth of T. nilotica is similar to the predicted heritability of 0.04 ± 0.14 and 0.04 ± 0.06 , (Tave and Smitherman 1980) for rapid growth in 45- and 90-day-old fish, respectively. Both indicate the impracticality of selecting for rapid early growth in the Auburn (Ivory Coast strain) stock of T. nilotica. An asymmetric response to selection in the positive and negative direction was found also in T. mossambica (Ch'ang 1971) and common carp (Moav and Wohlfarth 1976), though a symmetric response was found in T. aurea (Bondari 1983). A lack of response to selection in the positive direction indicates that a selection limit has been reached, but response to selection in the negative direction implies that there is still additive genetic variance within this population's genome (Hartl 1980; Roberts 1966). The establishment of an upper selection limit may have been influenced by several factors:

1. The Auburn stock of T. nilotica was founded with 100-200 individuals derived from a few pairs of spawners which in turn were produced by only a few pairs of spawners. These small founder populations may have lost, by chance, alleles favorable for growth rate (Hartl 1980). This would decrease the initial amount of variability on which selection could be based. Loss of additive variation due to inbreeding, following these population "bottle necks", is probably insignificant. As of 1982, an inbreeding coefficient (F) of 6 - 11% has been calculated for this stock, if one assumes an initial F of 5 - 10%. However, preliminary electrophoretic data indicates that Ivory Coast strain of T. nilotica is highly homozygous. Selection in the positive direction tends to occur inadvertently during the domestication process (Dunham 1981; Reisenbichler and McIntyre 1977). Eight years (generations) of domestication may have added to a reduction in potential response to selection in the positive direction. Domestication of tilapia at Auburn University probably involves selection for cold tolerance which might be positively correlated with fast growth.
2. Natural selection for fast growth and large size may also contribute to a reduction in additive variance as is speculated for common carp (Moav and Wohlfarth 1976; Kirpichnikov 1981).

The response to indirect selection for rapid late growth is similar to the response to selection for rapid early growth. In both cases, the mean weights of C and H fish were not different. This suggests that, as in channel catfish (Dunham and Smitherman 1983), growth superiority that is gained early, is not lost during late growth.

Mass selection in the positive direction for rapid prematuration growth with concomittant indirect selection for rapid late growth in the Auburn stock of T. nilotica (Ivory Coast strain) is not practical.

RESPONSE TO BIDIRECTIONAL SELECTION FOR BODY WEIGHT IN BLUE TILAPIA

Most of the breeding research for blue tilapia (Tilapia aurea) to date has been in the areas of sex reversal and hybridization between species for the production of all-male progeny (Guerrero 1975; Pruginin 1975; Jensen and Shelton 1979). Tave and Smitherman (1980) estimated heritability for body weight in the Ivory Coast strain of the related species, T. nilotica, to be $h^2 = 0.04 \pm 0.08$ at 90 days through full-sib, half-sib analysis. Teichert-Coddington (1983) confirmed these results in the positive direction but not in the negative direction. He found that h^2 was -0.10 for increased body weight and 0.36 for decreased body weight.

METHODS

Blue tilapia (Tifton strain), used in this study, were second generation offspring from a foundation stock transferred from Auburn, Alabama to the Coastal Plain Station, Tifton, Georgia in 1977. The Auburn strain originated from Israel in the early 1960's. The fish were hatched in the summer of 1979 and were maintained in indoor tanks for the first year. At 12 weeks of age, the mean body weight and total length ($\pm$ SD) determined from a random sample of 50 unsexed fish were 30 ± 12 g and 117 ± 15 mm.

In the summer of 1980, 3 cages of tilapia (250 fish/cage) were transferred to a 2-ha reservoir. Culture methods and facilities used have been described by Bondari (1980; 1982). The fish were placed indoors for overwintering in circular fiberglass tanks near the end of November, 1980. Mean $\pm$ SD for body weight and total length determined from a random sample of 30 females and 30 males were 269 ± 57 g, 238 ± 14 mm, 528 ± 96 g, and 290 ± 15 mm, respectively.

At 22 months of age (April, 1981), 3 body weight lines were developed from a base population of approximately 600 fish. A random sample of 25 females and 25 males was first taken to represent an unselected control line (C). The 25 largest females and 25 largest males were then selected from the remaining

tilapia to represent the High (H) body weight line. The 25 smallest females and 25 smallest males were also selected to be designated Low (L) body weight line. All 150 selected and control fish were anesthetized with tricaine methanesulfonate, the upper jaw (premaxilla) of each male was surgically removed with a pair of scissors according to Lee (1979), and the fish were individually weighed and measured for total length. Brood fish from each of the 3 body weight lines were spawned by mass and pair matings.

Two random samples of 75 fry each/line, produced from the mass mated fish, were transferred to 6 indoor rectangular tanks at 4 weeks of age. Two sets of full-sib fry/line, produced from the pair-mated fish, were also reared in indoor tanks (75 fry/tank) to pool the data for statistical analysis. The fry remained in these tanks to reach 40 weeks of age. All fish were grown equal lengths of time at equal temperatures. A random sample of 25 fish/tank were individually weighed and measured for total length at 16, 26, and 40 weeks of age. The fish were fed a commercial trout ration (37.5% crude protein) ad-libitum 3 times daily.

At 40 weeks of age, 60 fish from each of the 3 lines (30 ♀ and 30 ♂) were fin clipped and placed together in one 1.27 cm mesh Vexar® cage (86 x 89 x 122 cm). The cage was placed in the 2-ha reservoir. The fish were fed a commercial trout ration to satiation twice daily. All fish were individually weighed and measured for total length. A random sample of 6 females and 6 males/line were sacrificed at the end of the cage test to determine carcass yield.

A second test was conducted at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University. The 3 lines were initially stocked with 40 fish each in 4 replicate plastic pools (3 m in diameter and 1 m deep). On June 10, these fish were transferred to 4 replicate cages/line in a one-ha pond to allow faster growth by the tilapia. The fish were fed 36% protein floating catfish feed to satiation once daily. The fish were grown 9 weeks and then harvested.

Statistical analyses included least-square analysis of variance and comparison of mean (Barr et al., 1979). Line and tank were the sources of variation for the tank culture data; whereas, tank, line, sex, and line X sex interaction were sources of variation included in the model to analyze data from cage cultures. Standard errors for realized heritabilities were calculated using formulas of Prout (1962).

RESULTS

Results from fry culture in indoor tanks showed no significant differences in 16-week body weight or total length of fish in H and C lines, but differences were observed at subsequent ages (Table 3). A clear divergence occurred between H and L and between C and L lines by 40 weeks. Asymmetrical responses to selection in the low direction were strongly evident for body weight (25 vs. 7%) and total length (8 vs. 2%) at 16 weeks of age but were less pronounced thereafter. Changes in total length should be regarded as a correlated response to selection since no direct selection was carried out for this trait.

From 16 to 26 weeks of age, the H line continued as upward trend and the fish were 27% heavier and 7% longer than those in the C line. The L line, however, continued a downward trend in this period and the fish were 29% lighter and 11% shorter than in the C line. These percentages changed to 20 and 5 for the body weight and total length of the H line at 40 weeks and to 26 and 8 for the body weight and total length of the L line, respectively. The divergence rate between H and L lines during tank culture ranged from 0.68 to 0.99 SD for body weight and 0.67 to 1.05 SD for total length (Table 3). Realized heritability estimate for 40-week body weight of the fish in the H and L lines (sexes combined) were 0.14 and 0.17, respectively. Corresponding values for the 40-week total length were 0.22 and 0.17, respectively.

Cage culture. Females and males from the H line located at Tifton continued to respond and were 21 and 12% heavier than those in the C line.

Corresponding values for the total length were 5 and 6%. When the 2 sexes were combined, the fish from the H line were 16% heavier and 6% longer than those from the C line. A surprising result was that tilapia from the L line were comparable in body weight and total length to those from the C line at the end of the cage test. No significant line differences were observed for condition factor and dressing percentage of the female and male tilapia. Male tilapia were 58% heavier (278 vs. 176 g) and 14% longer (237 vs. 207 mm) than female tilapia. Condition factor and dressing percentage of the males were greater than females ($P < 0.05$). The increase in dressing percentage by males was found only in the control line.

H line results were similar at Auburn. L line results were quite different. When the 2 sexes were combined, weights of H, C, and L were 258, 224, and 156 g, respectively. Increase by males, 20%, was higher than that of females, 7%.

Realized heritability estimates, computed from the ratio of standardized response/standardized selection differential, are shown in Table 4 for body weight or total length. Heritability for increased body weight was relatively low. Heritability for increased body weight was 0.24 ± 0.07 and 0.23 ± 0.05 at Tifton and Auburn, respectively. Heritability for decreased body weight was 0.07 ± 0.06 and 0.43 ± 0.05 at Tifton and Auburn, respectively. Uniformity of size, measured by coefficient of variation, was greater in the H and L lines than the control line in both locations (Table 4). Range of size was greater in the control fish.

DISCUSSION

A significant response to selection was obtained, indicating mass selection in T. aurea will be effective in producing larger fish. This is in contrast to the heritability for increased body weight in T. nilotica, Ivory Coast strain, which is not significantly different from zero (Tave and Smitherman 1980; Teichert-Coddington 1983). An important consideration in contrasting the results of these experiments is the history of the strains used. T. aurea

(Tifton) and T. nilotica (Ivory Coast) have both been transferred twice to reach their present hatcheries. Loss of genetic variability through founder effects could be different in the two species. Isozyme variability of the basic Auburn stock of T. aurea is greater than that of the Ivory Coast strain of T. nilotica (Brummett 1982) and might be indicative of the additive genetic variance present for selection.

The divergence of the various lines occurred during the early growth stages. After this initial divergence, growth rates are similar. Dunham and Smitherman (1983) reported that differences emerged early between selects and controls and remained constant for the remainder of the growth period in some strains of catfish. This phenomenon has important implications for the development of selection procedures, experimental procedures, and the use of multiple rearing technique (Moav and Wohlfarth 1973; Dunham et al. 1982b). If procedure requires that fry or fingerlings are reared or nursed to similar sizes for the start of experimentation, early growth differences may be lost unless there is compensatory gain. However, in a practical fingerling production system such rearing practices may be standard.

SEX RATIOS OF PROGENY FROM MASS SPAWNINGS OF SEX REVERSED BROODSTOCK OF TILAPIA NILOTICA

Tilapias of the family Cichlidae have many desirable aquacultural characteristics but a major drawback is their ability to quickly overpopulate production ponds because they mature sexually at a small size, and many of their offspring survive to compete with their parents for available food and space (Hickling, 1963). One method of minimizing this unwanted reproduction is to produce monosex populations by breeding hormone sex-reversed broodstock (Jensen and Shelton, 1979). This procedure is based upon the theory that tilapias have a single set of sex chromosomes (Hickling, 1960; Chen, 1969; Jalabert et al, 1971); however, Avtalion and Hammerman (1978) and Hammerman and Avtalion (1979) suggested that tilapias have a more complex sex determining mechanism.

The use of sex hormones to sex reverse tilapias is well documented. In the present study, we tested spawning of normal T. nilotica females with sex-reversed males as a means of producing large numbers of monosex (female) offspring. ~~Production of all-female offspring was compared when different~~ genetic groups of female brooders were used.

METHODS

The stock of T. nilotica at Auburn University was obtained from Brazil in 1974; the Brazilian stock had been imported from the Ivory Coast in 1971. The Nile tilapia is native to fresh and brackish waters from Syria and Egypt, to parts of West Africa and much of East Africa to Zaire (Philippart and Ruwet, 1982).

Production of the sex-reversed male broodstock. To produce the sex-reversed male broodstock for the mass spawning experiment, enough all-female fry were needed for androgen sex reversal. A functionally sex-reversed male T. nilotica that was first tested by Hopkins (1979) was respawned in an aquarium with three normal females in the summer of 1979. All spawns were kept separate by removing the fertilized eggs from the mouth of the brooder after 3 days and were then incubated artificially in a water-filled bowl on a shaker table (Lee, 1979). After hatching and yolk-sac absorption, fry were transferred from the incubation bowl to a stainless steel nursery trough (127 l) with an inflow (4 l/min) of filtered water. One-hundred seventy fry obtained from two spawns of the same female (half-siblings) were subjected to the sex reversal treatment. Fry that were not selected for sex reversal were fed ground trout feed for later sexing by the gonadal-squash method (Guerrero and Shelton, 1974) or for use as female brooders in the mass spawning test. At least 45 fish from each spawn were sexed.

The methyltestosterone (MT)-treated feed (45 mg MT/kg feed) was prepared by the alcohol evaporation method (Shelton et al., 1978). Fry were fed three times

daily each day of the week at 12% of body weight per day; the amount fed was adjusted weekly. The androgen treatment ended after 21 days. Temperature throughout the experiment was $26 \pm 2^{\circ}\text{C}$.

After the treatment ended, the fry were transferred to outdoor plastic
pools (4,200 l) and fed pelleted trout feed. In October these fish were transferred to an overwintering tank in an indoor heated facility and were fed at 1% body weight/day.

Mass spawning in the sex-reversed broodstock in concrete tanks. In May, 1980, the overwintered androgen-treated fish were examined for abnormal male characteristics. Fish having poorly formed male papillae were rejected; gonads of the culled fish were examined for atypical characteristics. The selected sex-reversed males were stocked into six concrete tanks (22 sex-reversed males per tank) with a surface area of 18.6 m^2 and an average depth of 1.2 m. Unrelated female brooders were randomly selected from the University's population and stocked into three concrete tanks at 3:1 ratio to the sex-reversed males (66 females per tank). A group of females related (sibling or half-sibling) to the sex-reversed males, was stocked at the same density into the remaining three tanks. One control tank was stocked with normal females and males at the same ratio (3:1). Fish were fed to satiation daily with pelleted catfish feed.

In late June, when spawning began, fry from each of the seven tanks were collected over a 2-week period and stocked into separate plastic pools (7.3 m^2). After the collection period the tanks were drained, brood fish removed, and the remaining fry collected. The total numbers of fry in each tank were estimated volumetrically.

After the tanks were drained they were flushed and refilled. The same broodstock were placed back into the tanks. This entire procedure was repeated twice more until three samples of fry from separate mass spawns in each tank had been obtained.

In October, the 21 plastic pools containing the progeny from each of the three mass spawning periods were drained, and 100 fish from each pool were randomly selected and preserved for later sexing. All breeding tanks were also drained to examine the remaining brood fish.

In Fall 1980, the preserved fish were sexed using the aceto-carmin stain, gonadal-squash method. Progeny sex ratios were compared on the basis of percent female since spawns were expected to yield all-female populations. The percent females in the samples from the three different groups of brood stock--normal brood stock, sex-reversed males with non-related females, and sex-reversed males with related females (siblings) - were compared by analysis of variance (ANOVA; Snedecor and Cochran, 1967). Each group of sex-reversed brooders provided three replications; the controls were not replicated.

RESULTS

Progeny sex ratios from a confirmed sex-reversed male. In 1979, one androgen-treated male was confirmed as functionally sex-reversed (XX-"♂") by sexing his offspring from three unrelated females (XX-"♀"); five spawns were obtained in an aquarium from these fish. At least 45 fry from each spawn were sexed, and all were females. Half of the fry from two of these spawns from a single female were treated with androgen to provide the sex-reversed males for the mass breeding of monosex (female) fry.

Mass spawnings of sex-reversed broodstock. The androgen treatment with methyltestosterone at 45 mg/kg for 3 weeks, to produce the sex-reversed males, was very effective. Of the 150 fish overwintered as potential brood fish (20 fish died during the winter) only 18 were rejected because they had atypical papilla or poor male coloration. Nine of the fish had normal appearing testes, but nine had atypical, poorly formed male gonads.

The fry production from the tanks with sex-reversed male brood stock was about the same as for the control tank--about 25,000 fry per tank. Thus the

androgen treatment did not appear to have affected the potency of the fish that were sex-reversed. Higher fry production could be expected if the tanks were not drained after each spawning period since this activity caused females that were incubating eggs or fry to lose them.

A total of 300 fry from each tank covering the three separate spawning periods were sexed by using the aceto-carmin stain, gonadal-squash method. Since total average fry production for each tank was estimated to be 25,000 fry, the number of fry sexed was about 1.2% of the total production.

Of the 300 fish sampled from the control tank, 49% were females. These fish were offspring from non-hormone treated parents. The remaining 1,800 fry came from tanks with sex-reversed male brood stock. Half of these fry came from three tanks stocked with female brooders that were the full- or half-siblings of the sex-reversed males. The other half came from three tanks stocked with non-sibling female brooders. The tanks with the half or full-sibling brood stock yielded a higher percentage of female offspring (99.9) than did the non-sibling brood stock (94.7). Both groups of brood stock produced high percentage of female fry but there was also a significant difference ($P < 0.01$) in the percentage of females produced in the two groups, both of which differed significantly from the control group. No differences were found between replicates, tanks, or spawning periods within each group; therefore, variation between groups for the sex ratios of their progeny was caused by genetic differences in the two groups of female brooders.

DISCUSSION

The androgen treatment used was successful in producing large numbers of functionally sex-reversed males; normal reproductive ability was apparently not impaired. Breeding of these sex-reversed males produced large numbers of predominately female offspring. Since males were present among the offspring, some reproduction in these populations would have occurred, and the numbers of

young produced could have been sufficient to cause the usual overcrowding and stunting typical of tilapias (Anderson and Smitherman, 1978). Therefore, for optimal growth, production ponds stocked with offspring from sex-reversed parents would need to be augmented with predatory fish.

Sex ratios of progeny from sex-reversed brood stock were not fully explained by a simple monogenic theory of sex determination. Other factors (either autosomal or gonosomal) may be present that determine the sex of T. nilotica. If these factors are polygenic, then possibly selection similar to that described by Hulata et al. (1983) could be used to produce a population in which a predominance of either male or female factors occurred, which would yield a preponderance of male or female offspring.

Normalcy of 1:1 sex ratio. The genetics of sex determination, more specifically the normalcy of the 1:1 sex ratio in tilapia, affected the outcome of this and related experiments. Progeny testing has been a basis for identifying functionally sex-reversed tilapia for their use in breeding programs to produce monosex populations (Jensen and Shelton, 1979). The basic assumptions have been that: 1) sex-reversal affects the phenotype and not the genotype (Yamamoto, 1969), 2) sex determination in tilapia is based on sex chromosomes (Chen, 1969) and 3) a 1:1 sex ratio is normal. Shelton et al. (1978) present evidence of a 1:1 sex ratio for Tilapia aurea and T. nilotica. Wohlfarth and Hulata (1981) discuss various models of sex determination for tilapias and conclude that none satisfactorily explain all sex segregations of interspecific crosses. Progeny sex ratios from interspecific pair crosses between T. aurea and T. nilotica have been conducted by Hulata et al. (1981); here we examine the premise of normalcy of a 1:1 sex ratio from intraspecific pair spawnings of these species.

Tilapia aurea and T. nilotica were intraspecifically pair spawned. Progeny sex ratios for T. aurea ranged from 0-100% males with a mean of 49.4%; sex

ratios of 20% of the 126 progeny groups were statistically different from the expected 1:1 ($\alpha = 0.05$; $n = 60$). The sex ratios in repeat pair spawnings of brood fish were similar; this trend appeared more related to the female than male. Sex ratios for T. nilotica ranged from 31-83% males with a mean of 54.7%;

sex ratios of 21% of the 71 progeny groups were outside the expected range with 18% being skewed toward male predominance. Consecutive spawnings did not have the pattern of similarity that was apparent for T. aurea.

The basic objective in the discussion of these data was to address the normalcy of a 1:1 sex ratio from pair spawns of tilapias. Considerable variation in sex ratio was demonstrated between pair spawns, yet an overall population sex ratio of 1:1 results through random matings or the summation of pair spawnings. The sex ratios observed in this study may have been affected by differential mortality of sexes during incubation or early post-hatching. But the exact influence of early mortality cannot be determined if gonad sexing is required. Stock purity must be considered as a possible factor adding to variation, particularly in populations at locations where two or more species have been maintained for several years. In the present study, sex ratios of T. nilotica were not quite as variable as those of T. aurea, particularly with reference to extremes; however, two stocks of T. aurea (Auburn and Marion) had similarly aberrant sex ratios despite the fact that the Marion subpopulation was separated from the original Auburn stock early after acquisition.

The breeding approach to produce all-female populations in this study with T. nilotica included low numbers of males; the presumed brood stock genotypes involved sex-reversed males (XX) and normal females (XX). Wohlfarth and Hulata (1981) state that there is no evidence that a single pair of chromosomes determines sex in tilapias. Avtalion and Hammerman (1978) and Hammerman and Avtalion (1979) offer an alternative hypothesis to explain discrepancies in the chromosomal explanation of Chen (1969) for hybrid studies.

Control of sex determination by sex chromosomes in tilapias cannot be addressed solely on the basis of the present data. While the observed variation does not negate sex chromosome consideration, the breeding trends observed for individuals does reduce the acceptability of this explanation without including autosomal influence or multigene or multifactor involvement. Individuals with a pattern of aberrant sex ratios in their progenies suggests the possibility of selecting for sex ratios in a particular direction or with a desired composition on the interspecific basis. Hulata et al. (1981) have used this approach to select for desired sex ratios of tilapias through progeny testing.

Progeny testing of tilapias to identify sex-reversed individuals on the basis of presumed sex chromosome control should take into consideration the potential for sex ratio variation. Particularly, when attempting to differentiate a 3:1 sex ratio which assumes sex reversal of presumed heterogametic sex (androgen treatment in T. aurea; estrogen treatment in T. nilotica). Identification of the reciprocal, i.e., 100% male (estrogen treatment in T. aurea) or 100% female (androgen treatment in T. nilotica) is less precarious. Nevertheless, monosex progenies have resulted from pair spawning of apparently normal parents.

It seems that while many fishes appear to conform to the simpler sex chromosome model, the various species of tilapia that have been investigated, either through sex reversal and breeding or through hybridization, require a more complex explanation to adequately describe sex determination.

SPAWNING OF ESTROGEN-TREATED AND UNTREATED TILAPIA AUREA

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-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 brood stock. Selecting brood stock 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). The primary objective of this study was to compare spawning success of normal and estrogen-treated females stocked together and separately, with a normal male.

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% 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 photo-period 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 tranverse genital slit. Papillae were normal-appearing in 46% of the estrogen treated fish (genotypic males), slightly atypical in 24%, and moderately to highly aberrant in 30%. 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% male offspring with spawned with normal males. One of these 2 sex-reversed females (F_1) died early in the study. The other female was stocked 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.

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).

Spawning by untreated (normal) females occurred in every aquarium except one 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 ($P = .05$) than in those with other ratios. The mean number of spawns/untreated female/week was significantly higher ($P = .05$) 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. Three spawns within a 6-week period occurred in the pool containing the progeny-tested (F_1) sex-reversed female. All 3 spawns were fertilized by the same male and resulted in large numbers of viable and presumably 100% 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.

Similar problems resulted when genetic males of T. hornorum, another species where the male is homogametic, were fed hormone to induce sex-reversal to functional phenotypic females. Each of two synthetic androgens or a synthetic estrogen were fed to T. hornorum fry at the following rates and durations: ethynyltestosterone at 30 and 60 mg/kg of diet for 21 and 28 days; methyltestosterone at the same concentrations but for 18, 21 and 28 days; and ethynylestradiol plus methallibure diets at different combinations for 35 and 49 days. Each androgen treatment yielded over 92% males but all-male populations were produced only by two. Only one estrogen treatment significantly altered the sex ratio in favor of females. Urogenital papillae were more easily feminized but were not effective indicators of successful estrogen sex-reversal.

GROWTH COMPARISONS OF MONOSEX TILAPIA PRODUCED BY SEPARATION OF SEXES, HYBRIDIZATION, AND SEX-REVERSAL

Culture of monosex male populations of tilapia is desirable to eliminate unwanted reproduction and because tilapia males grow more rapidly than tilapia females (Pruginin, 1967; Shell, 1967; Hickling, 1968; Bardach et al, 1972; Stone, 1981). Three techniques are used to produce all male populations: separation of sexes, interspecific hybridation, and hormonal sex-reversal.

The objective of this study was to make growth comparisons of six monosex populations of tilapia produced through separation of sexes, interspecific hybridization, and sex-reversal.

METHODS

The experiment was conducted at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University, Alabama, U.S.A.

Six population of monosex tilapia were tested:

1. T. nilotica (genotypic male and female, phenotypic male), produced through methyltestosterone treatment (Shelton et al., 1978).
2. T. nilotica (genotypic female, phenotypic male), produced through the androgen treatment of all-female offspring resulting from breeding sex-reversed, androgen treated females with normal females (Shelton et al., 1978).
3. T. nilotica (genotypic male, phenotypic male), obtained through separation of sexes.
4. T. mossambica x T. hornorum (genotypic male, phenotypic male) (Khater, 1982).
5. T. nilotica x T. hornorum (genotypic male, phenotypic male) (Khater, 1982).
6. T. nilotica (genotypic female, phenotypic female) produced through the breeding of sex-reversed, androgen treated females with normal females (Shelton et al., 1978).

In late March, fish from the six treatments were moved from the overwintering facilities. For two months varying stocking densities and feeding regimes were used to adjust mean weights for all groups to 18 g.

Four replicates of each of the six fish groups were stocked into cages and concrete tanks. Random block design was implemented in positioning cages and complete random design was implemented in placement of fish groups in tanks. Water in concrete tanks was fertilized with 47 kg/ha of 20-20-0 (N-P-K) fertilizer three weeks prior to stocking to promote phytoplankton growth. Tanks were 2.7 m x 7.3 m x 0.8 m (0.002 ha). Forty fish per tank were stocked. Daily

food allowance for the tilapia groups was based on 3% of body weight of the heaviest group with 32% crude protein pelleted floating catfish feed (Lovell, 1979). Tanks with dissolved oxygen less than 1.0 ppm at 6:00 a.m. were aerated with compressed air for three hours.

Cylindrical cages (0.43 m³; 0.71 m depth, 0.91 m diameter, and 0.64 cm mesh), were placed in a 1.0 ha pond with 1.13 m average depth. One hundred fish per cage (152 per m²) were stocked. Each group was fed 3% of their body weight daily. Twenty-five percent of each population in tanks and cages was sampled every two weeks. Feed conversion (S) was calculated using the formula $S = \text{weight of feed} : \text{weight gained by fish}$.

The duration of the experiment in tanks and cages was 102 and 104 days, respectively. At harvest, fish were counted, weighed, and their sex determined by observation of genitalia and other secondary sex characteristics. Application of crystal violet dye to the genital area facilitated distinction of the sexes.

Data were analyzed with one-way analysis of variance, followed by Duncan's new multiple range test when differences were indicated.

RESULTS

The most rapidly growing fish in tanks were androgen-treated, sex-reversed T. nilotica (genotypic male and female, phenotypic male) ($P < 0.05$, Fig. 1). Sex-reversed T. nilotica (genotypic female, phenotypic male) and normal T. nilotica (genotypic male, phenotypic male) grew faster than the hybrids and T. nilotica females. T. mossambica x T. hornorum grows faster than either parent species (Hickling, 1968). Pruginin (1967) found that T. nilotica x T. hornorum all-male hybrids grew 30% faster than mixed-sex fingerlings of T. nilotica and 40% faster than mixed-sex fingerlings of T. hornorum. However, data of Lovshin and DaSilva (1975) and of the current study indicate that T. nilotica x T. hornorum grow at the same rate or slower than T. nilotica males. Therefore, when

sex is considered there is no expression of overdominance by the hybrid. As expected, T. nilotica females grew slower than normal T. nilotica males.

Genotype x environment interactions were indicated. Relative performance by T. nilotica was reduced in the cage environment (Fig. 1). This genotype x environment interaction was probably caused by different abilities of different tilapias to utilize phytoplankton and other natural foods. T. nilotica (genotypic male and female, phenotypic male) in cages was the fastest growing ($P < 0.05$) group at 88 days. Group means at 104 days in cages were equal except for T. nilotica females which had the slowest growth rate.

Net gains for both hybrids in cages and in tanks were similar ($P < 0.05$). Tanks had dense phytoplankton blooms, as measured by Secchi disk readings, and occasionally required aeration. The phytoplankton in the pond with cages was less dense and aeration was not necessary. Feed conversions of the hybrids in the tank environment were poor and probably caused by overfeeding. Feed conversions for T. nilotica groups in tanks were better as excess feed likely resulted in phytoplankton available to T. nilotica.

There was some reproduction in the tanks for all treatment groups, except for the hybrid T. nilotica x T. hornorum; the young found in a tank replicate of hybrid T. mossambica x T. hornorum was attributed to contamination of fry from adjacent tanks which had mixed-sex T. nilotica. Regression analysis indicated that weight of young fish had insignificant ($P < 0.05$) effects on mean weight of stocked fish. There was no reproduction in cages (Pagan-Font, 1975). Ranking of mean weights of fish in the cage treatments was similar to that for tanks.

DISCUSSION

Genotype had an important role in determining growth of sex-reversed phenotypic males (Fig. 1). Growth increased when there were genotypic males in the populations of androgen-treated tilapia.

Although not statistically different, mean weight of sex-reversed T. nilotica (genotypic females, phenotypic male) ranked higher than that of normal genetic males in both environments at the end of the experiment. This suggest that the effect of the methyltestosterone may be stronger than that of genotype.

The fact that androgen-treated T. nilotica grew faster than normal males conflicts with the findings of Katz et al. (1976) and Anderson and Smitherman (1978), but is in agreement with Guerrero (1974) and Owusu-Frimpong and Nijjhar (1981). This disagreement can possible be explained by difference in androgen treatment. Type of androgen and dosage is probably important. In our experiment and in those of Guerrero (1974) and Owusu-Frimpong and Nijjhar (1981), methyltestosterone resulted in increased growth rate over untreated fish. Katz et al. (1976) used andrenosterone, resulting in increased fry weight, but no difference between treated and untreated fish was evident at 3 g. Anderson and Smitherman (1978) administered ethynltestosterone to T. aurea and T. nilotica, resulting in decreased growth rates.

The methyltestosterone-treated T. nilotica groups apparently utilized food more efficiently than the untreated fish in this experiment. Similar results concerning methyltestosterone treatment and growth were obtained with salmon (Yamazaki, 1976; Fagerland et al., 1978).

Sex-reversed T. nilotica (genotypic male and female, phenotypic male) exhibited the fastest growth. This increased growth likely resulted from a combination of androgen and genetic effects. Breeding for all-male tilapia populations (Hopkins, 1979; Obi, 1982) has not yet been successful. Mass selection has improved growth rate in T. aurea (Bondari et al., 1983) and T. mossambica (Chang 1971), but has not improved growth rate in T. nilotica (Teichert-Coddington, 1983). Therefore, faster growing fish might be developed through a combination of androgen treatment and mass selection.

REPRODUCTIVE TRAITS OF THREE YEAR CLASSES OF TILAPIA NILOTICA

Regardless of the breeding program chosen, seed production is critical for commercial application. Considerable study has been conducted on the reproductive behavior of tilapia, but little on the spawning potential of females from different year classes throughout the spawning season. The reproductive efficiency for females is a product of spawning frequency, fecundity, hatchability of eggs, and survival of fry.

METHODS

Spawning rate, fecundity, egg length, egg weight, hatchability and sac fry length were studied for year classes I, II and III of Tilapia nilotica during three spawning periods June to August, 1982. Mean weights of females were 48.5, 156.5, and 293.5 g, respectively.

RESULTS

Spawning rates of females in each year class were 64%, 69%, and 80%; fecundity was 13,171, 7,607, and 6,106 eggs/kg body weight. Egg lengths were 2.08 mm, 2.28mm, and 2.35 mm; sac fry lengths were 4.28 mm, 4.55 mm and 4.61 mm. Hatchability averaged 78.3% for year class I, 85.5% for year class II and 87.5% for year class III. Fry survival was 91.3%, 96.7%, and 98.3%, respectively. Total fingerlings/kg of female were 6,026, 4,453, and 4,202. Egg and sac-fry size increased as the season progressed; other factors were relatively unchanged. Larger, older females produced larger eggs and fry. The maternal effect of age and size on length of young was evident at eight days but was no longer discernible for 20-day-old fry.

ISOZYME VARIABILITY IN TILAPIA

Populations of Tilapia hornorum, T. mossambica, T. nilotica, T. aurea (Auburn, Florida and Marion) and two crosses, ♀ T. nilotica x ♂ T. hornorum and ♀ T. mossambica x ♂ T. hornorum, were sampled at random from holding tanks at

the Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama in the fall of 1981. These fish were then compared electrophoretically for the activity of several enzymes in various tissues in order to choose tissues for use in a screening procedure. The relative mobilities of these same enzymes in the various populations were then compared in order to generate a key to the identification of the tilapia species examined and to collect data for possible correlation of desirable phenotypic traits with discernible polymorphisms in the genome.

METHODS

Fish samples were frozen alive to prevent the denaturation of their proteins. Just prior to electrophoresis, samples of tissue were dissected and homogenized in 0.1 M Tris-HCl buffer (pH 7.0) using a teflon-coated tissue grinder attached to an electric drill to rupture the cells and release their contents into solution. Wicks cut to 4 x 10 mm from Whatman #3 filter paper were soaked in the tissue homogenate for 15-20 minutes to insure adequate absorption of protein.

Variously buffered starch gels were prepared. Gels were then cooked for approximately 10 minutes and poured into plexiglass molds and allowed to cool in a refrigerator. By keeping the gels covered in plastic wrap both during cooling and electrophoresis, loss of moisture and subsequent deterioration of the gel was minimized.

When completely cooled, the starch gel was cut across its width and inserted with the protein laden wicks. During the process of insertion, any intact tissue that might have adhered to the wick was wiped off along with excess moisture.

Electrophoresis. Electrophoresis was performed on a plexiglass unit maintained in a large, glass-doored refrigerator. These units were constructed in the laboratory from 3.175 mm plexiglass with silicone covered brass bolts in

each end of the buffer chamber from which to suspend the wire electrode.

Platinum wire of approximately 20 gauge was essential to the proper operation of the unit. Other types of wire, primarily highly purified, gas free nickel-chromium, had some undefined adverse effect which resulted in either no migration of the proteins or in their failure to stain. Cloth diapers were used as conductive wicks to insure sufficient current through the gel. Folding these wicks to increase their cross-sectional area increased amperage at a given voltage.

Electrophoresis was performed at 20-25 mA (voltage was not allowed to exceed 225 V) for 12-15 hours. This strategum allowed sufficient migration to separate isozymes while preventing the heating of the gel due to the flow of electricity.

Upon completion of electrophoresis, the gels were removed to a plexiglass slicing frame where it could be cut horizontally and longitudinally into as many as seven slices, each 1.6 mm thick. Each slice was then stained for a single enzyme system according to the procedures of Shaw and Prasad (1970), as modified by Philipp et al. (1979).

Selection of tissues and enzymes. The enzymes were stained on slices in which one sample each of heart, liver, spleen, muscle, eye, gonad, kidney and brain from T. hornorum were inserted and electrophoresed. Based on the results of this survey, tissues of muscle, liver and eye were selected and the resolution of the enzymes compared for 10 specimens each of T. hornorum and T. nilotica and twenty of T. aurea (Marion population). Preliminary comparisons were made using samples of each of the three tissues selected, inserted into a single gel in order to determine the extent of tissue specificity of gene activity as well as to provide information for the interpretation of the data collected in species comparisons.

RESULTS

Eight of the examined loci; liver alcohol dehydrogenase, muscle and liver aspartate amino transferase, liver and eye esterase, muscle creatine kinase, eye glucose phosphate isomerase and liver superoxide dismutase, were found to have interspecific variability; five of these (liver and muscle aspartate amino transferase, liver alcohol dehydrogenase, muscle creatine kinase and liver superoxide dismutase) proved to possess intraspecific polymorphisms as well. This variability was studied and found to be sufficient to differentiate the species surveyed. Keys were developed for the identification of Tilapia species examined.

DISCUSSION

Although little intraspecific polymorphism was noted, the ADH polymorphisms in T. aurea (Auburn) and T. mossambica, and the CK, SOD and AAT variations in the Auburn T. aurea was indications that variability does exist and that they might be related to differences in performances of the corresponding phenotypes for production of all male progeny.

More biochemical genetic variation was found in T. aurea than in the Ivory Coast strain of T. nilotica. This may be related to T. aurea responding to mass selection and the Ivory Coast strain not responding and is consistent with the premise that the breeding history of the Ivory Coast strain indicates much inbreeding.

Ghana strain of T. nilotica had more isozyme variability than Ivory Coast and Nile strain more than either of these strains. Although mass selection was unsuccessful for increasing body weight in the Ivory Coast strain and minimally so in the Ghana strain, the greater genetic variability in Nile strain as indicated by electrophoresis warrants examination of mass selection to increase body weight in this strain.

The species-specific isozymes were used to determine parentage of Florida red tilapia at Auburn. The data indicated, as expected, parentage primarily from T. hornorum and T. mossambica with contamination of the genome by either T. aurea or T. nilotica at the SOD locus. Eleven generations of selection for red color resulted in an increased frequency of alleles from T. mossambica species.

Species differences in embryonic development of T. aurea and T. nilotica were also evident. Fertilized eggs, sac fry and swim-up fry collected daily through the first twenty days of life were included for analysis. Activation at the various enzyme loci occurred at different points in development. Some loci (e.g., malate dehydrogenase-A, lactate dehydrogenase-A, and glucosephosphate isomerase-B) were active throughout the process of development. In enzymes with multiple loci (e.g., GPI, LDH), activation of the enzyme at one tissue-specific locus often preceded that in another. Onset of expression at key biochemical loci was apparently related to morphological events in development.

CATFISH

RESPONSE TO SELECTION AND REALIZED HERITABILITY FOR BODY WEIGHT IN THREE STRAINS OF CHANNEL CATFISH, ICTALURUS PUNCTATUS, GROWN IN EARTHEN PONDS

Catfish production during 1980 was 57 million kg (United States Department of Agriculture, 1980). Even slight improvement in channel catfish, Ictalurus punctatus, body weight would result in millions of kilograms of additional production.

Heritability estimates from sib analysis have been determined for body weight of channel catfish. However, such estimates have not been determined in the pond environment and some estimates have high standard errors. The heritability of growth for channel catfish in cages has been estimated to be 0.61 ± 0.57 at 5 months of age and 0.74 ± 0.53 at 15 months of age (Reagan et al., 1976). Bean (1977) estimated the heritability of growth to 55 days in

aquaria to be 0.02. In a high-density tank study El-Ibiary and Joyce (1978) found heritability estimates for female body weight to be: $h^2_{\text{sire}} = 0.51 \pm 0.22$, $h^2_{\text{dam}} = 0.50 \pm 0.23$; for male body weight: $h^2_{\text{sire}} = 0.27 \pm 0.37$, $h^2_{\text{dam}} = 0.67 \pm 0.41$. Reagan (1979) estimated heritabilities in 3-year classes of the Tupelo (Mississippi) strain of channel catfish grown in tanks. Heritabilities for 30- to 180-day-old catfish ranged from 0 to 1 ± 1 . Heritabilities for 18-month-old catfish ranged from 0.40 to 0.87 ± 0.50 . These estimates indicate that mass selection for body weight in channel catfish might produce gains in body weight. The intent of this experiment was to determine the response to selection and realized heritability for body weight in three strains of channel catfish, Ictalurus punctatus, grown in earthen ponds.

METHODS

History and development of brood. The grandparent stock of the experimental fish used in this research were collected by Green et al. (1979) during the early 1970's. The fish were propagated at Auburn University and at Marion State and Federal Fish Hatcheries in Marion, Alabama. The history of these fish is given in Dunham and Smitherman (1981).

The brood populations used were:

Rio Grande - a strain from the Rio Grande River, Falcon Reservoir, Texas. Brood stock used were the F₂ generation from wild parents.

Marion - a domesticated strain from the U.S. National Fish Hatchery at Marion, Alabama. This group has been maintained in ponds at Marion for about 20 years (three to five generations).

Kansas - a domesticated strain from a private fish hatchery in Kansas. It has been maintained there for nearly 50 years (eight to 15 generations).

The channel catfish parent brood used in this study were hatched from four to eight spawns (20,000 - 30,000 eggs/spawn) of the grandparent fish in May, 1976 and evaluated for growth during the summer of 1977 (Chappell, 1979). The

fry were stocked at 148,260/ha. At 10 months of age the fingerlings were harvested and three random samples of 300 fish of each population were stocked into 0.04 ha experimental earthen ponds at Auburn University. Production stocking rate was 7,500 catfish/ha. In mid-October, 1977, the fish were

harvested. Mean weight was approximately 500 g. A random sample of 90 from each population was retained for the respective control populations. Then, the largest 10% of the remaining fish was kept for the selected population.

Management of experimental fish. In June 1979, Rio Grande brood were spawned. Three spawns from select brood fish and five spawns from random brood fish were obtained. Rio Grande swim-up fry (4 days post-hatch) were stocked at 148,260/ha in six replicate 0.04/ha ponds.

Fry were fed 45% protein ground trout food (Ralston Purina Company, St. Louis, Missouri) at 27 kg ha⁻¹ day⁻¹. Survival of fry to fingerlings was determined at 55 days, when fish weighed 1 to 2 g. They were then restocked in six replicate ponds (6,000 fish per treatment) at 24,710/ha. In March 1980, Rio Grande were harvested and restocked for production at 75,000/ha. The Rio Grande experiment had four replicates (1,200 fish per treatment).

In June 1980, Kansas and Marion brood had reached sufficient maturity to produce adequate numbers of spawns from select and random brood fish. There were seven Marion select spawns, four Marion random spawns, six Kansas select spawns, and eight Kansas random spawns. Kansas and Marion fry (7 days post-hatch) were stocked at 432,425/ha in recently filled ponds. There were 100,000 fry per treatment and six replicates per treatment. Fry survival was determined at 40 days, when the fish weighed approximately 1 g. Kansas and Marion channel catfish were then restocked for production in mid-July 1980. Stocking density was 7,500/ha. The Marion and Kansas experiment had six replicates (1,800 fish per treatment).

In each experiment, fish were fed all they would consume in 30 minutes with 35% protein, floating catfish food (Producers Feed Company, Belzoni, Mississippi). A subjective feeding vigor score was given during the feeding. A score of zero to 10 was assigned, weekly. Ten equalled extremely vigorous feeding, five equalled moderately vigorous feeding, and zero equalled no feeding activity. Feed consumption during the production period was recorded for Marion and Kansas strains and feed conversion calculated. Emergency aeration with one-third horsepower aerators was used for approximately 2 h when dissolved oxygen dropped to 2 ppm.

Sex ratios. Channel catfish males usually grow faster than channel catfish females (Beaver et al., 1966; Brooks, 1977; Benchakan, 1979). Sex was determined for both select and random populations to determine if any size differences were caused by skewed sex ratios. Sample size was 250.

Data analysis. Experimental fish were periodically sampled and measured from hatch to harvest. All comparisons were between selects and randoms within a strain.

Standard errors for realized heritability were calculated using the formula of Prout (1962). The variance of realized heritability equals $\frac{1}{D^2}((h^2(1 - h^2) \sigma_p^2/N_p) + \sigma_o^2/N_o)$ where D equals the selection differential; σ_p^2 equals the phenotypic variance in the parent generation; σ_o^2 equals the phenotypic variance in the offspring; N_p equals the number of parents; and N_o equals the number of offspring. The formula is only useful for one generation experiments.

RESULTS

Response to selection in the Rio Grande population. There was no significant difference in weight between the select progeny and random progeny to 35 days, 18 July, but select fingerlings grew faster to 137 days, 28 October, averaging 21.6 g versus 14.1 g for the random fingerlings. Select progeny grew faster than random progeny during the winter. During March through July the select

fingerlings and random fingerlings grew at the same rate. The size difference expanded during the last 45 days (1 August, 405 days, through 15 September, 450 days) of culture and the select fish were 63 g larger at harvest (Table 5). Rio Grande select fry and fingerlings had higher survival than random fry and fingerlings.

The cumulative survival of select Rio Grande through all life stages (70%) was higher than that of random Rio Grande (46%). Ichthyophthirius appeared to be the primary pathogen during the fingerling stage.

Response to selection in the Marion population. Marion selects grew faster than Marion randoms to 150 days (117 vs. 106 g), 1 November. On 26 March, 300 days, the Marion selects (166 g) were larger than the Marion randoms (132 g), and their percent gain during winter was higher than the Marion randoms.

The selects continued to grow faster than the randoms and at harvest, 403 days (8 July) selects averaged 484 g and the randoms averaged 413 g. Response was 73 g and realized heritability was 0.50 ± 0.13 (Table 5). No differences in survival were found between the selects and randoms.

Response to selection in the Kansas population. Selects were larger, 130 g, than randoms, 107 g, at 150 days (1 November). There was no significant difference in winter growth (1 November to mid-March) rate. At 300 days (26 March) selects were larger, 166 g, than randoms, 147 g. At harvest, 8 July, selects averaged 513 g, 54 g larger than randoms 459 g (Table 5). Realized heritability was 0.33 ± 0.10 (Table 5). Survival of fry, survival of fingerlings, and total survival from fry to harvest was not different between selects and randoms.

Response to selection in male and female populations. In the Kansas and Marion strains, realized heritability for body weight of males and females did not differ significantly ($P < 0.05$). Response for Kansas male body weight was greater than that for female body weight. Progress in male and female Marion catfish was the same.

Sex ratios for progeny of brood fish selected for body weight. Sex ratios that differ from 1:1 could bias the results since male channel catfish generally grow faster than female channel catfish (Beaver et al., 1966; Brooks, 1977; Benchakan, 1979). No strain in this experiment differed from the expected 1:1 ratio ($P < 0.05$).

Feed consumption, feed conversion and feeding vigor. Marion selects consumed 9.5% more food than Marion randoms during the production phase and Kansas selects consumed 8.0% more food than Kansas randoms ($P < 0.05$). Preliminary experiments indicated feed conversion of select fingerlings was lower than that of random fingerlings. Low feed conversion values (weight of feed consumed/body weight gained) are desirable in fish culture. However, no differences in feed conversion were found during the second year of growth. Fish size and water quality may have affected feed conversion in the second year of growth. Feeding vigor of Kansas selects was greater than randoms during the fingerling phase ($P < 0.05$). Marion selects fed more vigorously than randoms during the production phase ($P < 0.05$).

DISCUSSION

All three strains responded to selection. Average increase in body weight was 64 g (16%), and average realized heritability was 0.34 ± 0.07 . Since all three strains responded, mass selection should result in progress in most strains of channel catfish. Realized heritability and response differed among populations. Realized heritability was higher in the Marion strain than in the Rio Grande strain ($P < 0.01$). Response was higher ($P < 0.05$) in the Marion strain than in the Rio Grande and Kansas strains. Progeny from select brood fish had greater feed consumption, low feed conversion, and greater disease resistance than the random controls.

Realized heritability was less than the heritability estimates of Reagan et al. (1976), El-Ibiary and Joyce (1978), and Reagan (1979). Reagan et al.,

(1976) had predicted an increase in harvest weight of 100 g from their heritability estimates when selecting 10% of the population.

Realized heritabilities for female and male body weight were the same in both Marion and Kansas strains. However, response to selection for male body weight was higher for Kansas males than Kansas females. This contradicts the conclusion of El-Ibiary and Joyce (1978) that progress for female body weight would be higher than that for male body weight.

Domesticated strains grow faster than wild strains (Smitherman and Green, 1973; Burnside et al., 1975; Broussard, 1979; Chappell, 1979; Green et al., 1979; Dunham and Smitherman, 1981). The average increased growth performance of hatchery fish (five strains) over wild fish (six strains) is 3% per generation (Smitherman and Green, 1973; Smitherman and Pardue, 1974; Burnside et al., 1975; Broussard, 1979; Chappell, 1979; Green et al., 1979). Assuming slow turnover of brood and long generation intervals, 6% improvement per generation has occurred. Mass selection in one generation during the current study improved growth rate two to six times faster than that which had occurred during three to 15 generations of domestication (undirected selection).

The progress made through one generation of mass selection was similar to that of two alternative breeding programs for channel catfish, intraspecific crossbreeding and interspecific hybridization. Growth of catfish can be improved 18% by hybridizing (female) channel catfish with (male) blue catfish, Ictalurus furcatus (Giudice, 1966; Yant et al., 1975; Chappell, 1979). Intraspecific crossbreeding of channel catfish can result in a 10% increase in growth rate (Smitherman and Green, 1973; Smitherman and Pardue, 1974; Broussard, 1979, Chappell, 1979; Green et al., 1979).

However, problems in seed fish production for crossbreeding and hybridization (Giudice, 1966; Yant et al., 1975; Chappell, 1979; Bice, 1981) may limit their usefulness. Since significant responses to selection in all three

strains, Rio Grande, Marion, and Kansas, were found, mass selection should be the currently recommended breeding program. Realized heritability for body weight, 0.34 ± 0.07 , and the standard deviation body weight, 123.1 g, are sufficiently large that further mass selection for body weight should be practiced in channel catfish.

Findings of the present study could be of considerable significance to hatchery managers and commercial farmers with an interest in genetic improvement of catfish growth rate through individual selection. However, we have also found that the program of retaining the largest 10% of a juvenile catfish population for brood stock could result in all-male brood stock populations if applied to channel and white catfish, while grading for the largest juveniles of blue catfish would yield equal numbers of males and females. When selecting channel or white catfish in such populations, care is required to find the largest females. To find the largest 10% of channel catfish females, the largest 30% of the 6- and 18-month-old fish must be graded and sexed and for the white catfish the largest 30-50% of the 6- and 18-month-old fish must be graded and sexed.

CORRELATED RESPONSES OF REPRODUCTIVE TRAITS AND GROWTH IN CHANNEL CATFISH

Improvement in growth rate is of little utility if seed production becomes more difficult. Mass selection is one technique to improve body weight. If careful planning and selection are not implemented, inbreeding can increase. Inbreeding causes decline in reproductive performance (Lasley 1978). Even if inbreeding is avoided, the traits of fast growth and good reproductive performance could be antagonistic. For these reasons, the reproductive performance of channel catfish selected for increased body weight was compared to the reproductive performance of randomly selected channel catfish.

RESULTS

Data were analyzed for each strain then pooled and re-analyzed. No differences were found in spawning date, spawning rate, hatchability of eggs and survival of sac fry. Fecundity (eggs deposited) was increased ($P < 0.05$) in the pooled select brood fish. Fingerling output was also higher for selects ($P < 0.05$) in the Marion and pooled populations, but lower ($P < 0.05$) in the Kansas select population. Fecundity, sac fry survival, and fry survival had the largest effects on fingerling output. Selecting for growth rate did not decrease fingerling output in channel catfish, and some improvement in reproductive performance was evident.

CROSSBREEDING CHANNEL CATFISH FOR IMPROVEMENT OF BODY WEIGHT IN EARTHEN PONDS

Crossbreeding is one technique for improving body weight in livestock and other organisms (Moav and Wohlfarth 1973, Lasley 1978). The purpose of this study was to compare the results of these previous experiments and those of more recent research at Auburn University to determine the effectiveness of crossbreeding.

METHODS

Parent lines, stocking rate, and age of the fish are all variables that could affect the success of crossbreeding. Those variables were tested in these experiments. All experiments were conducted in 0.04 ha ponds. In Experiments 1-8 (Tables 6, 7) the catfish were fed 3% of their body weight daily with 36% protein floating catfish feed. The catfish were fed ad-libitum in Experiments 9-12 (Tables 6, 7).

Histories of the strains, except Uvalde, that were crossed are in Green et al. (1979), and Dunham and Smitherman (1981). The Uvalde strain was obtained from the federal fish hatchery in Uvalde, Texas.

All crosses were designated female x male. Means within an experiment were evaluated with Duncan's multiple range test. All means were evaluated,

$P = 0.05$. Mean weights in Experiments 5-8 are adjusted for initial size differences by regression equations of Dunham et al. (1982b). Auburn x Rio Grande F_1 populations are symbolized as AR and Marion and Kansas F_1 populations are symbolized as MK when they are used as brood fish. Heterosis will only be considered to exist if the mean of the crossbred is higher than both parents.

RESULTS

Domestic x wild strains. In Experiments 1 and 2 (Green et al. 1979, Table 6), Yazoo, a domestic strain, was crossed with two wild strains, Warrior and Tennessee. Warrior x Yazoo exhibited heterosis but Tennessee x Yazoo did not exhibit heterosis. Other crosses involving domestic and wild strains included the reciprocals of Auburn and Rio Grande (Broussard 1979, Chappell 1979). One reciprocal had positive heterosis and the other had negative heterosis.

Domestic x domestic crosses included reciprocals of Marion and Kansas, reciprocals of Auburn and Uvalde, (Broussard 1979) and Auburn x Kansas (Smitherman and Pardue 1974). Four of these crosses resulted in positive heterosis and one resulted in negative heterosis. Domestic x domestic crosses were more likely to give positive heterosis (80%) than domestic x wild crosses (50%). In the 5 cases where both parent lines were represented, Warrior-Yazoo, Yazoo-Tennessee, Marion-Kansas, Auburn-Rio Grande, Auburn-Uvalde crossbreds usually exhibited (+ or -) overdominance. Only one of 8 crossbreds, Yazoo x Tennessee, had a mean weight intermediate to its parent lines.

Effect of age. In Experiments 3, 5, 9, 11, and 12 (Tables 6, 7) the Marion x Kansas crossbred was compared to its parents. When grown to a larger stocker size of 50 to 100 g, Marion x Kansas grew 17.7-31.0 percent faster than its parent strains. When grown to a harvestable body weight, Marion x Kansas was 1.0-9.1 percent larger than its parent strains. The magnitude of the difference in body weight between crossbreds and parents was smaller at harvest body weight than at stocker body weight. This may have been caused by the relative growth

rate of channel catfish slowing with increased size or by the onset of early sexual maturity in crossbreds. Another explanation would be an age-genotype interaction; crossbreeding may be more effective for improving growth of younger fish than older fish.

Density. Marion x Kansas and its parents were stocked at 4 different densities. Stocking rates are generally higher for culture of fingerlings than for culture of food-size catfish because of available space. Age appeared to be more important than density for determining crossbred response. Heterosis of Marion x Kansas fingerlings was 17.7 and 31.0% compared to 1.0, 9.1, and 6.9% for food size Marion x Kansas (Table 6, 7). In Experiments 11 and 12 where fish of different ages, fingerlings and food size Marion x Kansas, were stocked at similar densities, heterosis was different 31% and 9.1%, respectively.

The large variation in heterosis between experiments for fish of the same age might be caused by different combining abilities of individuals within a strain with individuals of the second strain. Variability for individual body weight in catfish is high (Broussard 1979, Dunham 1981).

Reciprocal crossbreds. In Experiment 12 (Table 6) the growth of the reciprocal crossbreds, Marion x Kansas and Kansas x Marion, was compared to their parent strains. Reciprocal crossbreds did not grow at the same rate. Marion x Kansas and Kansas x Marion grew 31.0 and 8.4% faster than their parent strains.

The Auburn and Rio Grande strains have been crossbred at both Auburn University (Chappell 1979) and Texas A&M University (Broussard 1979). The Auburn x Rio Grande crossbred was produced at Auburn (Chappell 1979) and the reciprocal was produced at Texas A&M (Broussard 1979). When Auburn strain was the female, growth improved 7.9% and when Rio Grande was the female, growth was 18.0% less than that of the fastest growing parent strain. Reciprocal crossbreds did not grow at the same rate. When Auburn was the female, the crossbred grew more rapidly than the parents as was the result when crossed with

Uvalde (Broussard 1979). In Experiment 8 (Broussard 1979) Auburn x Uvalde and Uvalde x Auburn crossbreds were compared to their parent strains. Auburn x Uvalde was the only reciprocal expressing heterotic growth, 9.7%. The reciprocals did not grow at the same rate. In Experiment 4, Auburn x Kansas had heterotic growth rate. The Auburn female crosses well with other lines.

In all 3 experiments where reciprocal crossbreds were produced, the reciprocals did not grow at the same rate. Interspecific reciprocal hybrids are also different from each other (Tave et al. 1981, Dunham et al. 1982a). A maternal effect on inheritance was evident as demonstrated by crossbreds of Auburn females. If both parents are contributing equally to the genome, reciprocals should perform equally. The crossbred zygotes differ in the source of their cytoplasm and interactions within the cytoplasm or between the cytoplasm and nuclear genome may explain the differences between reciprocals. Also, the mitochondrial genome is maternally inherited.

Four-line crossbreds. Experiments 9, 10, and 11 indicated that the four line crossbreds AR x MK and MK x AR grew at the same rates as the F_2 , AR x AR and MK x MK. Four-line crosses in livestock result in heterosis (Lasley 1978). The amount of heterosis expressed in livestock crossbreds (Lasley 1978) is more than that expressed by the catfish crossbreds. Four-line catfish crossbreds grew slower than two-line catfish crossbreds. The four-line crossbred catfish may have represented a large assortment of genotypes that phenotypically averaged near mid-parent values. Catfish body weight is highly variable; coefficient of variation = 25-35% (Broussard 1979, Dunham 1981). This variability may explain why inbreeding depression is small (5-7%) when full-sibs are mated (Bondari 1980) compared to trout (10-20%, Kincaid 1976) and livestock (10-40%, Lasley 1978). This variation may also explain the lack of response by the four-line crossbreds.

Reproduction. The reproductive performance of crossbred and pure-strain channel catfish used in the growth studies were also compared for spawning date, spawning rate(percent of replicate pairs that spawned), fecundity, egg size and hatchability, and survival of offspring. Crossbred fish usually spawned earlier ($P < 0.05$) than pure-strain channel catfish. As 3-years-olds, crossbred fish had higher spawning rates and fecundity than purebred fish, and their output of juveniles (number surviving 45 days in ponds, per kilogram of female parent) was greater. As 4-year-olds, pure-strain fish improved their performances, and crossbreds lost most of their relative advantages. Brood stock derived from crosses of four strains spawned earlier than those from two-strain F_2 crosses, but their surviving offspring were no more numerous. The main value of cross-strain breeding is to produce channel catfish that mature earlier in life and spawn sooner in the season than purebreds.

EFFICIENCY OF CROSSING STRAINS OF CHANNEL CATFISH

The potential for improved growth cannot be utilized unless sufficient seed stock are produced. Spawning percentages between strains or species should be reasonably similar to that within strains or species, so as not to require excess brood or hatchery facilities. Fingerling output/kg female is a good measure of reproductive efficiency and equals spawning percentage x fecundity x hatchability x fry survival. Spawning percentage can be controlled by stripping gametes in some but not all aquatic organisms. The other variables -- fecundity, hatchability, and fry survival -- are not so easily controlled. Our objectives were to compare the reproductive performance of matings within and between two strains of channel catfish to determine the efficiency of reciprocal crossbred mating systems.

RESULTS

Pairings of channel catfish (female x male), Marion x Marion, Kansas x Kansas, Marion x Kansas, Kansas x Marion were evaluated in pens for spawning date, spawning percentage, fecundity, hatchability, fry survival, and total fingerling output. More four-year-old brood stock spawned ($P < 0.05$) than three-year-olds. Marion x Marion and Marion x Kansas pairings resulted in spawns earlier than Kansas x Kansas and Kansas x Marion pairings ($P < 0.05$). Strain of the female was important in determining spawning date and may impede crossbreeding success. Spawning percentage in the pure-strain pairings (52%) was higher ($P < 0.05$) than in crossbred pairings (33%). No differences were found in fecundity (eggs deposited) and hatchability. Total fingerling output was higher from pure-strain pairings (1,630 fingerlings/kg female) than from crossbred pairings (1,210 fingerlings/kg female) ($P < 0.005$). When the fish were six years old the reciprocal Marion x Kansas pairing was as successful as parental pairing but Kansas x Marion still resulted in fewer spawns.

INTERSPECIFIC HYBRIDIZATION OF CATFISH

The hybrid catfish channel ♀ x blue ♂ (I. furcatus) has proven a valuable commercial fish in a number of studies (Yant et al. 1975, Chappell 1979, Tave et al. 1981); most of these concern growth rate and carcass yield. This program also indicated that the hybrid exhibits heterosis for catchability and tolerance of low-dissolved oxygen. However, the hybridization rate between the species has been variable and remains the major obstacle to the commercial scale production of hybrid fingerlings. In 1980 genetic and behavioral experiments were conducted in an effort to overcome the mating block.

With the use of 4-year-old channel catfish female brooders, it was found that only crossbred females spawned with blue catfish males. Rate of spawning, using hormone injection for the females of different genetic groups was 0.55%. Surgical removal of olfactory organs from brooders had no effect on

hybridization rate. Subsequent hybridization attempts in 1981-83 have had limited success (10-20%). Timing of pairing, temperature, and imprinting appear to be important variables for further research.

The production of F₂ hybrid catfish and three-way crosses were unsuccessful. Chromosome incompatibilities appear to be the primary block. Three parental species and four hybrid combinations among them had the following diploid chromosome numbers: 2N = 48 white catfish (I. catus), 2N = 58 (channel, blue, channel x blue and blue x channel catfish) and 2N = 53 (channel x white and white x blue). Karyotypes of I. punctatus, I. furcatus and the reciprocal hybrid combinations were not distinguishable from one another yet F₂ eggs had minimal hatch. The karyotype of I. catus differed from those of I. punctatus and I. furcatus in diploid number and in arm number. Hybrids between I. catus and either I. punctatus or I. furcatus were intermediate in karyotype, and most obvious parental marker chromosomes were five I. catus chromosomes which were considerably larger than any element in the I. punctatus or I. furcatus karyotype. The three species had unique isozyme patterns that can be used as genetic markers if chromosome incompatibilities are overcome.

COMPARISON OF MASS SELECTION, CROSSBREEDING AND HYBRIDIZATION FOR IMPROVING BODY WEIGHT OF CATFISH IN FIELD TRIALS

Three major breeding programs have been used to increase growth rate of channel catfish, Ictalurus punctatus. These are mass selection, intraspecific crossbreeding, and interspecific hybridization.

One generation of mass selection for increased body weight has been successful in all channel catfish populations evaluated (Dunham and Smitherman 1983). Response to selection and realized heritability for body weight averaged 16% and $0.34 \pm .07$, respectively, for three strains of channel catfish. Both dominance variation and additive variation are present for body weight. Sixty-seven percent of intraspecific crossbreedings have indicated overdominance and

an average increase of 10% for body weight (Smitherman et al. 1983). Twenty-eight interspecific hybrids have been produced and evaluated for growth rate (Giudice 1966, Dupree and Green 1969, Yant et al. 1976, Smitherman et al. 1983). Only one interspecific hybrid, channel catfish female x blue catfish I. furcatus male, has shown overdominant growth in the pond environment with an average increase in body weight of 20% above that of channel catfish (Giudice 1966, Yant et al. 1976, Smitherman et al. 1983).

No experiments have directly compared the performance of the improved fish resulting from these three breeding programs. Our objective was to compare the best crosses resulting from mass selection, intraspecific crossbreeding, and interspecific hybridization.

METHODS

The experiment was conducted at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University. Kansas select, K (S), and Marion female x Kansas male, M x K, crossbred channel catfish (Dunham and Smitherman 1983b) were chosen for comparison because of their fast growth rate (Dunham and Smitherman 1983a, Smitherman et al. 1983). They were also of similar genetic background.

Three crosses of channel x blue catfish, C x B, were chosen for comparison to the mass selected and the crossbred channel catfish. These crosses were (M x K) x Commercial blue catfish, X x B-1; (M x K) x Auburn blue catfish select, C x B-2; and (Auburn x Rio Grande channel catfish) x Commercial blue catfish, C x B-3 (Dunham and Smitherman 1983b). Three different hybrid crosses were compared to determine if different families or strains of channel catfish and blue catfish have different combining abilities when crossed. This has not been previously studied. Brood fish were mated in 0.1 ha spawning ponds in June 1980. K (S), M x K, C x B-1, C x B-2 and C x B-3 produced 8, 9, 2, 6, and 1 spawns, respectively. Hatchery procedures are reported in Dunham and Smitherman (1983a). Fry were

treated equally. Fry were stocked in 0.04 ha earthen ponds at 494,000/ha and reared (Moav and Wohlfarth, 1974; Dunham et al. 1982a) for one month to a common size of 1.0 g.

Each group was then stocked in four replicate 0.02 ha ponds at 7,410/ha, Experiment 1 (Table 8). These fish were fed ad-libitum with 36% pelleted catfish feed until November 1980, harvested, enumerated and weighed.

Excess fingerlings from the first rearing phase were stocked in 0.04 ha ponds at 123,500/ha and reared seven additional months to a common size of 30 g. C x B-1 and C x B-2 were mixed prior to the second rearing phase. The 30 g fingerlings were then communally stocked (Moav and Wohlfarth, 1976; Dunham et al. 1982a) in four earthen ponds averaging 0.91 ha in March 1981. The advantage of communal stocking is the common environmental error for the genotypes being compared. Stocking rate was 9,880/ha. A mix of C x B-1 and C x B-2, 4,940/ha, was stocked in all four ponds. M x K was stocked at 4,940/ha in two ponds for comparison to C x B, Experiment 2 (Table 8). K (S) was stocked at 4,940/ha in two ponds for comparison to C x B, Experiment 3 (Table 8). The fish in all ponds were fed ad-libitum with 36% protein pelleted catfish feed until December 1981, harvested, enumerated and weighed. Emergency aeration with paddle-wheel aerators was implemented when dissolved oxygen was < 2.0 ppm. One hundred-fifty C x B-1 and 150 C x B-3 fingerlings from Experiment 1 were communally stocked into a 0.04 ha pond (7,410/ha), Experiment 4 (Table 8). These fish were fed ad-libitum with 36% protein pelleted catfish feed until October, 1981, harvested, enumerated and weighed. Emergency aeration was implemented when dissolved oxygen was < 2.0 ppm. Data were analyzed utilizing Student's t-test and Duncan's multiple range test.

RESULTS

M x K and K (S) fingerlings grew more rapidly ($P < 0.05$) to 100 g than any of the C x B crosses (Experiment 1, Table 8). Generally, the C x B hybrids had

higher survival than the channel catfish crossbreeds. C x B (1 and 2) grew more rapidly from fingerling size to food size than M x K (Experiment 2, Table 8). Although exhibiting slower fingerling growth, C x B fingerlings had the same mean body weight as M x K by the time the fish reached 575 g.

C x B (1 and 2) also grew more rapidly from fingerling to food size than K (S) (Experiment 3, Table 8). Mean body weight of C x B had surpassed ($P < 0.01$) that of K (S) by harvest. The C x B hybrid had higher ($P < 0.05$) survival than either K (S) or M x K. In general, the best crosses from the three breeding programs—mass selection, crossbreeding and hybridization—had similar growth rates to harvest weight of 500 g.

DISCUSSION

Both K (S) and M x K had faster early fingerling growth than C x B. However, the C x B hybrids had either attained or surpassed the body weight of the mass-selected and crossbred channel catfish at 500 (+) g. The relative growth rates of the channel catfish declined more rapidly than that of the hybrid catfish, which perhaps reflected the growth pattern of blue catfish, the male parent species.

Channel catfish mature at an earlier age than blue catfish. Sexually dimorphic growth is evident in channel catfish as early as six months of age (Brooks et al. 1982). However, sexual dimorphism of body weight for blue catfish is not evident at 11 months of age (Brooks et al. 1982) or at three years of age (Smitherman et al. 1983). Channel-blue hybrids exhibit paternal predominance (Dunham et al. 1982b), characteristics more like their paternal parent than the maternal parent. Growth rate of channel catfish may decline at an earlier age than the C x B hybrid because of paternal predominance for the onset of sexual maturity. This would also explain why the channel x white hybrid grew rapidly in the early fingerling stage (Dupree and Green 1969) but grew slowly in the second year of growth (Smitherman et al. 1983) since white catfish mature earlier than either blue or channel catfish.

Similar to results in this experiment, sexual maturation or lack of sexual maturation was an explanation (Wolters et al. 1982) for increased growth rate of triploid catfish. The triploid catfish did not gain advantage until the later stages of growth. Sexual maturation and growth are related through timing of gene expression. This is a genotype x age interaction.

Growth rate of C x B-1 was $> C \times B-3 > C \times B-2$ ($P < 0.05$) to 100 g (Experiment 1, Table 8) C x B-1 also attained a body weight 500 g before C x B-3. There were strain, family or line differences for combining ability when channel and blue catfish were hybridized. This indicates parental strain is important in determining hybrid performance and combinations of breeding programs such as mass selection followed by hybridization may yield the best results.

No genotype x age interaction was found within C x B since C x B-1 ranked first at 100 g (6-9 months) and at 500 g (16 months). No genotype x age interaction was found for mass-selected catfish (Dunham and Smitherman 1983a) or for crossbred catfish (Smitherman et al. 1983). This is probably related to sexual maturation since similar timing of sexual maturation for fish produced through the same breeding program would be expected.

ISOZYME ALLELE FREQUENCY CHANGES IN SELECT LINES OF CHANNEL CATFISH

Genetic improvement of aquaculture stocks has great potential importance in the economic future of aquaculture. Most genetic improvement programs are based on selection, crossbreeding or some combination of these basic approaches. The effect of all these approaches is to change the frequency of performance-related genotypes in the offspring generation relative to that in the parent generation. Successful breeding practice increases the frequencies of genes or gene-complexes coding for desired traits, and decreases those for undesired traits. The geneticist may indirectly infer changing allele frequencies by recognizing correlations between performance-related gene complexes and other loci encoding more discernible traits.

Quantitative genetics has made use of biochemical techniques, notably electrophoresis and histochemical staining, to detect the action of single genes which encode specific proteins, including enzymatic proteins. The concerns of the biochemical geneticist are the correlation of enzyme-encoding loci with performance-related traits, use of these biochemical loci as markers to monitor changing allele frequencies, and use of data from the marker studies and from the performance of the animals to make informed judgements of alternative breeding practices as the breeding program progresses.

The objectives of this study were to use the allele frequency data to determine the relative importance of differentiation within and between stocks, levels of genetic variability in these stocks, changes in allele frequencies among the generations, and the importance of selection or random genetic drift in observed allele frequency changes.

METHODS

Using horizontal starch gel electrophoresis and histochemical staining techniques, the isozyme phenotypes at 28 biochemical loci were determined for channel catfish from 15 genetic stocks. These groups were derived from five genetic strains, and included one wild progenitor strain, four of the founder strains (one represented twice), five select lines, and four lines which were crossbred and selected.

RESULTS

Of the 28 loci examined, twelve were observed to be polymorphic (Table 2). At six of the polymorphic loci, EST-B, EST-C, EST-D, and a-GPDH-A, IDH-B, and 6-PGDH-A, variant alleles were observed in only a few individuals in certain stocks. The remaining six loci (GPI-A, GPI-B, LDH-A, MDH-A, PED-A, and PGM-A) were polymorphic with variant alleles observed at frequencies greater than 0.05 in some stocks.

A strong degree of differentiation was evident among stocks in terms of frequencies of alternative alleles and in terms of genetic variability within stocks. Related stocks did, however, tend to exhibit similar biochemical genetic character. Significant differences in allele frequencies were noted between founder and select lines in as little as one generation. Crossbred lines exhibited minimal biochemical genetic variability.

Seventy allele frequency changes were observed in the nine select lines, 25 of 70, or 36% of the values of t proved significant. The heterogeneity of the collection of $F(ST)$ values involved the comparison of the observed variance of $F(ST)$ with the theoretical variance (Lewontin and Krakauer, 1973) to determine if these allele frequency changes were due to selection or random genetic drift.

The analysis of $F(ST)$ values indicated that the observed variance was sufficiently greater than its theoretical expectation to invoke selection as causal in affecting allele frequencies at some loci.

DISCUSSION

It is possible to make inferences regarding which loci were subject to selection. For a given allele, the magnitude of $F(ST)$ would be expected to be low if the various alleles were selected uniformly in all populations (Spiess, 1977). It would be high if there were considerable selection restricted to certain stocks. Neutral alleles would be expected to give intermediate values.

The distribution and heterogeneity of $F(ST)$ values indicated that all three such cases were implicated regarding changes in allele frequencies among the various stocks. Values of $F(ST)$ for many alleles, including all three EST-C alleles, were low, suggesting a uniform selection pressure in all stocks where the loci in question were polymorphic. Skow (1975) also concluded uniform selection for certain EST alleles in catfish. Values of $F(ST)$ for the PEP-A-2 and A-3 alleles were high, suggesting selection that was restricted to certain stocks. Values of $F(ST)$ for many alleles, including PGM-A-1 and A-2, were in

the intermediate range, suggesting that the alternative allelic forms of this enzyme were selectively neutral.

The majority of F_{ST} values were in the moderate to low range. That the range of F_{ST} values was broad and heterogeneous indicated that while both selection and random drift were apparent, neither was predominant to the other in changing allele frequencies among founder and derived stocks.

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Table 1. Summary of harvest data for Tilapia nilotica
(F₂ Generation-1982) at 59 days of age.

Treatment	Total Weight (kg)	Fish/Pond	Mean Weight (g)	Adjusted Mean Wt. (g)
H	34.4	4,703	7.4	7.1
C	36.9	5,166	7.2	7.6
L	32.8	4,765	6.9	6.7

Table 2. Mean weights (g) of control and high line fish at sampling and harvest with corresponding standard deviation (SD).

Day	C Wt/SD	H Wt/SD
43	175/1	164/16
109	310/25	281/37

Table 3

Estimates of genetic trend in body weight (Wt) and total length (TL) of first generation tilapia at 16, 26, and 40 weeks of age.

Line	16 wks		26 wks		40 wks	
	Wt	TL	Wt	TL	Wt	TL
Mean ^d						
High	16.3 ^a	92.4 ^a	30.1 ^a	117.2 ^a	77.5 ^a	154.8 ^a
Control	15.3 ^a	90.2 ^a	23.7 ^b	109.1 ^b	64.6 ^b	147.1 ^b
Low	11.5 ^b	83.1 ^b	16.8 ^c	96.8 ^c	47.9 ^c	134.7 ^c
Mean as % of Control line						
High	107	102	127	107	120	105
Low	75	92	71	89	74	92
Standard Deviation (SD)						
	7.1	13.2	13.4	19.3	41.5	30.0
Divergence						
	0.68	0.70	0.99	1.05	0.71	0.67

^{a,b,c} Means in the same column with different superscripts differ (P < 0.05).

^d Body weight (g) and total length (mm) means were determined from a random sample of 25 fish/tank/line at each age.

^e $(\bar{H} - \bar{L}) / SD$, where $\bar{H}$ and $\bar{L}$ represent means for High and Low lines, respectively (SD = $\sqrt{MS}$ Error).

Table 4

Realized heritability estimate (h^2) and least-squares means for various traits of Tilapia aurea from control (C), high (H), and low (L) body weight lines grown in cages in Tifton and Auburn

	Female			Male			Combined		
	H	C	L	H	C	L	H	C	L
Tifton fish									
Body weight (g)	195 ^a	161 ^b	171 ^b	311 ^a	278 ^b	246 ^b	253 ^a	219 ^b	209 ^b
Total length (mm)	214 ^a	203 ^b	205 ^b	247 ^a	234 ^b	229 ^b	230 ^a	218 ^b	217 ^b
Condition factor	1.96 ^a	1.91 ^a	1.95 ^a	2.03 ^a	2.08 ^a	2.03 ^a	2.00 ^a	1.99 ^a	1.99 ^a
Dressing %	61.5 ^a	59.6 ^a	61.4 ^a	61.4 ^a	63.3 ^a	61.5 ^a	62.5 ^a	61.5 ^a	61.4 ^a
h^2 for weight	0.38		-0.17	0.20		0.14	0.24		0.07
h^2 for length	0.08		0.12	0.09		0.07	0.07		0.06
h^2 for length	0.87		-0.09	0.40		0.07	0.53		0.02
SE of h^2	0.20		0.11	0.19		0.08	0.24		0.11
CV (%) for weight	19.8	21.9	18.3	24.9	36.7	17.5	32.8	43.0	24.9
Auburn fish									
Body weight (g)	182 ^a	170 ^b	145 ^c	334 ^a	279 ^b	168 ^c	258 ^a	224 ^b	156 ^c
h^2 for weight	0.10		0.34	0.27		0.41	0.23		0.43
SE of h^2	0.06		0.08	0.07		0.07	0.05		0.05
CV (%) for weight	12.4	26.6	18.6	19.9	34.4	28.0	16.2	30.5	24.0

^{a,b,c} Means in same row and within a sex group with different superscripts differ ($P < 0.05$).
^d Determined as 10^5 (body weight)/(total length).

Table 5

Response to selection for body weight in Rio Grande, Marion and Kansas channel catfish grown in earthen ponds at 7500/ha (S = select; R = random)

Population	Mean weight (S.D.) (g)	N	Response (g)	Selection differential (g)	Realized heritability ± S.E.
Rio Grande	(S) 431 (136)*	1044	63	263	0.24 ± 0.06
	(R) 368 (109)	1068			
Marion	(S) 486 (146)*	1674	73	145	0.50 ± 0.13
	(R) 413 (99)	1656			
Kansas	(S) 513 (142)*	1692	54	163	0.33 ± 0.10
	(R) 459 (107)	1764			
Pooled	(S) 477 (156)*	4410	64	190	0.34 ± 0.07
	(R) 413 (117)	4488			

*Significantly different ($P < 0.001$).

Table 6. Mean weights of parent strain and crossbred channel catfish fingerlings and the relationship of the crossbred to the best parent.

Experiment	Strain	$\bar{x}$ weight (g)	Percent Increase or decrease
1	Warrior X Yazoo	127 a	14.4
	Yazoo X Yazoo	111 b	----
	Warrior X Warrior	75 c	----
2	Yazoo X Tennessee	89 b	20.0
	Yazoo X Yazoo	111 a	----
	Tennessee X Tennessee	57 c	----
12	Marion X Kansas	142 a	31.0
	Kansas X Marion	118 b	8.4
	Kansas X Kansas	109 c	----
	Marion X Marion	96 d	----
9	Marion X Kansas	51 a	17.7
	AR X MK	44 b	2.3
	Kansas X Kansas	43 b	----
	Marion X Marion	43 b	----
	MK X MK	39 b	----
10	AR X AR	20 a	----
	MK X AR	20 a	0.0
	AR X MK	18 a	10.0
	MK X MK	18 a	----
	Rio Grande X Rio Grande	8 b	----

^{abc}Means followed by the same letter were not significantly different ($P > 0.05$) Duncan's multiple range test.

Table 7. Mean harvest of parent strain and crossbred channel catfish and the relationship of the crossbred to the best parent strain.

<u>Experiment</u>	<u>Strain</u>	<u>$\bar{x}$ weight (g)</u>	<u>Percent increase or decrease</u>
3	Marion X Kansas	294 a	1.0
	Marion X Marion	291 a	---
	Kansas X Kansas	261 b	---
4	Auburn X Kansas	294 a	13.0
	Kansas X Kansas	261 b	---
11	Marion X Kansas	336 a	9.1
	Marion X Marion	308 b	---
	Kansas X Kansas	300 b	---
	AR X MK	310 b	0.0
	MK X MK	308 b	---
5	Marion X Kansas	694 a	6.9
	Marion X Marion	649 b	---
	Kansas X Kansas	620 b	---
6	Auburn X Rio Grande	494 a	7.9
	Rio Grande X Rio Grande	458 b	---
	Auburn X Auburn	454 b	---
7	Auburn X Auburn	489 a	---
	Rio Grande X Rio Grande	413 b	---
	Rio Grande X Auburn	398 b	18.0
8	Auburn X Uvalde	639 a	8.2
	Uvalde X Auburn	497 c	15.9
	Uvalde X Uvalde	591 b	---
	Auburn X Auburn	514 c	---

abc Means followed by the same letter were not significantly different ($P > 0.05$) Duncan's multiple range test.

Table 8. Mean body weights and the survival rates of Kansas select, K (S), Marion female x Kansas male, M x K, channel catfish and channel x blue (C x B) hybrid catfish.

Experiment	N	$\bar{X}$ body weight (g) at harvest	Survival (%)
1 M x K	600	110 ^d	88 ^e
K (S)	600	114 ^d	94 ^{de}
C x B-1 ^a	600	95 ^e	99 ^d
C x B-3 ^b	600	87 ^f	94 ^{de}
C x B-2 ^c	600	78 ^g	96 ^d
2 M x K	9900	578 ^d	80 ^e
C x B (1&2)	9900	574 ^d	98 ^d
3 C x B (1&2)	9600	530 ^h	97 ^d
K (S)	9600	523 ⁱ	88 ^e
4 C x B-1	150	526 ^d	97 ^d
C x B-3	150	472 ^e	97 ^d

^aMarion x Kansas channel catfish female x Auburn select blue catfish male.

^bMarion x Kansas channel catfish female x Commercial blue catfish male.

^cAuburn x Rio Grande channel catfish female x Commercial blue catfish male.

^{defg}Means followed by different letters are different (P < .05).

^{hi}Means followed by different letters are different (P < .01).

Figure 1

Figure 1. A comparison of mean net gains (g) and feed conversions (S) among six monosex populations of tilapia grown in cages and tanks: (1) *T. nilotica* (genotypic male and female, phenotypic male), (2) *T. nilotica* (genotypic female, phenotypic male), (3) *T. nilotica* (genotypic male, phenotypic male), (4) *T. mossambica* female x *T. hornorum* male (genotypic male, phenotypic male), (5) *T. nilotica* female x *T. hornorum* male (genotypic male, phenotypic male), (6) *T. nilotica* (genotypic female, phenotypic female). Means followed by different letters are significantly different, using Duncan's multiple range test ($P < 0.05$). Comparisons for cages are indicated in parentheses.

II. EVALUATION OF RESEARCH ACHIEVEMENTS

Research in Israel and the United States was directed (1) to test the feasibility of producing monosex male tilapia fry through direct hormone sex reversal, breeding of hormone sex-reversed brood fish, and interspecific hybridization; (2) to compare crossbreeding and hybridization with mass selection of tilapias and catfishes for improvement of growth; and (3) to apply electrophoresis for stock identification, maintenance of stock purity, and tracing parentage.

All research objectives were met in the collaborative studies in Israel and the United States. Hormone sex reversal of tilapias was achieved and applied in both countries. Hormone sex-reversed brood fish were produced through androgen treatment of Tilapia nilotica. The potential for breeding monosex populations was indicated through the successful production of all-female progenies. Interspecific hybridization of tilapia resulted in essentially all-male populations in both Israel and the United States, however, it was difficult to produce commercial quantities of fry. In Israel, a selection program was initiated with the goal to identify "proven" individuals for producing all-male hybrid progenies. Initial results were promising and base stocks have been established.

Crossbreeding of tilapia strains was initiated in the United States, results of which are incomplete. Mass selection in T. aurea resulted in 15% improvement in growth; there was no response by the Ivory Coast (U.S.) or Ghana (ISRAEL) strains of T. nilotica. Hybrid T. nilotica x T. hornorum was tested in both Israel and the United States, heterosis was indicated in both growth and survival.

Breeding programs for crossbreeding, hybridization and mass selection to improve growth in channel catfish were compared. Crossbreeding was effective in some 50% of crosses averaging 10% yield improvement. Hybridization of the

III. DESCRIPTION AND RESULTS OF COOPERATION

Cooperation between the grantee (Auburn University International Center for Aquaculture) and cooperating (Dor Fish and Aquaculture Research Station, ARO) institutions began with joint development of specific research plans through correspondence and by a 1-week visit to Dor by the principal investigator from Auburn. The co-principal investigator from Auburn visited Dor and several kibbutzim during 1982 to share data on sex reversal of tilapia through hormone administration. The co-principal investigator from Dor (ARO) visited Auburn during 1981 to provide expertise in experimental design of selection experiments with tilapia and catfish.

The collaboration between the institutions resulted in coordinated selection schemes in Tilapia aurea and T. nilotica at both research stations and has influenced experimental design in both countries. This has provided the data for obtaining the first realized heritability on growth of tilapia in the world.

The exchange has included information from both stations on sex ratios in single-pair, intraspecies spawns of tilapia, providing a new viewpoint on the sex determination mechanism in tilapias.

A most important result was the exchange of genetic material in tilapia. Israel provided T. aurea stock to Auburn, and Auburn has provided T. hornorum and T. nilotica (Ivory Coast strain) to Dor Station. There was further exchange in 1982 involving T. nilotica (Ghana strain) to Auburn and T. nilotica (Nile strain) to Israel.

Exchange of ideas influenced the interpretation of the results in both countries and similar results in both countries verified conclusions.

IV. BENEFITS TO AGRICULTURE

This research has demonstrated that hormone sex-reversal of tilapia is a valuable new tool in aquaculture, benefitting through both sex control and anabolic effects on growth. Hybridization resulted in heterosis for growth and survival, and the combination of hybridization and hormone treatment will likely benefit a large area of the world.

The catfish research demonstrated the effectiveness of selective breeding to fish farmers and resulted in the first known release of genetically improved fish to American farmers. The positive results also suggest that research should begin to evaluate the simultaneous use of more than one of these successful breeding programs to maximize rate of genetic improvement in farm-raised fishes.

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