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Hormonal Sex Reversal and Evidence of Female Homogamety in Black Crappie

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Abstract.—This study reports the results on hormone-induced sex reversal in black crappie *Pomoxis nigromaculatus* and the sex ratios of progenies obtained from test crosses of androgen-treated males with normal females. The androgen 17α-methyltestosterone (MT) was orally administered to fish with an artificial diet (30 mg/kg) for 30 d beginning 37 d after hatching; mean fish lengths were 35 and 55 mm at the beginning and conclusion of the treatment, respectively. The MT-treated group consisted of 95% males and 5% intersex fish, while the sex ratio in the control group was not significantly different from 1:1. Four males from the androgen-treated group were individually test-crossed with normal females to identify sex-reversed males according to the sex ratios in progenies and to reveal the chromosomal mechanism of sex determination in black crappie. Two out of three analyzed progenies consisted of females only, while one progeny had a sex ratio not significantly different from 1:1. The appearance of all-female progenies in test crosses of MT-treated fish indicated the existence of female homogamety (females, XX; males, XY) in black crappie.

The black crappie *Pomoxis nigromaculatus* and white crappie *P. annularis* are popular game fishes in the United States and have potential as aquaculture species. One of the main obstacles to successful management of their populations in small impoundments is their high rate of reproduction, which leads to overcrowding and subsequent stunting (USDA 1983; Martin 1988; Hooe 1991). Management suggestions have included production and rearing of interspecies hybrids (Hooe et al. 1994) or triploid fish (Baldwin et al. 1990; Parsons 1993; Parsons and Meals 1997).

Another possible solution is the production of monosex crappies. Al-ablani and Phelps (1997) reported the first study on the masculinization of black crappies by androgen treatment. The objective of this investigation was to further develop the androgen treatment; in particular, we tested the possibility of inducing sex reversal by the treatment of larger fish.

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Hormonal sex reversal may also be used for indirect production of monosex populations (Purdom 1993; Donaldson 1996). The indirect (genetic) method involves crossing normal fish with previously sex-reversed fish. This method is optimal because hormone-treated parental fish will not be consumed by humans. In the case of female homogamety (genotype of females, XX; genotype of males, XY), all-female progenies may be obtained by crossing sex-reversed males (neomales, XX) with normal females (XX). Thus, another objective of the present study was to test the sex determination mechanism in black crappie by analyzing sex composition in the progenies of males from an androgen-treated group (Hunter and Donaldson 1983; Purdom 1993).

Methods

The experiments were conducted at the Aquaculture Research Center, Kentucky State University, in 1999 and 2000. The fish used for the sex reversal experiment were obtained by artificial spawning (Gomelsky et al. 2000). Briefly, sexually mature black crappies were sorted by sex, paired (one male and one female), and placed into separate 115-L aquaria. Males were given a single intramuscular injection of luteinizing hormone releasing hormone analog ($LHRH_a$; 50 μg/kg of body weight) or human chorionic gonadotropin (HCG; 1,000 IU/kg). Females were given a total dose of $LHRH_a$ (100 μg/kg) or HCG (1,000 IU/kg) in two injections (10% and 90%, respectively, of the total dose) 12 h apart. We closely observed the fish in each aquarium, and when spawning behavior was first observed the female was removed and eggs were stripped. Because the males did not release sperm by stripping, they were killed and the testes dissected and washed with a 0.85% saline solution. The suspension obtained in this way was used for egg insemination. The inseminated eggs were spread evenly on shallow glass dishes, which were then submerged in 60-L plastic tanks. After transition to active feeding (6 d after hatching), the larvae were stocked in a nursery pond.

TABLE 1.—Fish growth (mm; mean ± SE) and survival (%) during the period of treatment with 17α-methyltestosterone (MT) and sex distribution in experimental groups of black crappie.

Group	Lengths during period of MT treatment		Survival	Length of fish analyzed	Number of fish analyzed	Sex (%)		
	Initial	Final				Male	Female	Intersex
MT-treated	35 ± 0.3	55 ± 0.8	94.0	150 ± 3.3[a]	38[b]	94.7	0	5.3
Control	35 ± 0.3	65 ± 0.9	89.3	126 ± 0.6	94	41.5[c]	58.5[c]	0

[a] Data from 9-month-old fish.
[b] Combined data from 9-month-old (n = 18) and 11-month-old (n = 20) fish.
[c] Not significantly different from 1:1 ($P > 0.05$).

Reared fry (33 d after hatching) were collected from the pond and stocked into two round 0.8-m^3 tanks supplied with flow-through water (150 fish per tank). During the first 3 d, fish were fed a combination of frozen *Artemia* nauplii and a dry diet. During the next 30 d (i.e., from 37 to 67 d after hatching), fish in one tank were fed an artificial diet containing 30 mg/kg of 17α-methyltestosterone (MT) while those in another tank (the control) were fed an androgen-free diet. The MT-containing diet was prepared according to a technique used in experiments on hormonal sex reversal in striped bass *Morone saxatilis* and their hybrids (Gomelsky et al. 1999). The 17α-methyltestosterone (Sigma Chemical Company, St. Louis, Missouri) was suspended in a small quantity of edible vegetable oil (2–3% of the weight of the diet) and mixed with commercial fish diet (FryFeed Kyowa, Kyowa Hakko Kogyo Co., Ltd., Tokyo). Oil without MT was also added to the control diet. The water temperature was 26–28°C.

After hormonal treatment, fish were raised in tanks on an androgen-free diet or in earthen ponds on natural foods. The sex ratio in the control group was determined by inspection of 9-month-old fish. The effectiveness of the androgen treatment was determined by inspection of both 9- and 11-month-old fish. The gonads were sexed by examination of their morphological structure and color, examination under a microscope using the "squash" method (Guerrero and Shelton 1974), as well as histological examination. Gonad samples were fixed in a 10% solution of buffered formalin and embedded in paraffin; cross sections (7 μm) were stained with hematoxylin and eosin.

Four 11-month-old males from the MT-treated group were test-crossed individually with normal black crappie females. These test crosses were performed by artificial spawning according to the technique described above. The larvae obtained by these crosses were stocked in separate earthen ponds. Sex ratios in progenies were determined by analysis of 6-month-old fish. Fish were sexed according to the methods described above, including macroscopic, microscopic, and histological methods. Deviations of the sex ratios from 1:1 were assessed by means of a chi-square test.

Results

The growth and survival rates of fish during the period of androgen treatment are presented in Table 1. Survival was high in both groups, though the mean final length of fish in the MT-treated group was less than that of fish in the control group. Since fish of each variant were raised only in one tank, this difference in lengths could not be statistically evaluated.

The sex ratio in the control group was not significantly different from 1:1 ($P > 0.05$; Table 1). Males and females from the control group had well-developed, maturing testes and ovaries. Histological analysis of testes showed that the lumens of the seminiferous tubules were filled with agglomerations of spermatozoa, which is typical of maturing fish testes; in the ovaries, the oocytes were at different stages of vitellogenesis.

Investigation of the MT-treated group showed that the androgen treatment was effective: no females were found. Of the 38 fish analyzed, 36 (94.7%) were males and the remaining 2 were intersex fish. Histological analysis of testes revealed a normal process of spermatogenesis, and the lumens of the seminiferous tubules were filled with agglomerations of spermatozoa. The gonads of the two intersex fish contained both male and female generative tissue.

Survival of the larvae obtained from test crosses was low in some ponds. Only three progenies survived (Table 2), and only 11 fish were collected and analyzed from male number 3. The fish obtained from male number 1 had well developed testes or ovaries (Figure 1) and a sex ratio that was not significantly ($P > 0.05$) different from 1:1. The progenies obtained from males number 2

TABLE 2.—Sex distribution of progenies obtained in test crosses of black crappie males treated with 17α-methyltestosterone with normal females.

Male number	Length of fish analyzed (mm; mean ± SE)	Number of fish analyzed	Sex (%)		Proposed genotype of male
			Male	Female	
1	124 ± 0.8	130	54.6[a]	45.4[a]	XY
2	111 ± 0.7	206	0	100	XX
3	126 ± 2.2	11	0	100	XX

[a] Not significantly different from 1:1 ($P > 0.05$).

and 3 consisted only of females; these fish had well-developed ovaries that were similar to those observed in the females from the mixed-sex progeny of male number 1.

Discussion

Al-ablani and Phelps (1997) reported up to 90% males after a 30-d MT treatment that started when the fish were 40 d old. The mean lengths at the beginning and conclusion of the hormonal treatment were 23 and 27 mm, respectively, and survival during the treatment was 47%; the same hormonal treatment was ineffective in 60-d-old fish of similar size. In the present study, we treated fish with MT from 37 to 67 d after hatching, which was close to the optimal period according to the data of Al-ablani and Phelps (1997); however, the fish in our experiment were much larger, with initial and final mean lengths of 35 and 55 mm, respectively. The positive results of our experiment (95% males and no females in MT-treated group) support the suggestion by Al-ablani and Phelps (1997) that in black crappie age at the initiation of the androgen treatment is more important than size. The raising of larger fish with an artificial diet was technically simpler and provided higher survival (94% in the MT-treated group).

It is known (Hunter and Donaldson 1983; Purdom 1993) that the type of chromosomal sex determination mechanism in a given species may be revealed by the sex composition of the progenies obtained in test crosses of males from androgen-treated groups with normal females. Female homogamety (genotype of females, XX; genotype of males, XY) is revealed when sex-reversed males (XX) are crossed with normal females (XX) and all-female progenies are produced. In the case of male homogamety (genotype of males, ZZ; genotype of females, WZ), mixed-sex progenies are produced with a male:female ratio about 1:3 (Hunter and Donaldson 1983) when the sex-reversed males (WZ) are crossed with normal females (WZ). We analyzed three progenies obtained in test crosses. The progenies of males number 2 and 3 consisted of only females. The appearance of all-female progenies in test crosses indicated that the genotype of these males was XX (Table

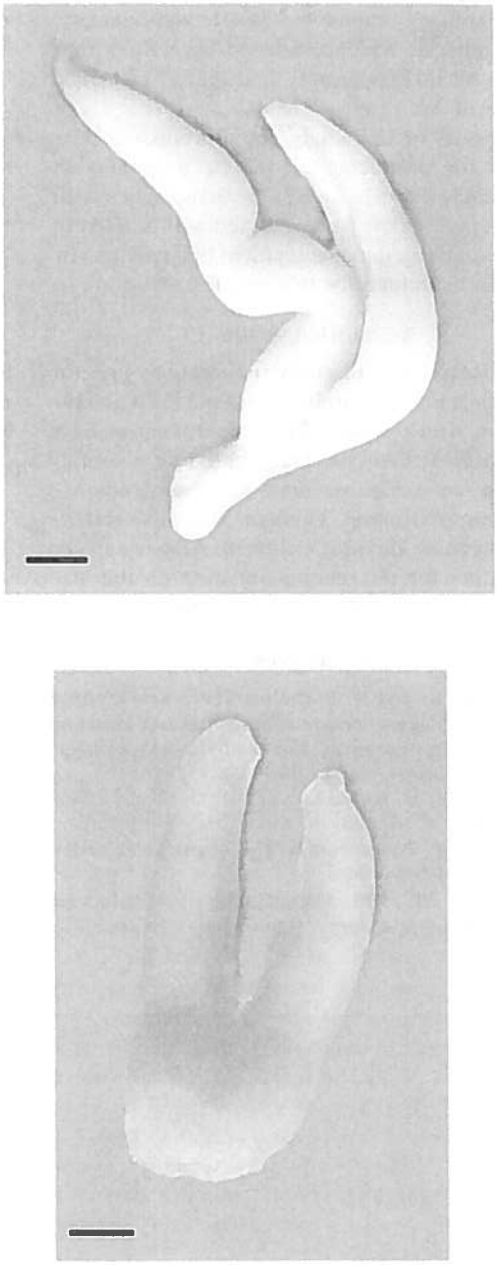

FIGURE 1.—Gonads of fish from progeny obtained from male number 1. The upper panel shows a testis, the lower panel an ovary; bar = 2.5 mm.

2) and that black crappie has female homogamety. Male number 1, which produced progeny with a sex ratio of 1:1, apparently had the normal male genotype of XY (Table 2).

The results of this study show promising possibilities for producing all-female progenies in black crappie by crossing sex-reversed males with normal females. The data obtained will be used in further studies on the development of a genetic sex regulation technique for this species.

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