Reviews in Fisheries Science, 17(1):1–7, 2009

ISSN: 1064-1262 print
DOI: 10.1080/10641260802003073

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Progress in Studies on Hormonal Sex Reversal and Genetic Sex Control in Black Crappie

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Sex control can solve the problem of stunted black crappie populations in small impoundments. The main objectives of the present study were (1) to identify sex-reversed males of black crappie from a previously obtained androgen-treated group using test crosses, and (2) to develop broodstock of sex-reversed males by masculinization of fish from those crosses. An additional objective of the study was to try to identify sex-specific RAPD markers, which might be used for identification of sex-reversed males. The progenies resulting from test crosses were divided into two groups: Group I (control) fish were raised without hormonal treatment, while Group II fish were subjected to androgen (MT) treatment. Seven progenies were obtained from the cross of preliminary androgen-treated males with normal females. Six of seven progenies had sex ratio in Groups I close to 1:1, and one progeny consisted of females only. This shows that only one male, which generated this progeny, was a sex-reversed homogametic fish (XX) while the other males were heterogametic normal (XY) fish. In Group II the androgen 17α-methyltestosterone (MT) was orally administered to crappie with an artificial diet (30 mg/kg) for 40 days beginning 35 days post hatching; androgen-treated groups consisted of 95–100% males. Sex-specific random-amplified polymorphic DNA markers were not identified in black crappie.

Keywords black crappie, sex reversal, methyltestosterone, RAPD markers

INTRODUCTION

The black crappie *Pomoxis nigromaculatus* is a popular sportfish in the United States and has potential as an aquaculture species. One of the main obstacles to successful management of their populations in ponds or small impoundments is their high rate of reproduction, which leads to overcrowding and subsequent stunting (USDA, 1983; Mitzner, 1984; Martin, 1988; Hooe, 1991). Rearing of monosex progenies obtained by genetic sex regulation, including the crossing of normal and sex-reversed fish, might solve this problem. This method of genetic sex regulation allows the production of monosex populations on a large scale by a simple breeding process, eliminates the need for continued use of hormones, and ensures that hormone-treated fish will not be consumed by humans (Hunter and Donaldson, 1983; Devlin and Nagahama, 2002).

Gomelsky et al. (2002) successfully induced hormonal sex-reversal in black crappie and demonstrated female homogamety (females XX, males XY) in this species. For species with female homogamety, the most practical way of genetic sex regulation is crossing sex-reversed (XX) males (neomales) with normal females (XX) for the production of all-female progeny.

Previous attempts to produce monosex male black crappie populations through direct hormonal sex reversal yielded variable results. Al-Ablani (1997) achieved up to 79% masculinization by oral administration of trenbolone acetate (TBA). Al-Ablani and Phelps (1997) obtained 90% masculinization by feeding fish with feed containing 17α-methyltestosterone (MT). Gomelsky et al. (2002) achieved 95% masculinization by oral administration of MT. Finally, Arslan and Phelps (2004) accomplished 100% masculinization by immersing fry in TBA and MT solutions. One objective of this present investigation was to further develop the masculinization technique in black

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crappie with special attention on identifying proper size and age as critical factors to the initiation of androgen treatment.

After the masculinization of the normal progeny, the second step for genetic sex regulation is to distinguish the sex-reversed males XX from genotypic males XY. Test crossing of males from the androgen-treated groups is usually used for this purpose (Hunter and Donaldson, 1983; Purdom, 1993). Sex-reversed males (XX), when crossed with normal females (XX), produce all-female progenies, while normal males (XY) give mix-sex progenies. Another objective of the present study was to identify sex-reversed males of black crappie in a previously obtained androgen-treated group and to develop broodstock of sex-reversed males by masculinization of the XX progenies obtained in test crosses.

Progeny testing to identify sex-reversed males can be tedious and requires extensive facilities. Another approach to identify sex-reversed males is to develop sex-specific DNA markers. Segments of the Y chromosome may have distinctive DNA sequences that differ from those found in the X chromosome (Dunham, 1990). If a molecular marker is located in the sex chromosomes, it can be used for the identification of genetic sex. One of the first systems to identify genetic sex in fish was elaborated by Devlin et al. (1994) for Chinook salmon *Oncorhynchus tshawytscha*. Since this study, additional species have had sex genetic markers identified, including rainbow trout *Oncorhynchus mykiss* (Iturra et al., 1998) and African catfish *Clarias gariepinus* (Kovács et al., 2001) using random amplified polymorphic DNA (RAPD). Amplified fragment length polymorphism (AFLP) was used for DNA sex identification in the three-spined stickleback *Gasterosteus aculeatus* (Griffiths et al., 2000), the white shrimp *Litopenaeus vannamei* (Pérez et al., 2004), and the puffer fish *Takifugu rubripes* (Cui et al., 2006). The application of the RAPD assay (Welsh and McClellan, 1990; Williams et al., 1990) together with bulked segregant analysis (Michelmore et al., 1991) has created an efficient approach to identify genetic markers associated with specific regions of the genome. Thus, the third objective of the present study was to attempt to identify sex-specific RAPD markers, which might be used for identification of sex-reversed males.

MATERIALS AND METHODS

The experiments were conducted at the Aquaculture Research Center, Kentucky State University, Frankfort, in 2004 and 2005. Mature males obtained from a preliminary androgen-treated group in 2002 (Gomelsky, unpublished) were test crossed with normal females. The males represented 56% of this androgen-treated group as compared with 24% in the control group. The general scheme of the experiment was the following (Figure 1): males from the 2002 preliminary androgen-treated group were individually crossed with normal females to identify neomales. To move toward development of neomale broodfish and establish sex reversal efficiency, the resulting progenies were divided into two groups. Group I (control) fish were raised without any hormonal treatment while the fish in Group II were subjected to androgen (MT) treatment to achieve fish masculinization. The final step was the investigation of fish sex ratios in control and androgen-treated groups. The sex ratio in Group I would indicate whether the male genotype of XX or XY was used for production of given progeny. The sex ratio in the androgen-treated group would show the effectiveness of hormonal sex reversal.

Average weights of males used in test crosses were 120 ± 34 g (mean $\pm$ SD) and 162 ± 38 g in 2004 and 2005, respectively. Average weights of females were 105 ± 39 g and 143 ± 40 g in 2004 and 2005, respectively. For induction of spawning, fish were injected intramuscularly with human chorionic gonadotropin (HCG) at a dosage level of 1,000 IU/kg BW. Injected breeders were paired (one male:one female) and placed into separate 115-L aquaria for natural spawning. After spawning, brooders were removed from the aquaria. In 2004, the males used in test crosses were sacrificed after spawning, and the testes were observed macroscopically and fixed in a 10% solution of buffered formalin for further histological examination. After transition to active feeding (beginning 6 days post hatching), the larvae were subsequently stocked to nursery ponds or tanks.

A total of seven progenies were produced in 2004 and 2005 (Table 1). Survival of progeny 1-2004 was low, and therefore it was not split into two groups and MT treatment was not applied. The other six progenies (one from 2004 and five from 2005) were divided, as planned, into control (Group I) and MT-treated (Group II) groups. For MT treatment, fish collected from nursery ponds or tanks were placed into round 0.8-m^3 tanks. During the 40-day treatment period, fish were fed with an automatic belt feeder (Fiap Fish Technik, Hohenburg, Germany), continuously releasing a prepared diet (INVE Aquaculture, Dendermonde, Belgium) containing 30 mg/kg of 17α-methyltestosterone (MT). The MT-containing diet was prepared according to a technique used in experiments on hormonal sex-reversal of striped bass, *Morone saxatilis*, and their hybrids (Gomelsky et al., 1999). The 17α-methyltestosterone (Sigma, St. Louis, MO, USA) was suspended in a small quantity of edible vegetable oil (2–3% of the weight of the diet) and mixed with the prepared diet. The water temperature during MT treatment was 26–28°C. After hormonal treatment, fish were stocked in earthen ponds and raised on natural foods.

The fish of Group I from progeny 2-2004 were fed an androgen-free diet in a 0.8-m^3 tank over the 40-day period and later stocked into an earthen pond. In other progenies, the control groups of fish were stocked directly to ponds from nursing tanks or ponds.

The effectiveness of the androgen treatment was determined by inspection of 5-month-old fish, except for the progeny from 2-2004, which were sexed at 13 months (Table 1). The gonads were sexed by examination of their morphological structure and color under a microscope using the "squash" method (Guerrero and Shelton, 1974). Further, histological preparation and examination were performed. Gonad samples were fixed in a 10%

Table 1 Fish lengths (mean ± SD) and survival (%) during the period of treatment with 17 α-methyltestosterone (MT) and sex distribution in control (Group I) and MT-treated (Group II) groups of progenies obtained in test crosses

Progeny Year	Group	Initial Number of fish	Lengths (mm) during period of MT treatment		Survival	Length of fish analyzed	Number of fish analyzed	Sex (%)		Genotype of male
			Initial	Final				Males	Females	
1-2004	Control	75			60.0	155.7 ± 0.7	40	—	100.0	XX
2-2004	MT-treated	81	21.2 ± 1.3	28.1 ± 0.2	88.9	149.2 ± 10	27	100.0	—	
	Control	82	21.2 ± 1.3	33.9 ± 0.4	95.1	122.6 ± 2.9	23	54.5	45.5	XY
1-2005	MT-treated	40	16.0 ± 1.5	33[a]	67.5	104.1 ± 0.7	8	100.0	—	
	Control	35			N/R	157.8 ± 0.6	13	53.9	46.1	XY
2-2005	MT-treated	450	20.2 ± 1.8	26.1 ± 3.9	46.0	100 ± 0.4	21	95.2	4.8	
	Control	600			N/R	126.3 ± 0.5	49	44.9	55.1	XY
3-2005	MT-treated	400	20.6 ± 1.4	29.9 ± 4.1	41.3	116.2 ± 0.6	18	100.0	—	
	Control	300			N/R	140.3 ± 0.5	75	57.3	42.7	XY
4-2005	MT-treated	100	17.1 ± 1.1	35.4 ± 4.1	89.0	106.6 ± 0.7	20	95.0	5.0	
	Control	65			N/R	129.3 ± 0.9	44	38.6	61.4	XY
5-2005	MT-treated	600	21.1 ± 0.2	29.5 ± 0.5	62.2	97.5 ± 0.8	31	100.0	—	
	Control	500			N/R	125.8 ± 0.4	77	51.9	48.1	XY

[a]Data for one fish.
N/R = not recorded.

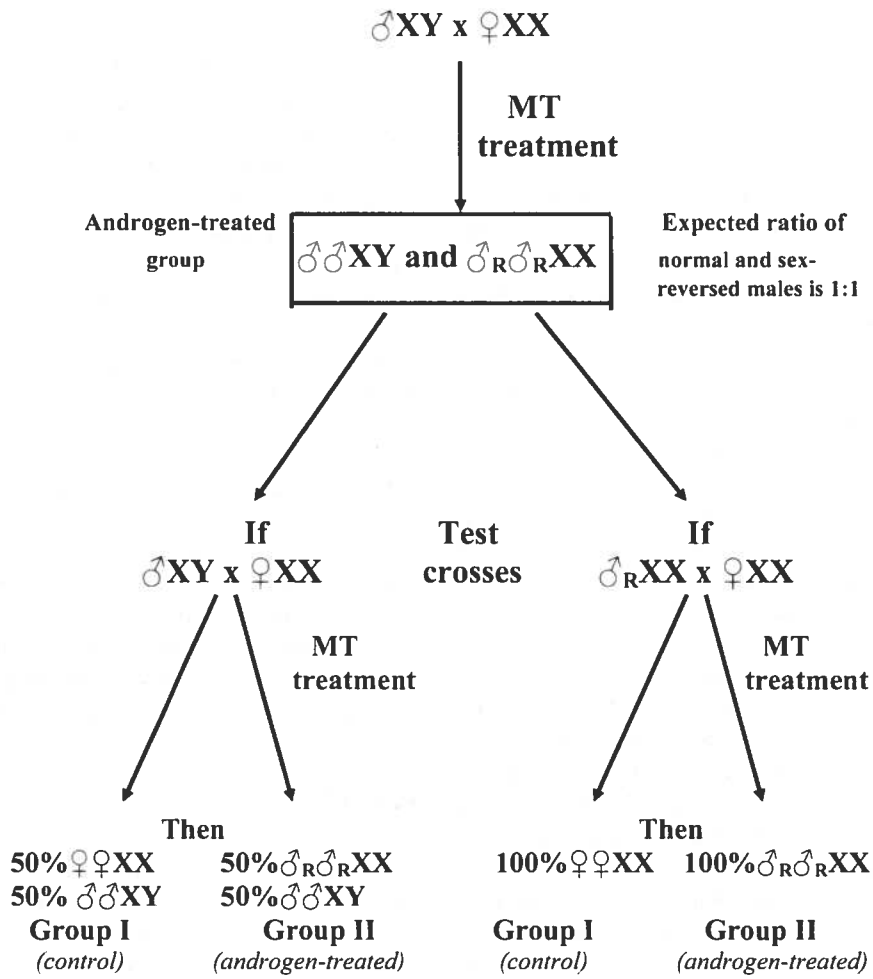

Figure 1 Schematic diagram of the protocol used to identify sex-reversed males ($\sigma^{\prime}_{R}$) that are capable of producing all-female progenies.

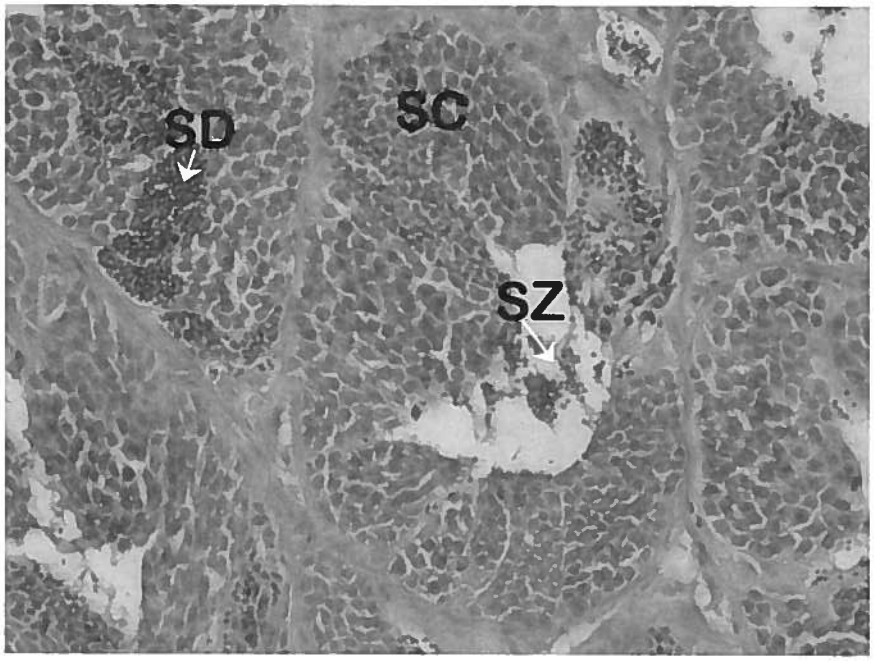

Figure 2 Normal testis of the sex-reversed male, which produced all-female progeny. The gonad showed various stages of spermatogenesis (sc: spermatocytes; sd: spermatids; sz: spermatozoa).

solution of buffered formalin and embedded in paraffin; cross sections (7 μm) were stained with hematoxylin and eosin. Deviations of the sex ratios from 1:1 were assessed by means of a chi-square test.

DNA extraction and the RAPD marker system used were conducted using previously published protocols (Lowe, 2005). For identification of sex-specific DNA markers, the normal mix-sex progeny obtained in a study on inheritance of predorsal black stripe in black crappie (Gomelsky et al., 2005; progeny group 3) were used. Samples (i.e., fin clips) were collected from 5-month-old fish; fish mean length was 10.58 $\pm$ 0.5 cm. The sex of each fish was identified. The fin clips were preserved into 95% ethanol until processed. DNA was extracted from the samples with the Promega Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA) using the protocol for mouse tail extraction with the exception that the tissue was initially incubated at 65°C instead of 55°C. Approximately 18–39 mg of tissue was used for each extraction. Following extraction, the DNA concentration was determined using a Pharmacia Biotech GeneQuant Pro Spectrophotometer (Amersham Biosciences, Piscataway, NJ, USA). Following quantification, portions of the DNA stocks were diluted to a concentration of 1 ng/μl and held at 4°C until used as a template source for all PCR reactions. Conditions for PCR reactions were 1X PCR buffer, 0.02 mmol dNTPs, 0.195 pmol/μl primer, 0.1 U/μl Taq polymerase, 2.5 mmol $MgCl_2$, 1 ng/μl DNA template, and 10.8 μl H_2O, for a total volume of 20 μl. Two pairs of pools (males vs. females) were generated from individual DNA samples; the number of individuals per pool was six and contained an equal amount of DNA from each individual. These resulting two pools were screened against the father and mother (i.e., parents which produced this progeny). A total of 47 RAPD primers from Operon and University of British Columbia (UBC) primers sets were screened against two pools of full sibs of six females (daughters), six males (sons), and their parents (father and mother). All PCR reactions were performed in a Gene Amp® System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) using the same program, which consisted of an initial period of 94°C for 5 min, followed by 45 cycles of 1 min at 95°C, 1 min 30 sec at 36°C, 30 sec at 54°C, and 2 min at 72°C, with a final extension period of 15 min at 72°C. Following amplification, PCR reactions were electrophoresed on 300 ml 1.4% agarose gels in 1/2X TBE buffer at 120 V for approximately 4 hr to separate PCR products. After electrophoresis, gels were stained using ethidium bromide and visualized on a UV transilluminator. A digital photo using a Kodak DC120 digital camera (Kodak, Rochester, NY, USA) was taken for subsequent gel analysis. Gels were scored based on the presence or absence of specific products for each primer. Promising primers that amplified a band shared only by either the pool of sons and the father, or by the pool of daughters and the mother, were examined in individual males and females.

RESULTS

The data on fish survival during the period of hormonal treatment and sex distributions in the control (Group I) and MT-treated groups (Group II) are given in Table 1. In six of the seven progenies, the sex ratios in the control (intact) groups did not differ significantly from a 1:1 ratio ($p > 0.05$). In the progeny 1-2004, the control group consisted of all females (Table 1).

Fry survival in Group II during MT treatment ranged from 41–89%. Investigation of fish sex ratio in the MT-treated groups showed that the androgen treatment was highly effective. The MT-treated groups in four progenies (2-2004, 1-2005, 3-2005, and 5-2005) consisted of males only; in two progenies (2-2005 and 4-2005) the percentage of males in MT-treated groups was 95%.

Histological analysis confirmed sexing of fish in progenies based on examination of morphological structure and color of gonads and revealed a normal process of spermatogenesis in the testes. The testes of the male, which produced all-female progeny (1-2004), also had normal histological structure (Figure 2). Male sexual cells of different stages (spermatogonia, spematocytes, and spermatids) were developed in cysts located in the walls of seminiferous tubules; agglomerations of spermatozoa were located in the lumens of seminiferous tubules.

A total of 47 different RAPD primers were tested. A total of 324 bands were examined and each primer produced eight bands on average. Several primers produced bands that were observed only in the sons' bulked and father's DNA samples but not in the daughters' and mother's DNA samples (Figure 3). Further analysis of individual fish samples showed that these differences were not sex-specific but resulted from occasional segregation of fish with and without bands among "female" and "male" groups. Finally, it was concluded that none of the 47 tested primers produced sex-specific bands.

DISCUSSION

From the seven progenies obtained in test-crosses, six had a sex ratio in control groups of approximately 1:1 and only one (1-2004) consisted of females only. This demonstrates that only the male, which generated this progeny, was a genotypic sex-reversed fish (XX), while the other males were genotypically normal (XY). This is the first report of the ability of a sex-reversed male black crappie to reproduce naturally and to produce all-female progeny. In a previous study on sex reversal in this species, the progenies from sex-reversed males were obtained artificially by sacrificing the fish and macerating their testes (Gomelsky et al., 2002). Unfortunately, the number of fish in progeny 1-2004 was low and, supposedly, not sufficient for androgen treatment. Therefore, we have not produced sex-reversed males in larger quantity for further development of sex-reversed broodstock. The reason for the observed low frequency of sex-reversed males (one from seven) in the tested group is not clear. As mentioned above, the tested androgen-treated group had 56% males, while the control group had a sex ratio shifted to the prevalence of females (24% males:76% females). Therefore we expected that the ratio of normal (XY) and sex-reversed (XX) males in the tested androgen-treated group should be close to 1:1. It is possible that sex-reversed males have less profound secondary sexual traits (spawning color, etc.) and therefore have not been chosen for spawning. This hypothesis should be tested in further studies.

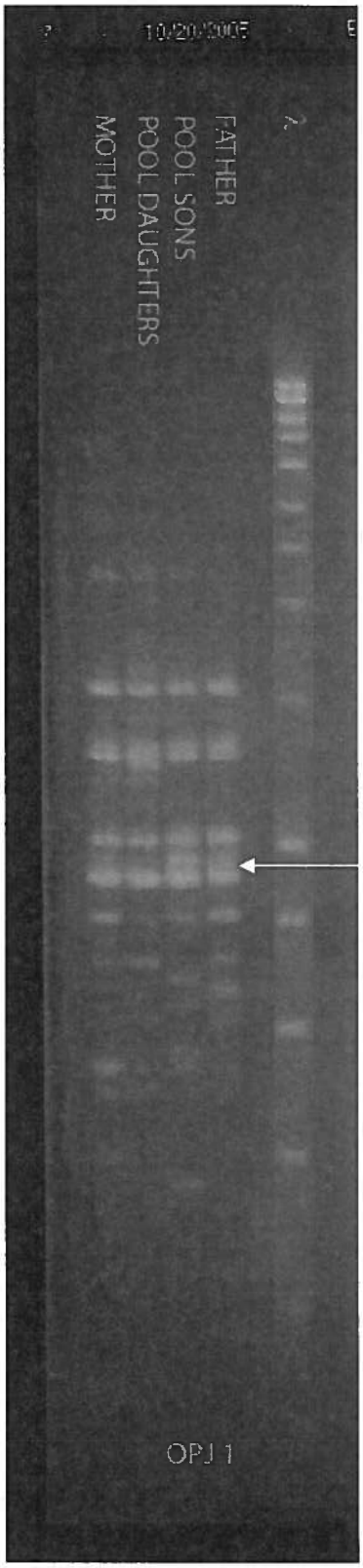

Figure 3 Agarose gel (1.4%) of RAPD-PCR amplification products using primer OPJ1. The pooling of sons and daughters were screened against the father and mother. The arrow indicates an amplification product which thought to be specific to the male DNA.

Table 2 Comparison of the different studies of masculinization of black crappie by oral administration of MT

MT dosage mg/kg	MT treatment duration[a]	Age at the start of MT treatment[b]	Lengths (mm) during period of MT treatment		% Survival	% Males	References
			Initial	Final			
30	30	40	23 ± 2 .2	28 ± 3.7	40	71	Al-Ablani and Phelps, 1997
60	30	40	23 ± 2 .2	27 ± 3.4	47	90	Al-Ablani and Phelps, 1997
60	30	60	24 ± 3.4	30 ± 3.1	57	57	Al-Ablani and Phelps, 1997
30	30	37	35 ± 0.3	55 ± 0.8	94	95	Gomelsky et al. 2002
30	30	45	35 ± 2.1	53 ± 0.5	73	56[c]	Gomelsky unpublished data
60	45	45	20 ± 1.4	38 ± 4.0	60	49	Arslan and Phelps, 2004
60	45	45	26 ± 2.0	40 ± 3.5	64	50	Arslan and Phelps, 2004
30	40	35	20 ± 2.5	29 ± 4.6	55	95–100	Present Study

[a]Days.
[b]Days post hatch.
[c]Sex ratio in the control group was significantly different ($P < 0.025$) from a 1:1 ratio.

Oral administration of 30 mg 17α-methyltestosterone per kg of feed for 40 days, starting with 35-day-old black crappie, is a feasible technique for masculinization. Four of the six trials in this study resulted in 100% masculinization. The other two trials resulted in 95% males. Overall, this method resulted in 98.7% male progeny. This method generates the highest percentage of males compared with previous studies that used oral administration of MT in black crappie (Table 2). Al-Ablani and Phelps (1997) achieved 90% males starting with an older fry (40 days old), higher dosage (60 mg MT/kg), and shorter MT duration (30 days). Gomelsky et al. (2002) used the same dosage as the present study, but in an older fry (37 days old) and produced 95% males. In a second attempt, Gomelsky (unpublished data) tried to achieve sex reversal in older fry (45 days old) but attained only 56% males (vs. 24% males in control). Finally, Arslan and Phelps (2004) decided to prolong the treatment for 45 days using 60 mg MT/kg diet to 45-day-old crappies without success (50% males). Thus, the labile period, during which endocrine sex reversal is possible, appears to be between 35 to 40 days post-hatch for black crappie. It is important to mention that, in this study, the feeding of fish was done by automatic feeders. Crappie fry only eat artificial feed when pellets are falling, but do not eat from the bottom. With the use of an automatic feeder, the fish were exposed to the hormone constantly, which ensured a sufficient dosage. This differs from Al-Ablani and Phelps (1997) and Arslan and Phelps (2004), who fed fish four times a day.

One possible disadvantage of the current method is overall low survival of the MT-treated groups (from 41 to 89%). Pandian and Kirankumar (2003) stated that hormonally sex-reversed fish suffered from low survival and functional deficiencies. However, in these experiments, all dead fish were weak and thin, indicating that the probable cause of mortality was starvation. Treated crappie presented normal gonadal development, and no intersex fish were found. The present data suggest that the age of the fish (rather than their size) may be used as a practical criterion for determining the appropriate period of androgen treatment.

A promising method for distinguishing sex-reversed males (XX) from normal males (XY) is the development of DNA sex-specific markers. These markers have been identified in guppy *Poecilia reticulata* (Nanda et al., 1990), Chinook salmon *Oncorhynchus tshawytscha* (Devlin et al., 1991), jackdaw *Corvus monedula*, zebra finch *Taenopygia guttata* (Griffiths and Tiwari, 1993), rainbow trout *Oncorhynchus mykiss* (Iturra et al., 1998), platyfish *Xiphophorus maculatus* (Coughlan et al., 1999), and several other species. It should be noted that the attempts to detect a molecular marker for the identification of sex in several fish species such as Nile tilapia *Oreochromis niloticus* (McConnell et al., 1996; Bardakci, 2000), Atlantic salmon *Salmo salar* (McGowan and Davidson, 1998), the green spotted pufferfish *Tetraodon nigroviridis* (Li et al., 2002), and sturgeons *Acipenser* spp. (Wuertz et al., 2006) failed. In the present study, we screened only 47 RAPD primers and, despite observed DNA marker polymorphisms, did not identify any sex-related markers. Screening of additional primers may still result in the identification of sex-related RAPD markers in crappie.

In summary, these studies clearly demonstrated the potential to achieve high rates of masculinization in black crappie when androgens are applied by oral administration to 35-day-old fry. The dosage and duration of treatment within a particular age are critical to treatment effectiveness. The results of this study show the promising possibility for producing all-female progenies in black crappie by naturally 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.

ACKNOWLEDGMENTS

Support for this study was provided by Kentucky's Regional University Trust Fund to the Aquaculture Program as Kentucky State University's Program of Distinction. The authors give special thanks to James Tidwell for reviewing this manuscript, Noel

Novelo for assistance during this project, and Linda Hayden for making available interlibrary loans.

REFERENCES

Al-Ablani, S. A. Use of synthetic steroids to produce monosex populations of selected species of sunfishes (Family: Centrarchidae). Dissertation, Auburn University, Auburn, AL (1997).

Al-Ablani, S. A., and R. P. Phelps. Sex reversal in black crappie *Pomoxis nigromaculatus:* Effect of oral administration of 17α-methyltestosterone on two age classes. *Aquaculture,* **158:** 155–165 (1997).

Arslan, T., and R. P. Phelps. Production of monosex male black crappie, *Pomoxis nigromaculatus,* populations by multiple androgen immersion. *Aquaculture,* **234:** 561–573 (2004).

Bardakci, F. The use of random amplified polymorphic DNA (RAPD) markers in sex discrimination in Nile tilapia, *Oreochromis niloticus* (Pisces: Cichlidae). *Turk. J. Biol.,* **24:** 169–175 (2000).

Coughlan, T., M. Schartl, U. Hornung, I. Hope, and A. Stewart. PCR-based sex test for *Xiphophorus maculatus. J. Fish Biol.,* **54:** 218–222 (1999).

Cui, J.-Z., X.-Y. Shen, Q.-L. Gong, G.-P. Yang, and Q.-Q. Gu. Identification of sex markers by cDNA-AFLP in *Takifugu rubripes. Aquaculture,* **257:** 30–36 (2006).

Devlin, R. H., B. K. McNeil, T. D. Groves, and E. M. Donaldson. Isolation of a Y-chromosomal DNA probe capable of determining genetic sex in Chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.,* **48:** 1606–1612 (1991).

Devlin, R. H., B. K. McNeil, I. I. Solar, and E. M. Donaldson. A rapid PCR-based test for Y-chromosomal DNA allows simple production of all-female strains of Chinook salmon. *Aquaculture,* **128:** 211–220 (1994).

Devlin, R. H., and Y. Nagahama. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture,* **208:** 191–364 (2002).

Dunham, R. A. Production and use of monosex or sterile fishes in aquaculture. *Rev. Aquat. Sci.,* **2:** 1–17 (1990).

Gomelsky, B., N. B. Cherfas, A. Gissis, and G. Hulata. Hormonal sex inversion in striped bass and white bass X striped bass hybrids. *N. Am. J. Aquacult.,* **61:** 199–205 (1999).

Gomelsky, B., S. D. Mims, R. J. Onders, and W. B. Bean. Hormonal sex reversal and evidence of female homogamety in black crappie. *N. Am. J. Aquacult.,* **64:** 66–69 (2002).

Gomelsky, B., S. D. Mims, R. J. Onders, and N. D. Novelo. Inheritance of predorsal black stripe in black crappie. *N. Am. J. Aquacult.,* **67:** 167–170 (2005).

Griffiths, R., and B. Tiwari. The isolation of molecular genetic markers for the identification of sex. *Proc. Natl. Acad. Sci. USA,* **90:** 8324–8326 (1993).

Griffiths, R., K. J. Orr, A. Adam, and I. Barber. DNA sex identification in the three-spined stickleback. *J. Fish Biol.,* **57:** 1331–1334 (2000).

Guerrero, R. D., and W. L. Shelton. An acetocarmine squash method for sexing juvenile fishes. *Prog. Fish Cult.,* **36:** 56 (1974).

Hooe, M. L. Crappie biology and management. *N. Am. J. Fish. Manage.,* **11:** 483–484 (1991).

Hunter, G. A., and E. M. Donaldson. Hormonal sex control and its application to fish culture. **In:** *Fish Physiology, Vol. IX, Reproduction, Part B: Behavior and Fertility Control,* pp. 223–303. (W. S. Hoar, D. J. Randall, and E. M. Donaldson, Eds.). New York: Academic Press (1983).

Iturra, P., J. F. Medrano, M. Bagley, N. Lam, and J. C. Marin. Identification of sex chromosome molecular markers using RAPDs and fluorescent *in situ* hybridization in rainbow trout. *Genetica,* **101:** 209–213 (1998).

Kovács, B., S. Egedi, R. Bártfai, and L. Orbán. Male-specific DNA markers from African catfish (*Clarias gariepinus*). *Genetica,* **110:** 267–276 (2001).

Li, Y., J. A. Hill, G. H. Yue, F. Chen, and L. Orban. Extensive search does not identify genomic sex marker in *Tetraodon nigroviridis. J. Fish Biol.,* **61:** 1314–1317 (2002).

Lowe, J. D. Randomly amplified polymorphic DNA (RAPD) and microsatellite markers in paddlefish, *Polyodon spathula.* Dissertation, Kentucky State University, Frankfort, KY. (2005).

Martin, M. Black and hybrid crappie culture and crappie management. *Aquacult. Mag.,* **14(3):** 35–41 (1988).

McConnell, S. K. J., D. O. F. Skibinski, and J. A. Beardmore. A search for sex-specific DNA regions in *Oreochromis niloticus,* pp. 349–353. **In:** *Proceedings of the Third International Symposium on Tilapia in Aquaculture Conference ICLARM 41* (R. S. V. Pullin, J. Lazard, M. Legendre, J. B. Amon Kothias, and D. Pauly, Eds.). Manila, Philippines (1996).

McGowan, C., and W. S. Davidson. The RAPD technique fails to detect a male-specific genetic marker in Atlantic salmon. *J. Fish Biol.,* **53:** 1134–1136 (1998).

Michelmore, R. W., I. Paran, and R. V. Kesseli. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregation populations. *Proc. Natl. Acad. Sci. USA,* **88:** 9828–9832 (1991).

Mitzner, L. Crappie management: Problems and solutions. *N. Am. J. Fish. Manage.,* **11:** 534–542 (1984).

Nanda, I., W. Feichtinger, M. Schmid, J. H. Schröder, H. Zischler, and J. T. Epplen. Simple repetitive sequence are associated with differentiation of the sex chromosome in the guppy fish. *J. Mol. Evol.,* **30:** 456–462 (1990).

Pandian, T. J., and S. Kirankumar. Recent advances in hormonal induction of sex-reversal in fish. *J. Appl. Aquacult.,* **13:** 205–230 (2003).

Pérez, F., C. Erazo, M. Zhinaula, F. Volckaert, and J. Calderón. A sex-specific linkage map of the white shrimp *Penaeus* (*Litopenaeus*) *vannamei* based on AFLP markers. *Aquaculture,* **242:** 105–118 (2004).

Purdom, C. J. Genetics and fish breeding. *Fish and Fisheries, Series 8.* London: Chapman and Hall (1993).

USDA. National Aquaculture Development Plan, Vol. 2. Washington, DC: United States Department of Agriculture (1983).

Welsh, J., and M. McClellan. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.,* **18:** 7213–7218 (1990).

Williams, J. K. G., A. R. K. Kubelik, J. L. Livak, J. A. Rafalski, and S. V. Tingey. DNA polymorphism amplified by random primers are useful as genetic markers. *Nucleic Acids Res.,* **18:** 757–763 (1990).

Wuertz, S., S. Gaillard, F. Barbisan, S. Carle, L. Congiu, A. Forlani, J. Aubert, F. Kirschbaum, E. Tosi, L. Zane, and J. Grillasca. Extensive screening of sturgeon genomes by random screening techniques revealed no sex-specific marker. *Aquaculture,* **258:** 685–688 (2006).