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The Effect of the Absence or Presence of Channel Catfish Males on Induced Ovulation of Channel Catfish Females for Artificial Fertilization with Blue Catfish Sperm

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Abstract.—Female channel catfish *Ictalurus punctatus* were induced to spawn with carp pituitary extract either singly paired with channel catfish males in aquaria (males present) or stocked with multiple females in aquaria or tanks (males absent). There was no difference between ovulation rates of females induced to spawn in the presence or absence of channel catfish males ($P = 0.57$). Females hand-stripped with males absent produced more eggs per kilogram of body weight (4,585) than females with males present (2,942; $P = 0.07$). When females in the males-present treatment deposited eggs in the aquaria, which allowed observation of the readiness of females, the number of eggs available for stripping was reduced. There was no difference in fertilization rate between treatments when using sperm from blue catfish *I. furcatus* ($P = 0.35$). Females that ovulated with males absent produced a similar ($P = 0.12$) number of viable eggs per kilogram of female body weight as females that ovulated with males present (3,809 and 2,291, respectively). It may be commercially feasible to artificially produce hybrids of channel and blue catfish by using the methods demonstrated in this experiment. This study has shown that pairing channel catfish females with males is unnecessary to induce ovulation in females and that more eggs are available to the culturist for hybrid production when males are absent.

The catfish industry accounts for over 50% of U.S. aquaculture production and has become one of the major fish-farming enterprises worldwide. Channel catfish *Ictalurus punctatus* accounts for virtually all of the catfish production in the U.S. The hybrid between the female channel catfish and the male blue catfish *I. furcatus* exhibits several traits that are superior to those of the channel catfish, including increased growth, tolerance of low oxygen, disease resistance, seinability, hook-and-line vulnerability, and dress-out and fillet percentage (Yant et al. 1976; Tave et al. 1981; Dunham et al. 1983a, 1983b; Smitherman and Dunham 1985; Argue 1996). Commercial production of this hybrid would greatly enhance the catfish industry. Unfortunately, reproductive isolating mechanisms

have prevented widespread commercial production of this hybrid (Dunham and Smitherman 1987). An alternative to circumvent this problem is artificial fertilization of channel catfish eggs with blue catfish sperm. Recently, techniques have been developed to increase the efficiency of fertilization of channel catfish eggs with blue catfish sperm (Bart 1994; Bart and Dunham 1996). However, ovulation and stripping of eggs from channel catfish needs to be more efficient to maximize the feasibility of producing this hybrid.

Earlier experiments on the hybridization of ictalurid catfishes indicated that it was necessary to pair individual females with individual males of the same species and then use hormone induction to obtain ovulation of these females in aquaria (Dupree and Green 1969). The pairs of catfish were observed, and the culturists would only remove the female for stripping after approximately 25% (10–50%) of the eggs were deposited and fertilized in the aquarium. It was believed that the female could only be stripped after egg deposition had begun (Dunham 1993). At that point, the female was removed and anesthetized, and the remaining eggs were stripped. This procedure is inefficient in the use of space and the need for channel catfish males and because a percentage of the eggs are unavailable for producing hybrids. Research since that time has indicated that the timing of ovulation can be predicted within a few hours when carp pituitary extract (CPE) is used as the ovulating agent (Dunham 1993; Argue 1996). Therefore, if multiple channel catfish females could be induced with CPE to ovulate without males, the artificial propagation of hybrids would be more efficient. The objectives of this study were to compare the survival, ovulation rate, and egg yield of channel catfish females ovulated in the presence and absence of male channel catfish. These eggs were fertilized with blue catfish sperm to determine if the presence or absence of channel catfish males affected egg quality.

Methods

Channel catfish females (mean weight = 3.04 kg, range = 1.07–5.44 kg) were selected for spawn-

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TABLE 1.—Mean \pm SE body weight, percent survival, percent ovulation, eggs/kg female body weight, percent fertilization, and viable eggs/kg female body weight for female channel catfish ovulated with or without male channel catfish present. Eggs were fertilized with blue catfish sperm.

Variable	Male absent	Male present	<i>P</i>
Female body weight (kg)	2.99 \pm 0.20	3.07 \pm 0.17	0.65
Percent survival of females	87.5 \pm 0.5	97.5 \pm 2.5	0.06
Percent ovulation (surviving females)	76 \pm 9.0	66.0 \pm 12.5	0.57
Percent ovulation (all females)	66 \pm 9.0	65.0 \pm 12.7	0.78
Eggs/kg body weight (ovulated females)	5,649 \pm 557	4,730 \pm 638	0.28
Eggs/kg body weight (all females)	4,585 \pm 39	2,942 \pm 446	0.07
Percent fertilization	81 \pm 4.1	70 \pm 7.2	0.35
Viable eggs/kg body weight (all females)	3,809 \pm 405	2,291 \pm 443	0.12

ing readiness (well-rounded abdomen and swollen and reddish urogenital area). Fish judged to be ready for spawning were randomly divided between the two treatments. In the first treatment, one female was stocked in a 160-L aquarium with a channel catfish male of similar size. In the second treatment, two females were stocked in a 160-L aquarium without a male. Fifteen aquaria were stocked in the first treatment, and nine aquaria were stocked in the second treatment (one aquarium was stocked with only one female), for totals of 15 and 17 females per treatment, respectively. Reservoir water flowed through the aquaria at a rate of 20 L/min for both treatments. Additionally, aeration was provided to maintain dissolved oxygen above 6.0 mg/L. Initially, water temperature was 26°C. All females were injected with 2.0 mg CPE/kg body weight. Twelve hours later, a resolving dose of 8.0 mg CPE/kg was administered. When males were present, stripping was delayed until females exhibited ovulation and mating with the males. If the female never began to deposit eggs, she was removed for stripping at the end of the day. The females were anesthetized in a 250-mg/L solution of tricaine methanesulfonate (MS-222), and the eggs were manually stripped into a bowl containing Hanks' balanced salt solution (HBSS; Bart 1994; Tiersch et al. 1994; Bart and Dunham 1996). Females in the second treatment (without males to help induce ovulation) were manually stripped of their eggs by the same procedures as in treatment 1, 35–48 h after the initial dose of CPE. Blue catfish males were sacrificed to remove their testes for artificial fertilization of the eggs. Testes were macerated by hand into 5 mL HBSS for each gram of testes. The resulting sperm solution was mixed with the eggs (2.5 mL for every 100 mL eggs), and pond water was added to activate spermatozoa and ova. The eggs were then water hardened for 1 h, transferred to paddlewheel troughs, and incubated at 28°C. Percent fertile eggs

was recorded approximately 48 h after fertilization. A sample of eggs was placed in a clear petri dish and a light shown through the bottom to view the eggs and estimate the percent that was developing. Number of viable embryos were estimated by multiplying fertilization rate and number of eggs. Eggs were treated with formalin to prevent fungus growth.

The experiment was repeated a second time with the same treatments and the same ovulation, stripping, fertilization, and incubation procedures. The male-present treatment was repeated as in the first set, but with 19 aquaria. However, in the second set, the 15 females for the male-absent treatment were all stocked together in one tank containing 1,000 L of water. Flow rate in this tank was 80 L/min.

The survival of the females before ovulation, the number of fish ovulating, number of eggs, fertilization rates, and number of viable embryos were determined. The two treatments were compared with a Student's *t*-test.

Results and Discussion

Mean survival rates of females in the male-present experiment was higher (97%) than in the male-absent treatment (87%; $P = 0.06$; Table 1). The difference in survival was the result of more fighting in the communal female aquaria and tanks compared with the aquaria with females paired with similar-sized males. This may be alleviated by stocking together females of similar size (females ranged from 1.20 to 5.0 kg in the male-absent treatment and from 1.07 to 5.44 kg in the male-present treatment).

The ovulation rate of the male-present treatment (66%) was not significantly different than the male-absent treatment (76%; $P = 0.57$; Table 1). However, the number of eggs produced per kilogram of all females (including females that did not ovulate) was higher ($P = 0.07$) for the male-absent

treatment (4,585 eggs/kg) than for the male-present treatment (2,942 eggs/kg female body weight). One explanation for this difference is the fact that in the male-present treatment the females were allowed to ovulate eggs before hand stripping was initiated. Females in the male-present treatment deposited an average of 1,298 eggs/kg female body weight before hand-stripping. Previous observations indicated that if this initial deposition of eggs was not allowed to take place, the hand stripping of the females was often difficult (Dunham 1993). No discernable differences were observed for ease of stripping of eggs from females of either treatment. Fertility was not significantly different ($P = 0.35$) for females without males (81%) compared with females of the male-present treatment (70%). Despite the cumulative differences in ovulation rate, eggs/kg, and fertilization, there was no significant difference ($P = 0.12$) in number of viable embryos/kg produced in the male-absent treatment (3,809 embryos/kg) compared with the male-present treatment (2,291 embryos/kg). This was due to a large variation among individuals despite a calculated 66% difference between the treatments. There was no difference between trials 1 and 2 for any of the variables tested ($P > 0.2$). Also, the use of aquaria or tank had no effect on any of the variables in the male-absent treatment ($P > 0.25$).

The ovulation rate, yield of eggs, and fertilization rates for the male-absent treatment indicate great promise for the application of this procedure to the catfish industry. There are several advantages to the male-absent procedure. The ovulation rate, egg yield, and fertilization rates were the same or superior to the traditional males-present treatment, resulting in almost twice (1.7) the number of viable embryos. Additionally, this procedure reduces the need for male channel catfish broodstock and hatchery facilities to pair broodstock, allowing more space for channel catfish females, blue catfish males, or production. Also, the absence of the males made fish handling and injection of CPE 33% quicker and more efficient. It took the same amount of time to inject 15 females from the male-absent treatment as it did to inject 10 females in the male-present treatment. Obviously, even if researchers interpret these treatments as equal ($P \leq 0.05$), the male-absent treatment is more efficient than the male-present technique in producing hybrids.

Artificial fertilization for hybrid production with the male-absent technique approaches the efficiency of natural spawning of channel catfish in

the farm environment. In pond conditions, approximately 30–50% of females will spawn (Hatch et al. 1987). These females should produce approximately 7,800 eggs/kg female body weight (Walser and Phelps 1993). This results in about 3,510 eggs/kg of female body weight if 90% are viable (Hatch et al. 1987). If 30% rather than 50% of the females spawn, this figure is lowered to 2,110 eggs/kg. The 2,290–3,800 viable eggs/kg produced artificially is comparable with the pond spawning results. Although artificial fertilization procedures appear to require more labor, the labor of checking spawning containers is eliminated. Additionally, hormone spawning allows synchronization of spawning and shortening of the spawning season, which should result in more efficient use of labor. Therefore, labor costs would be predicted to be equal between traditional pond spawning in channel catfish and the artificial propagation of hybrids, and perhaps actually more efficient for the artificial fertilization procedure. However, one may argue that it requires more skilled labor to execute hand-stripping than checking spawning cans. Our observation has been that unskilled labor quickly becomes adept at hand-stripping and that workers respond in a positive, motivated manner to the increase in job diversity and responsibility.

Tieman (1995) compared costs of producing fingerling hybrid catfish and channel catfish. The cost of producing hybrids was 6% lower because it was more efficient than producing fingerlings in ponds. Of course, there is an incidental cost of the hormones, and there is additional capital investment for the spawning aquaria or tanks (aquaria are not needed for the male-absent procedure, which will result in cost savings) associated with the artificial fertilization procedure. This is balanced by a reduction of pond space required for holding broodstock. The ovulation procedure may be further improved to make the cost per embryo less than that for natural spawning. Only 72% of the expected 7,800 eggs/kg (Walser and Phelps 1993) were hand stripped from the ovulating females in the male-absent treatment. Additional experimentation is needed to adjust dosage rate of the CPE, anesthesiology procedures, and the hand-stripping procedure to increase the yield of eggs per female.

The application of these artificial fertilization procedures for the production of channel \times blue catfish hybrid embryos will allow large-scale commercial production of this hybrid. This will have a major economic impact in all aspects of the catfish industry because this hybrid grows faster, tolerates lower water quality, has higher disease re-

sistance, higher seinability, increased hook-and-line vulnerability, and increased dress-out and fillet percentage (Yant et al. 1976; Tave et al. 1981; Dunham et al. 1983a, 1983b; Smitherman and Dunham 1985; Dunham and Argue 1998) compared with the traditionally grown channel catfish. One problem, however, will be the time needed to obtain and grow an adequate number of blue catfish for the industry to produce this hybrid.

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