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Comparison of Manual Stripping and Pen Spawning for Production of Channel Catfish × Blue Catfish Hybrids and Aquarium Spawning of Channel Catfish

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Abstract.—We compare manual stripping to pen spawning for production of hybrids of female channel catfish *Ictalurus punctatus* × male blue catfish *I. furcatus*, as well as to aquarium spawning of channel catfish. The ovulation rate for manually stripped females (91%) was not significantly different from that of females spawned in aquaria with channel catfish males (67%; $P > 0.05$); however, ovulation rate of manually stripped females during week 10 (80%) was superior to that of females spawned in pens (15%; $P < 0.05$) with blue catfish males. Female channel catfish spawned in aquaria with male channel catfish produced more ($P < 0.05$) eggs per kilogram body weight (6,607) than manually stripped females (4,587). Pen spawning of female channel catfish and male blue catfish also produced significantly more ($P < 0.05$) eggs per kilogram (7,950) than hand-stripping females (3,448) during week 10. Hatching rate (21%) and fry per kilogram (1,065) of female body weight were higher ($P < 0.05$) for channel catfish spawned with channel catfish in aquaria than for channel catfish manually stripped and fertilized with blue catfish sperm (8% and 325 fry/kg). This difference in means was a result of spawning technique because we have previously demonstrated that blue catfish and channel catfish sperm fertilize channel catfish eggs at equivalent rates. Hatching rate (66%) was also higher ($P < 0.05$) for pen-spawned hybrid embryos than for hybrid embryos produced by manual stripping and artificial fertilization (21%). However, fry per kilogram of female body weight was not different ($P > 0.05$) for manual stripping and pen spawning (851 and 821, respectively). The results from both experiments indicate that the quantity and quality of eggs and embryos obtained by manual stripping and artificial fertilization are inferior to eggs and embryos produced naturally but with hormone injections. Artificial fertilization procedures need to be improved. Hybrid fry produced per kilogram of female body weight for manual stripping and pen spawning were almost identical because of the low hatch rate for manually stripped eggs and the low spawning rate for pen spawning. Therefore, neither technique is better for commercial production of channel catfish × blue catfish embryos; improved hatching rates for manual stripping or higher ovulation rates for pen spawning would enhance production.

Introduction

Use of hybrid fish (female channel catfish *Ictalurus punctatus* × male blue catfish *I. furcatus*; hereafter referred to as CB hybrid) could have a significant impact on the catfish industry. Giudice (1966) first reported heterosis of channel catfish and blue catfish hybrids for potential in aquaculture. The CB hybrid has outperformed all other commercially raised catfish (Yant et al. 1976; Dunham and Smitherman 1987; Argue 1996; Wolters et al. 1996; Dunham and Argue 1998; Dunham and Devlin 1998). This is the only hybrid among 28 interspecific hybrids evaluated within the family Ictaluridae that exhibits overdominant traits desirable for intensive aquaculture (Dupree et al.

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1966; Dunham et al. 1982; Dunham and Smitherman 1983). The hybrid has great potential, but in the commercial catfish industry large-scale production of fingerlings has been impeded by reproductive isolating mechanisms between parent species (Tave and Smitherman 1982).

Dupree et al. (1966) elaborated on the techniques that overcome natural reproductive isolating mechanisms and facilitate channel catfish and blue catfish hybridization. Various efforts have been made to increase production of hybrid seed, including pen spawning following administration of hormone injections of channel catfish females (Tave and Smitherman 1982) and separation of channel catfish males and females during final oocyte maturation periods following hormone injections (Dunham et al. 1998). Research has also focused on optimal sperm concentrations needed for the artificial fertilization of eggs stripped from channel catfish (Bart et al. 1998) and on optimal hormone dosage regimes and multiple strippings to maximize production of viable hybrid gametes (Kim 1996).

The objectives of our research were to compare production capabilities of manually stripped and pen-spawned CB hybrids and to compare those methods with production of channel catfish in aquaria.

Methods

Experimental fish.—Female and male channel catfish and male blue catfish used in this experiment were maintained at the Fish Genetics Research Unit, Alabama Agricultural Experimental Station, Auburn University. Broodstock were fed 36% protein floating catfish feed from June 1996 until January 1997, and then fed 48% protein floating catfish feed two times per week until the spawning season. This diet was supplemented with raw chicken liver one time per week from January until May. Feeding rate was 3% of the total biomass of broodfish stocked per pond. Stocking densities for female broodstock were between 625 and 1,250 fish/ha.

Females of several channel catfish strains were used: Kansas (K), Marion (M), and Auburn (A), and lines derived from their crosses. Male blue catfish used to artificially produce hybrids were Commercial, D & B, Auburn-Commercial, and Tombigbee strains (Dunham and Smitherman 1984). Male blue catfish strains used in pens were Rio Grande, Auburn × Rio Grande, and Tombigbee. Data from all strains of channel catfish and blue catfish were pooled because no significant strain effects were observed for any trait (Lambert 1998). Body weights were 1.0–4.5 kg for female channel catfish, and 2.0–8.0 kg male blue catfish. Fish were spawned eight times over an 11-week period. Artificial spawning was conducted during each of the eight spawning trials, aquarium spawning during the first three trials, and pen spawning during the only the seventh trial.

Preparation of pituitary solutions.—Whole carp pituitary glands (Stollers, Iowa) were homogenized in saline solution using a Tissue Tearor (Biospec Products). After centrifugation (2 min at 3,000 revolutions/min, 20°C), the tissue residue was discarded and the prepared supernatant diluted to 1 mg carp pituitary/mL and 4 mg carp pituitary/mL were stored and refrigerated at 4°C until used (up to 20 h later).

Administration of hormone injections.—Females were given a priming injection of carp pituitary extract (CPE) followed 12 h later by a resolving injection. The regimes used (mg CPE/kg female body weight, priming: resolving injections) were 1:4 ($N = 16$), 2:4 ($N = 28$), 1:8 ($N = 18$), and 2:8 ($N = 49$). The regimes were not significantly different for inducing ovulation (as supported by Lambert et al. 1999), so data were pooled in the data analysis. Two days before anticipated spawning, females were seined from ponds and transported to an indoor holding facility. They were held together in a single, fiberglass tank supplied with continuous flow-through water maintained at 28°C. Individual females were randomly chosen, weighed, and administered the hormone. We used 18-gauge needles for intraperitoneal injections. Females from the same treatment were held together in tanks.

Artificial fertilization.—At 24 h after the resolving injection, females that expressed eggs when handled were removed from tanks and anesthetized in 250 mg/L tricaine methanesulfonate (MS-222). After all experimental females had been examined for ovulation and test-stripped, they were placed in a holding tank until restocked in outdoor ponds. Females that did not express eggs at the first attempt were checked one more time. Stripping ceased when all females had been stripped or attempts to strip them failed.

Anesthetized females were dried with a clean towel and eggs were stripped into a dry plastic bowl lubricated with vegetable shortening. Quantity of eggs was volumetrically enumerated. A sample of 5 mL of eggs removed from each mass was enumerated. Then the volume of the entire egg mass was determined. Strain and weight of

each female were recorded before stripping. Then Hanks balanced salt solution (HBSS; 160.0 g NaCl, 8.0 g KCl, 2.8 g $CaCl_2$, 4.0 g $MgSO_4 \cdot 7H_2O$, 2.4 g $Na_2HPO_4 \cdot 7H_2O$, 1.2 g KH_2PO_4, 7.0 g $NaHCO_3$, 20.0 g glucose/20 L reverse osmosis dH_2O, pH 6.9) was added and eggs were held until fertilization. Eggs were fertilized within 15 min of stripping.

Male blue catfish were sacrificed and their anterior testes surgically removed and weighed. To fertilize the manually stripped eggs, sperm was removed from the testes by macerating them in HBSS (Bart 1994). At least 0.5 g of testes in a 2.5-mL suspension was used per 100 mL of eggs. Testes from two or more males were macerated together in HBSS. The HBSS–sperm suspension was mixed with individual egg masses, after which approximately 1 L of water was added. Activated gametes were left undisturbed for 2 min, then an additional 5 L of water was added. Every 15 min water was decanted from developing egg masses, and an equivalent amount of water was replaced. After egg masses developed an adhesive matrix and had water hardened for 1 h, they were placed in wire mesh baskets suspended in paddlewheel incubation troughs. Egg masses were treated with 100 ppm formalin three times per day until 1–2 d before hatch. All water used to activate gametes and incubate eggs originated from surface runoff sources. Water flowing into the hatchery was maintained at 28°C. Total hardness and alkalinity of the holding water were 34 and 28 ppm, respectively.

Fertilization rates and the quantity of eggs remaining were determined 48 h after activation. Eggs were considered fertilized if neural ridge development and tail bud movement of the embryo was visible in a glass petri dish held above a 75-W bulb. Fertilization rate was based on volumetrically enumerated eggs. That is, a sample of 5 mL of eggs was removed from each mass and enumerated, and that count was expanded by the volume of entire egg mass. Hatching percentage was estimated in the same volumetric manner.

Aquaria spawning.—Production of CB hybrids by manual stripping was compared with two techniques that were more natural but included hormone induction: aquarium spawning and pen spawning. In pen spawning, blue catfish males were paired with channel catfish females. However, in aquarium spawning, channel catfish males were used because in the past we had found hybrid spawning in aquaria to be problematic. Additionally, we had previously observed (Dunham et al. 1999) no difference in blue catfish and channel catfish sperm for fertilizing channel catfish eggs. We therefore believed this treatment would increase the data quality and quantity for eggs and embryos obtained from manual stripping versus more natural techniques.

Female and male channel catfish were placed together for spawning in 160-L aquaria immediately after females ($N = 12$) received CPE priming injections. For the first three spawning trials, embryos from aquarium spawning of channel catfish females × males was compared with artificially produced catfish CB hybrid embryos. Female channel catfish receiving one of the four CPE treatments (three matings per treatment) were paired in aquaria with a male channel catfish of similar weight. Aquaria were situated over tanks holding females that received the same CPE treatment. This facilitated monitoring of ovulation for each treatment. When a female deposited eggs in an aquarium mounted over a treatment holding tank, we attempted to strip females held in the tank below that aquarium. Fertilized eggs were removed from the aquaria, volumetrically enumerated, then placed in paddlewheel incubation troughs. Fertilization and hatch of the naturally spawned eggs from the aquaria were compared with artificially fertilized eggs.

Pen spawning.—In pen spawning, blue catfish males were placed in pens (1.5 × 1.5 × 2.0 m) in an earthen pond 3 d before the females; this allowed them time to clean plastic spawning containers. These matings were conducted during the seventh spawning trial at week 10 and were compared with manually stripped eggs in week 10. Spawning cans were placed in each pen. Channel catfish females ($N = 47$) were given different rates of CPE (2 mg/kg with no resolving injection, 2 mg/kg, and 8 mg/kg body weight, priming: resolving) and paired with male blue catfish. Cans were checked for eggs 3 d after females were placed in the pens with males. When eggs were present, they were gently removed from spawning cans, transported back to the hatchery, volumetrically enumerated, and placed in paddlewheel troughs for incubation.

Statistical analysis.—We compared fertilization rates, eggs and fry produced per kilogram of female body weight, and hatching rates for the aquarium-spawned, manually stripped, and pen-spawned catfish. All data were analyzed using SAS 6.12 (1996). Percentages were transformed using the arcsine of their square root to normalize the distributions before statistical analysis (Zar 1984). Analysis of variance (ANOVA) was used to com-

TABLE 1.—Entire spawning period comparison (means ± SE) between manually stripped female channel catfish and those induced to spawn in aquaria. Eggs laid by females in aquaria were fertilized by male channel catfish. Stripped females include those induced to ovulate with carp pituitary extract at 1:4, 2:4, 1:8, or 2:8 mg/kg female body weight (priming: resolving injections); ovulation results were not significantly different (Lambert et al. 1999). Stripped females were then fertilized with sperm from either male channel catfish or male blue catfish. Means sharing the same letter in the same row are not significantly different ($P > 0.05$).

	Aquaria-spawned females ($N = 12$)	Stripped females ($N = 111$)
Ovulation rate (%)	67 z	91 z
Percent fertilization	75 ± 6 y	49 ± 4 z
Eggs/kg	6,607 ± 1,274 y	4,587 ± 390 z
Percent hatch	21 ± 4 y	8 ± 2 z
Fry/kg	1,065 ± 526 y	325 ± 67 z

TABLE 2.—Comparison (means ± SE) of week-10 fertilization rates, eggs produced per kilogram of female channel catfish, hybrid catfish (channel catfish ♀ × blue catfish ♂) embryo hatching rate, and hybrid fry produced per kilogram of female induced to spawn in pens or artificially spawned and hand-stripped. Females were induced to ovulate using carp pituitary extract (2 mg/kg body weight : 8 mg/kg body weight, priming : resolving injections). Means followed by the same letter in the same row are not significantly different ($P > 0.05$).

	Artificial spawning ($N = 10$)	Pen spawning ($N = 47$)
Ovulation rate (%)	80 y	15 z
Fertilization rate (%)	45 ± 14 y	79 ± 8 z
Eggs/kg	3,488 ± 972 y	7,950 ± 1,027 z
Hatching rate (%)	22 ± 8 y	66 ± 9 z
Fry/kg ovulated ♀	1,064 ± 526 y	5,474 ± 1.272 z
Fry/total kg ♀	851 y	821 y

pare effects (Zar 1984). Effect of female weight on eggs per female kilogram was examined with regression analysis. Comparisons were made between manual stripping and aquarium spawning and between manual stripping and pen spawning.

Results and Discussion

Aquarium Spawning versus Manual Stripping

No differences were observed in ovulation rate between female channel catfish manually stripped (91%, $N = 111$) and those in the aquarium trials (67%, $N = 12$; $P > 0.05$; Table 1) during the entire spawning period. In contrast, female channel catfish manually stripped in week 10 (80%, $N = 10$) had significantly higher ovulation rates than female channel catfish spawned with male blue catfish (15%, $N = 47$) in pens during week 10 ($P < 0.05$; Table 2). Channel catfish females allowed to spawn naturally in aquaria with male channel catfish produced significantly more eggs per kilogram (6,607) than channel catfish females artificially spawned (4,587 eggs/kg; $P < 0.05$; Table 1). Hatching rate and fry per kilogram of female body weight (21% and 1,065 fry/kg) was greater ($P < 0.01$) for aquarium-spawned channel catfish females than for those manually stripped and fertilized with male blue catfish sperm (8% and 325 fry/kg).

Contrast of Pen Spawning and Manual Stripping

Female channel catfish manually stripped (80%, $N = 10$) had higher ovulation rates than those spawned with male blue catfish in pens (15%, $N = 47$; $P < 0.05$; Table 2). The pen-spawned female channel catfish produced more eggs per kilogram of body weight (7,950/kg) than did females manually stripped (3,488/kg; $P < 0.05$; Table 2) during week 10. Female channel catfish spawned in pens with blue male catfish produced more fry per kilogram (5,474) body weight of ovulated female than females manually stripped (1,064 fry/kg; Table 2; $P < 0.05$). Female body weight did not influence eggs per kilogram ($r^2 = 0.04$, $P = 0.81$). Hatching rates of egg masses collected from pens were higher (66%) than egg masses produced by females artificially spawned (22%; $P < 0.05$); fertilization rates were different between the two treatments ($P < 0.05$; Table 2). Spawning frequency or ovulation rate of channel catfish females paired with blue catfish males in pens was lower ($P < 0.05$; Table 2) than that for manually stripped channel catfish females. Although number of fry produced per ovulated female was higher for pen-spawned females ($P < 0.05$); the number of fry produced per total kilogram of females used during the experiment was not different ($P = 0.47$; Table 2).

Artificial spawning techniques using CPE may ensure consistently high spawning frequencies; however, egg quality and fry production is compromised by factors such as mechanical damage of eggs during manual stripping, inconsistent carp pituitary quality, unnatural hormone levels and ratios, and stress. In contrast, female channel catfish given priming: resolving injections of CPE and paired with blue catfish males in pens have lower ovulation frequencies but produce better quality spawns and more fry per kilogram female than females ovulated and stripped (Table 2). However,

fry per kilogram of female body weight was not different for manually stripped and pen-spawned females (851 and 821 fry/kg, respectively). Manual stripping of females may produce eggs that are not yet fully mature or eggs that are physically damaged, hence, leading to death of embryos and a greater frequency of infected egg masses. The results from both experiments indicate that the quantity and quality of eggs and embryos obtained by manual stripping and artificial fertilization are inferior to those from females that spawn naturally after hormone injection. Artificial fertilization procedures need to be improved. Hybrid fry produced per kilogram of female for manual stripping and pen spawning were almost identical because of the low hatching rate for manually stripped eggs and the low spawning rate for pen spawning, so neither technique is better than the other for commercial production of CB hybrid embryos. An improvement in these shortcomings—i.e., hatching rate for manual stripping and ovulation rate for pen spawning—could enhance production of these hybrids, so future research should address both these areas.

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