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Preliminary Studies of Artificial Spawning of Channel Catfish as Male-Female Pairs or All-Female Groups in Recirculating Systems

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Abstract.—Channel catfish *Ictalurus punctatus* are commonly spawned for research purposes by pairing of a hormonally treated female with a male in flow-through aquaria. A technique that allows hormonal induction of ovulation in females without pairing would accelerate genetic improvement and production of hybrid catfish. Over a 3-yr period (1994, 1995, and 1996) we conducted a series of trials to demonstrate the potential for artificial spawning in recirculating systems, and in 1996 we included trials with grouped females in addition to male-female pairs. Females were induced to spawn with injection of synthetic leuteinizing hormone-releasing hormone, and those that ovulated were stripped and the eggs were artificially fertilized. During 1994 and 1995, all fish were spawned by pairing, and in 1996, half of the females were spawned by pairing and half were grouped in tanks without males. Spawning success (percent of females that produced eggs), latency (time between injection and ovulation), and percent fertilization were observed for the paired and grouped trials. Spawning success was 36% in 1994 (N = 36), 22% in 1995 (N = 54), 41% in 1996 (N = 27), and 58% for grouped females (N = 26). The latency period was 113 ± 69 h in 1994, 109 ± 57 h in 1995, 44 ± 8 h in 1996, and 50 ± 9 h for grouped females. Percent fertilization was 16 ± 26% for eggs stripped in 1994, 72 ± 25% in 1995, 43 ± 20% in 1996, and 16 ± 37% for grouped females. In 1995, water quality problems were associated with high mortality of females (24 of 44 females; 4 of 44 males). The metabolic demands of final oocyte maturation in combination with methemoglobinemia caused by high nitrite levels could account for the increased vulnerability of females. These trials indicate that with adequate biofiltration, artificial spawning is possible in recirculating systems and with females grouped rather than paired. Further research on hormone dosage and timing of egg stripping will increase the utility of grouped spawning of channel catfish.

Channel catfish *Ictalurus punctatus* have been cultured for more than 70 yr for food production and recreational purposes (Huner and Dupree 1984). There are three methods recognized for spawning of channel catfish: 1) natural (pond) spawning; 2) pen spawning; and 3) aquarium or artificial spawning (Busch 1985). The channel catfish industry relies exclusively on pond spawning to supply fry and fingerlings for grow-out. Farmers provide containers (e.g., milk cans, wooden boxes, metal drums) in ponds where spawning is desired (Steeby 1987) to simulate natural sites selected by channel catfish (Clapp 1929). The male chooses a spawning site, attracts a female, and the pair spawns until completion of egg laying by the female. The eggs form a mass held together by a proteinaceous matrix (Ringle et al. 1992). The male guards the egg mass by driving other fish away, including the female, once spawning is completed.

Pen spawning involves providing suitable spawning containers within wire mesh enclosures in ponds. The enclosures are often 120 cm × 180 cm minimum, with sides extending ≥30 cm above water level. The pens allow pairing and control of timing of spawning (Dupree and Huner 1984). A brood pair is selected, and the female can be injected with hormone to induce ovulation. The female is removed after spawning, and egg masses can be collected and placed in a hatchery or left with the male to hatch in the pond (Huner and Dupree 1984).

Aquarium spawning allows control over pairing and time of spawning, and allows

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collection of unfertilized eggs from females. This offers benefits including crosses with more than one male, production of hybrids, and manipulation of ploidy. The aquarium method is currently used only in research because it is time-consuming, complicated, and relatively expensive. Channel catfish have been spawned in aquaria as small as 38 L, although 120 to 200-L aquaria or tanks are recommended (Tucker and Robinson 1990). In this method, a brood pair is selected and the female is injected with a suitable hormone to induce ovulation. Occasionally male channel catfish are also injected to induce or increase spermiation. The pair is allowed to begin spawning in the tank before eggs are hand-stripped and artificially fertilized. Because sperm cannot be stripped from male channel catfish (Bart and Dunham 1990) testes are removed surgically and crushed in buffer (about 10 to 20 mL/g of testis) to produce a sperm suspension (Tiersch et al. 1994). The buffer solution must have an osmotic pressure of more than 275 mOsmol/kg to ensure that sperm are not activated prior to use for fertilization (Bates et al. 1996).

Production of polyploid and hybrid fish requires collection of unfertilized eggs. In addition, unfertilized eggs are desirable for production of transgenic channel catfish because they are not adhesive which allows easier handling and counting. The greatest disadvantages of the aquarium spawning method are that it is difficult to predict when fish will spawn or how many injections will be required, necessitating constant monitoring of broodfish. Another method of artificial spawning, hormonal induction of grouped (unpaired) females, is commonly used in fishes (Piper et al. 1983) including other species of catfish. In this method, several females are injected with hormone and grouped in a common tank until ovulation occurs. The Indian catfish *Heteropneustes fossilis* has been spawned using this method within 14 to 18 h after injection of salmon gonadotropin-releasing hormone analog (Gn-RHa; 25 μg/kg of body weight) (Alok et al. 1993). The Asian catfish *Clarias macrocephalus* has also been spawned in groups by hormonal induction, with eggs remaining viable for as long as 10 h after ovulation when hand-stripped (Mollah and Tan 1983). Although previously studied (R. Dunham, Auburn University, personal communication), there are no published reports on the use of grouped spawning with channel catfish.

Genetic improvement of channel catfish and commercial production of catfish hybrids would be aided by a hormonal induction technique that could reliably trigger synchronized ovulation of high-quality eggs from multiple females (Nwadukwe 1995). In our study, the aquarium method was used during the 1994 and 1995 spawning seasons to produce eggs for genetic experiments. In 1996, approximately half of our female brood fish were spawned by the aquarium method and half were spawned by grouping of unpaired females. Our objectives were to evaluate: 1) spawning success (percent of females producing eggs); 2) latency (time between hormone injection and spawning or stripping of eggs); and 3) percent fertilization for the 3 yr of the study and for paired and grouped trials from the 1996 season. In addition to the main objectives, percent fertilization data were plotted against time and temperature to make inferences about spawning success at different stages (early, middle, late) of the spawning season. Problems encountered with spawning fish in recirculating systems were identified.

Materials and Methods

Fish Collection and Hormone Injection

Pond temperature was measured 4 to 5 d per wk from a reference 0.1-ha earthen pond located at the LSU Ben Hur Aquaculture Research Station (ARS). On days that pond temperature was not measured, temperature was estimated from ambient air temperature taken at the Ben Hur farm by

the Southern Regional Climate Center (Louisiana State University, Baton Rouge, Louisiana). Comparison revealed that pond temperatures for the 1995 spawning season (mid-May to mid-June) were always 2 to 6 degrees (average of 4.5 C) lower than the daily high temperatures. Therefore, a correction factor of 4.5 C was used for days when pond temperature was not measured. In spring, when pond temperatures stabilized above 21 C for at least 3 d, mature (≥3 yr old) broodfish were collected. Males were selected based on secondary sexual characteristics (Tucker and Robinson 1990), and females were selected if they possessed a soft distended abdomen and red swollen urogenital area. Broodfish were moved to the hatchery in a hauling tank within 30 min of capture. Fish were segregated by sex and placed in a recirculating system for temporary holding. All fish were anesthetized with tricaine methanesulfonate (MS-222; Argent Chemical Laboratories, Redmond, Washington), weighed, and measured before pairing or grouping in tanks for spawning. Females were injected intramuscularly (inferior to the dorsal fin) with synthetic leuteinizing-hormone releasing-hormone (LH-RHa, Peninsula Laboratories, Belmont, California) at a dosage of 50 or 100 μg/kg of body weight (Busch and Steeby 1990). Males were not injected in this study. Broodfish of similar size were paired to limit injury from aggressive spawning behavior.

Spawning Systems

Paired spawning trials were conducted in a system consisting of eight, rectangular, 120-L fiberglass tanks. Each tank was constructed with a plexiglass viewing window to allow monitoring of broodfish for spawning behavior and egg release. The system was equipped with a 0.30-m^3 upwelling biofilter (Armant Aquaculture Inc., Vacherie, Louisiana) for function as a recirculating system. The system could also be used for flow-through (with dechlorinated city water) when water quality variables were outside of the desired range (described below).

Grouped female trials were conducted in a system consisting of four, round, fiberglass tanks (three 1,000 L and one 2,500 L), equipped with a 0.60-m^3 upwelling biofilter (Water Garden Gems Inc., Marion, Texas). Females were grouped in one of the 1,000-L tanks after injection. A mature, male channel catfish was placed in a separate tank within the system, although the contribution to female reproduction of waterborne factors from males is unknown. Desired water quality parameters for both systems were: ≤0.5 mg/L ammonia-nitrogen (NH_3-N); ≤0.5 mg/L nitrite-nitrogen (NO_2-N); 100–400 mg/L alkalinity as $CaCO_3$; 50–100 mg/L hardness as $CaCO_3$; and 7.5–8.0 pH. Water quality was monitored daily with a freshwater test kit (Model No. FF-1, Hach Co., Loveland, Colorado).

Sperm Storage Solutions

Mature channel catfish males were killed by overdose with MS-222, and testes were removed surgically. Testes were dissociated to yield a homogenate of tissue and sperm cells which was filtered through a 100-μm tissue sieve. The sperm were suspended in Hanks' balanced salt solution (HBSS) at a ratio of 20 mL of HBSS for each g of testis (Christensen and Tiersch 1996). Solutions were stored at 4 C, and sperm quality was evaluated by motility estimates: 2 μL of sperm suspension were diluted with 20 μL of deionized water to activate the sperm cells (Bates et al. 1996). Percent motility was estimated using darkfield microscopy (200 × magnification) and suspensions with motility ≥25% (typically > 75%) were retained for use in artificial fertilization. Sperm suspensions were monitored daily and were discarded when estimated motility was <25%, or when bacterial contamination was evident (Jenkins and Tiersch 1997).

Hand-Stripping of Eggs

When a female was spawning readily (frequent release of eggs), she was removed

from the tank, anesthetized with MS-222, and dried thoroughly with paper towels to ensure that eggs would not come in contact with water. The female was cradled in one arm while sufficient pressure was applied to the abdominal area to strip eggs (Tiersch et al. 1994).

Grouped females were checked for ovulation by netting and applying gentle pressure to the abdominal and urogenital region; if eggs were released, the female was removed for stripping. Eggs were stripped into food-grade plastic bowls that contained HBSS and were coated with silicone grease (Dow Corning, Midland, Michigan) to prevent eggs from adhering to the bowl (Goudie et al. 1992). When stripping was complete, blood clots (if present) were removed by pipetting and the eggs were poured into a graduated conical flask to measure volume.

Artificial Fertilization

Eggs were divided into ~10-mL aliquots (200–300 eggs) for fertilization. The eggs were placed into 400-mL plastic beakers (Tri-pour, Oxford Labware, St. Louis, Missouri) coated with silicone grease, and excess HBSS was decanted. For fertilization, 0.5 mL of sperm suspension and 50 mL of tank water were added to activate the gametes, and the beaker was swirled to facilitate mixing. An additional 75 mL of water was added to the beaker after 2 to 3 min to facilitate water hardening and adhesion of eggs. After fertilization, the eggs formed gelatinous masses which were transferred to screened containers and placed in a hatching trough for incubation. Females were considered successful spawners if they produced eggs that appeared normal in color (yellow) and size (about 5 mm) (Markmann and Doroshov 1983) and formed a single cohesive mass following fertilization. Development to the neurulation stage (~24 h at 28 C) was used as the criteria for fertilization success.

1994 Spawning Season

During the 1994 spawning season (16 May to 18 June) mature broodfish were selected from earthen ponds at the LSU ARS. Males used in 1994 weighed 2.8 ± 1.1 kg (mean ± SD) and females weighed 2.9 ± 1.0 kg (N = 36 for each sex). Females were injected with 100 μg LH-RHa/kg of body weight at the time of pairing.

1995 Spawning Season

During the 1995 spawning season (12 May to 17 June) three groups of channel catfish were spawned: LSU channel catfish; channel catfish collected from Lake Maurepas (LM) (Manchac, Louisiana) that were held in earthen ponds at the ARS for 1 to 2 yr; and channel catfish collected from Lake Maurepas and placed into spawning trials within a week of collection. Forty-four pairs of LSU channel catfish were collected for spawning; the males weighed 2.7 ± 0.8 kg and the females weighed 2.4 ± 0.9 kg. Six pairs of pond-held LM channel catfish were used for spawning; the males weighed 1.0 ± 0.3 kg and the females weighed 0.8 ± 0.4 kg. Four pairs of newly captured LM channel catfish were used for spawning; the males weighed 0.1 ± 0.1 kg and the females weighed 0.2 ± 0.2 kg. All females were injected with a priming dose of 100 μg LH-RHa/kg body weight at the time of pairing, followed by a resolving dose of 50 μg LH-RHa/kg body weight 24 h later.

1996 Spawning Season

During the 1996 spawning season (17 April to 15 July) 27 sets of LSU channel catfish were paired for spawning; males weighed 2.3 ± 0.6 kg (N = 25) and females weighed 2.4 ± 0.5 kg (N = 27, two males were mated twice). Females were injected with a single dose of 100 μg LH-RHa/kg of body weight at the time of pairing. Females used in grouped spawning experiments weighed 2.6 ± 1.1 kg (N = 26). These females were injected with a single dose of 100 μg LHRHa/kg of body weight

TABLE 1. *Summary for females spawned during the 1994, 1995, and 1996 spawning seasons. Number injected is the total number of females injected with synthetic leuteinizing-hormone releasing-hormone (LHRHa). Number spawned is the number of fish that produced eggs. Number stripped is the number of fish that were stripped by hand. Latency is the time (in h) between injection with LHRHa and observed release of eggs. Percent fertilization is the percent of eggs that reached the neurulation stage.*

Treatment	Number injected	Number spawned	Number stripped	Latency (h) (mean ± SD)[1]	Percent fertilization (mean ± SD)[1]
1994 paired	36	13	10	113 ± 69[a]	16 ± 26[a]
1995 paired	30	12	8	109 ± 57[a]	72 ± 25[b]
1996 paired[2]	27	11	11	[x]44 ± 8[b]	[x]43 ± 20[b]
1996 grouped[2]	26	15	15	[x]50 ± 9	[y]16 ± 37
Total	143	51	44	—	—

[1] Means for paired spawns within a column followed by a common superscript letter were not significantly different ($P > 0.05$) as determined by a Fisher's least significant difference (LSD) means separation test.

[2] 1996 means within a column preceded by a common superscript letter were not significantly different ($P > 0.05$) as determined by Student's t-Test.

and placed in tanks in groups of two to seven.

Statistical Analyses

We performed statistical analyses for the purposes of data organization and presentation only. Because of potential weakness in statistical power, results of these analyses were not used for the purpose of testing hypotheses. Spawning success data for paired spawning in 1994, 1995 and 1996 were tested by chi-square analysis using a 3 × 2 contingency table, and differences between the paired and grouped data (1996 only) were tested using a 2 × 2 contingency table. For paired females that were hand-stripped, latency (time in h between hormone injection and ovulation) and percent fertilization were analyzed with a multivariate analysis of variance (MANOVA) with yr as the factor, and latency and percent fertilization as dependent variables. The Fisher's protected least significant difference (LSD) test was used for means separation. For paired and grouped females that were hand-stripped in 1996, latency and percent fertilization were compared with a t-Test. Percent fertilization values were arcsine-square-root-transformed for analysis. The level of statistical significance was set at $P < 0.05$. Analyses were performed with the Data Desk statistical analysis program (version 4.2, Data Description, Inc. Ithaca, New York).

Results

1994 Spawning Season

Of the 36 pairs, 13 (36%) successfully spawned (Table 1). Three pairs spawned completely in the spawning tanks, and these females were not available for stripping. The latency period (mean ± SD) for the females that were hand-stripped was 113 ± 69 h (minimum = 36 h; maximum = 200 h; $N = 10$). Fertilization of hand-stripped eggs was attempted within 108 min after stripping (mean ± SD = 66 ± 24 min; minimum = 42). Fertilization percentages were 16 ± 26 (minimum = 0; maximum = 80; $N = 10$). Pond water temperature dropped below 21 C, the minimum temperature for final gonadal maturation, several times during the spawning season (Fig. 1a). Water temperature in the hatchery spawning system ranged from 23 to 28 C. Water quality was maintained within desired parameters throughout the season.

1995 Spawning Season

During the spawning season, we experienced mortality of female broodfish. The proportion of females that died (24/44) was significantly higher ($P = 0.0001$) than the number of males that died (4/44) as deter-

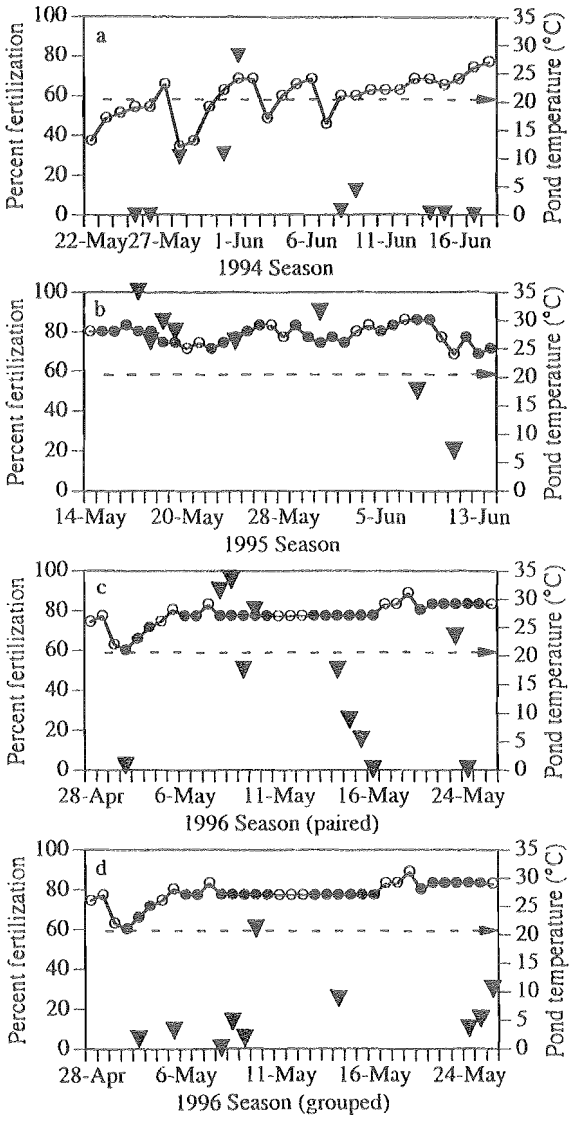

FIGURE 1. *Percent fertilization of channel catfish eggs (triangles) and average pond temperatures (solid line) during the spawning seasons of 1994 (a), 1995 (b), paired 1996 (c), and grouped 1996 (d). Closed circles represent actual pond temperatures: open circles represent estimated pond temperatures based on air temperatures. The dashed reference line represents the minimum temperature (21 C) for final gonadal maturation and spawning of channel catfish.*

mined by chi-square analysis with a 2 × 2 contingency table. The cause of mortality appeared to be related to water quality problems. The spawning system biofilter was not functioning adequately during the spawning season which resulted in periodic instances of high ammonia (1–5 mg/L) and nitrite (1–3 mg/L) in the system. This required switching the system to flow-through (dechlorinated city water) several times each day for about 3 wk. Because Baton Rouge city water has a low hardness (~16 mg/L hardness as $CaCO_3$) tanks were equipped with a drip system to deliver $CaCl_2$ to increase hardness, and NaCl to reduce stress.

Although formal necropsies were not performed on dead fish, ovaries were examined. Females that died were close to ovulation or had ovulated before dying (eggs were 4–5 mm in diameter and yellow in color). Females that died were eliminated from statistical analysis. Of the 20 remaining pairs of LSU channel catfish, seven (35%) spawned successfully (Table 1). Of the six pairs of pond-held LM channel catfish, three (50%) spawned successfully. Two of these pairs spawned completely in the tanks and the females were not stripped. Of the four pairs of LM channel catfish captured during spawning season, two (50%) spawned successfully. These pairs spawned completely in the tanks and females were not stripped. The average latency period for the stripped females was 109 ± 57 h (minimum = 28; maximum = 162 h; N = 8). Fertilization of hand-stripped eggs was attempted within 108 min after stripping (mean ± SD = 66 ± 24 min; minimum = 42). Fertilization rates for the artificially fertilized eggs were 72 ± 26% (minimum = 20%; maximum = 100%; N = 8). Pond water temperature remained above 21 C during the spawning season (Fig. 1b). Water temperature in the hatchery spawning system ranged from 26 to 30 C.

1996 Spawning Season

Of the 27 pairs of LSU channel catfish, 11 (41%) spawned successfully (Table 1). The average latency period for paired females was 44 ± 8 h (minimum = 32 h; maximum = 51 h). Fertilization of hand-stripped eggs was attempted within 90 min after stripping (mean ± SD = 40 ± 19 min; minimum = 18). Fertilization rates for eggs

from paired females were 43 ± 37% (minimum = 0; maximum = 95%).

Of the 26 grouped (unpaired) females, 15 (58%) were successfully stripped of eggs. The latency period for grouped females was 50 ± 9 h (minimum = 38 h; maximum = 70 h). Fertilization of hand-stripped eggs was attempted within 66 min after stripping (mean ± SD = 38 ± 17 min; minimum = 18 min). Fertilization rates were 16 ± 20% (minimum = 0%; maximum = 60%). Pond water temperature remained above 21 C throughout the spawning season (Fig. 1c, 1d). Water temperature in the hatchery spawning system ranged from 26 to 33 C. Water quality was maintained within desired parameters throughout the season.

Statistical Analyses

Analysis by chi-square (3 × 2 contingency table) did not detect differences (P = 0.17) in the proportion of successful spawns among the three data sets for male-female pairings (1994, 1995 and 1996). Analysis by chi-square (2 × 2 contingency table) did not detect differences (P = 0.64) in the proportion of successful spawns between paired and grouped females. The latency period for fish spawned in 1994 was not significantly different (P = 0.88) from that of fish spawned in 1995; however, the latency period for 1996 females was significantly shorter than those of 1994 (P = 0.004) and 1995 (P = 0.01). The latency period for grouped 1996 females was not significantly different (P = 0.10) from that of paired 1996 females (Table 1). Percent fertilization for eggs stripped in 1994 was significantly lower than that of eggs stripped in 1995 (P = 0.0005), but not significantly different than that of eggs stripped from 1996 females (P = 0.05). Percent fertilization was not significantly different (P = 0.05) for eggs stripped from 1995 and 1996 paired females. Percent fertilization was significantly higher (P = 0.02) for eggs stripped from paired females than for eggs stripped from grouped females (Table 1).

Discussion

The variation in spawning success, latency and percent fertilization that we observed is probably due to female variation in reproductive readiness, differential response to the hormone, stage of spawning season (early, middle, late) when the fish were injected, and water quality problems. Nutritional state and health of females also affect reproductive performance, egg quality, and larval survival. Burton (1994) found in winter flounder *Pleuronectes americanus* that an increase in non-reproductive females resulted from reduced rations during any part of the first half of the normal 6-mo feeding cycle. When channel catfish are produced using the natural or pond method, only those fish physiologically prepared to spawn will enter spawning containers (Huner and Dupree 1984). However, in artificial spawning, fish are selected if they appear ready to spawn. Because broodfish are commonly returned to ponds after spawning in the hatchery and are not seen again until the following spawning season, their health and nutritional state can go unmonitored. Therefore, it is possible that some broodfish have not fed well during the year or have been diseased during important parts of the feeding cycle. Accordingly, broodstock will be at different states of reproductive readiness when harvested.

Another important factor in reproductive readiness of channel catfish is temperature. Water temperatures must remain at or above 21 C long enough for females to undergo final gonadal maturation. If water temperature drops below 21 C or rises much above 30 C, egg quality will be reduced (Tucker and Robinson 1990) and some channel catfish may not spawn (Huner and Dupree 1984). Although not tested statistically, fish collected more than 3 wk after pond temperatures stabilized above 21 C seemed to be more likely to produce eggs of low quality. Kelly and Kohler (1996) were able to spawn small numbers of channel catfish out

of the normal season by holding them at cool temperatures (~17 C) and warming when spawning was desired. A modification of this technique might be used to spawn broodfish in groups over an extended period.

A variety of hormones have been used to induce final maturation and ovulation of eggs in channel catfish. Pituitary glands of common carp *Cyprinus carpio* or, more commonly, carp pituitary extract (CPE) are available in powdered form and delivered in a dosage of 4.5 mg of pituitary/kg of body weight at 24-h intervals for up to 10 d (usually requiring three to four injections). Human chorionic gonadotropin (HCG) can be used at a dosage of 1,760 IU/kg of body weight in one or more injections administered until ovulation occurs (Busch and Steeby 1990). Synthetic LHRH and GnRH have gained popularity as reliable means to induce ovulation in teleost fishes. Commonly, one or two injections of 50 to 100 μg LHRHa/kg of body weight are required for channel catfish (Busch and Steeby 1990). Hormone-induced ovulation followed by stripping of eggs is widely used in many species of finfish. However, uncertainty surrounds the proper protocol to reliably induce ovulation in channel catfish (Busch and Steeby 1990), and to properly time the stripping of eggs to prevent underripeness or over-ripeness (Dunham 1993).

Some species of teleost fishes have a long period of time between ovulation and over-ripening of eggs. Ovulated eggs retained *in vivo* in rainbow trout *Oncorhynchus mykiss* reared at temperatures of 10 C or less, remained viable for 1 to 2 wk (Sakai et al. 1975). Fertilization of eggs of the Asian catfish *Clarius macrocephalus* was not significantly affected until 12 h post ovulation (Mollah and Tan 1983). However, in cyprinids, the time between ovulation and over-ripening at a rearing temperature of 20 C can be as short as 30 min (Horvath 1978), and in striped bass *Morone saxatilis,* eggs can be unfertilizable in as little as 15 min post-ovulation (Rottman et al. 1991). The time between ovulation and over-ripening of eggs of channel catfish is undocumented, although multiple hand-strippings over a 10-h period from a single channel catfish female induced to ovulate with CPE yielded fertilization rates of 90 to 100% (Dunham 1993).

Egg quality can vary throughout the spawning season. During the 3 yr of this study, channel catfish (LSU population) were spawned as early as 1 May and as late as 17 June. However, the period of greatest likelihood of inducing ovulation and stripping eggs of high quality was a 4-wk period from early May to early June. Ninety-three percent of paired strip-spawns obtained in this period exhibited percent fertilization greater than or equal to 50%. In addition, 68% of the grouped female spawns occurred within this same period (Fig. 2). Therefore, to make best use of broodstock and time, effort should be concentrated in the early part of the spawning season (May in southern Louisiana). Successful production of desired numbers of fish early in the season would also avoid problems (e.g., fungus) associated with the warmer temperatures of late season.

Water quality was an issue particularly during the 1995 spawning season. Ammonia and nitrite in the spawning system accumulated to levels that were sublethal to the males but were apparently lethal to the ovulating females resulting in a selective loss of the females most likely to provide eggs. In flow-through systems, clean water is added to the culture system at a constant rate. Waste accumulation is not a factor because it is discharged continuously from the system. The high nitrite levels in the recirculating system could have resulted in brown blood disease or methemoglobinemia (Bowser et al. 1983; Wise et al. 1988) which in combination with the metabolic demand of final oocyte maturation caused a reduction in blood levels of oxygen (females were injected with LHRHa in this study, which could have exacerbated the oxygen deficit). Possible reasons for the

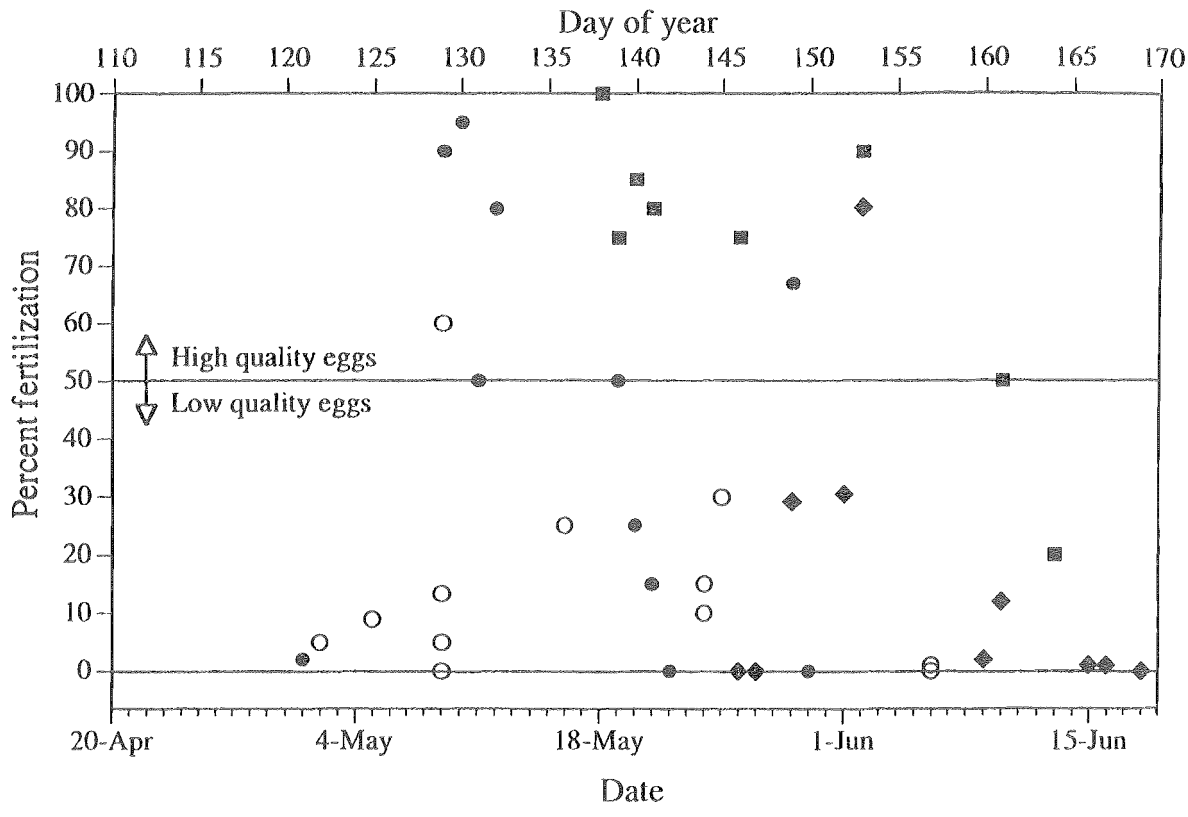

FIGURE 2. *Overlay of 3 yr of percent fertilization data: diamond, 1994; square, 1995; closed circle, paired 1996; and open circle, grouped 1996. Time is shown as day of the yr (upper horizontal axis) and date (lower horizontal axis).*

poor water quality during the 1995 season were: 1) insufficient colonization of the biofilter by nitrifying bacteria prior to stocking; 2) reduced bacterial activity in the biofilter due to seasonal increase in temperature; 3) overwhelming of the filtering capacity by large biomass at stocking (~48 kg when stocked); and 4) regurgitation of stomach contents upon stocking. To reduce water quality problems in recirculating spawning systems we recommend: 1) maintenance of a biological load on the filter year round; 2) maintenance of constant temperature in the hatchery; 3) a gradual increase of biomass when stocking; and 4) purging of fish prior to stocking.

Artificial spawning of grouped channel catfish females without pairing with males appears to be a viable technique if further research can optimize hormone dose and the timing required to consistently strip high-quality unfertilized eggs. If the channel catfish industry is to realize the degree of genetic improvement enjoyed by the cattle, pork, and poultry industries, artificial spawning must be developed into a reliable tool for use at the commercial level.

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Literature Cited

Alok, D., T. Krishnan, G. P. Talwar and L. C. Garg. 1993. Induced spawning of catfish, *Heteropneustes fossilis* (Bloch), Using D-Lys(6) salmon gonadotropin-releasing-hormone analog. Aquaculture 115:159–167.

Bart, A. N. and R. A. Dunham. 1990. Factors affecting survival of channel catfish after surgical removal of testes. The Progressive Fish-Culturist 52:241–246.

Bates, M. C., W. R. Wayman and T. R. Tiersch. 1996. Effect of osmotic pressure on the activation and storage of channel catfish sperm. Transactions of the American Fisheries Society 125:798–802.

Bowser, P. R., W. W. Falls, J. VanZundt, N. Collier and J. D. Phillips. 1983. Methemoglobinemia in channel catfish: methods of prevention. The Progressive Fish-Culturist 45:154–158.

Burton, M. P. M. 1994. A critical period for nutritional control of early gametogenesis in female winter flounder, *Pleuronectes americanus*. Journal of Zoology 233:405–415.

Busch, R. L. 1985. Channel catfish culture in ponds. Pages 13–84 *in* C. S. Tucker, editor. Channel catfish culture. Elsevier, Amsterdam, The Netherlands.

Busch, R. L. and J. A. Steeby. 1990. An evaluation of a leuteinizing hormone-releasing hormone analog to induce spawning of channel catfish *Ictalurus punctatus*. Journal of the World Aquaculture Society 21:10–15.

Christensen, J. M. and T. R. Tiersch. 1996. Refrigerated storage of channel catfish sperm. Journal of the World Aquaculture Society 27:340–346.

Clapp, A. 1929. Some experiments in rearing channel catfish. Transactions of the American Fisheries Society 59:114–117.

Dunham, R. A. 1993. Observations on controlled artificial spawning of channel catfish. The Progressive Fish-Culturist 55:60–61.

Goudie, C. A., B. A. Simco, K. B. Davis and N. C. Parker. 1992. Reproductive performance of pigmented and albino female channel catfish induced to spawn with HCG or ovaprim. Journal of the World Aquaculture Society 23:138–145.

Horvath, L. 1978. Relation between ovulation and water temperature by farmed cyprinids. Aquacultura Hungaria 1:58–65.

Huner, J. V. and H. K. Dupree. 1984. Methods and economics of channel catfish production, and techniques for the culture of flathead catfish and other catfishes. Pages 44–82 *in* H. K. Dupree and J. V. Huner, editors. Third report to the fish farmers. U.S. Fish and Wildlife Service, Washington, D.C., USA.

Jenkins, J. A. and T. R. Tiersch. 1997. Preliminary bacteriological study of refrigerated sperm of channel catfish. Journal of the World Aquaculture Society 28:282–288.

Kelly, A. M. and C. C. Kohler. 1996. Manipulation of spawning cycles of channel catfish in indoor water-recirculating systems. The Progressive Fish-Culturist 58:221–228.

Markmann, C. and S. I. Doroshov. 1983. Ovarian catheterization of the channel catfish, *Ictalurus punctatus*. Aquaculture 35:163–169.

Mollah, M. F. A. and E. S. P. Tan. 1983. Viability of catfish (*Clarias macrocephalus*, Gunther) eggs fertilized at varying post-ovulation times. Journal of Fish Biology 22:563–566.

Nwadukwe, F. O. 1995. Hatchery propagation of five hybrid groups by artificial hybridization of *Clarias gariepinus* (B) and *Heterobranchus longifilis* (Val.) (Clariidae) using dry, powdered carp pituitary hormone. Journal of Aquaculture in the Tropics 10:1–11.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler and J. R. Leonard. 1983. Fish hatchery management. U. S. Fish and Wildlife Service, Washington, D.C., USA.

Ringle, J. P., J. G. Nickum and A. Moore. 1992. Chemical-separation of channel catfish egg masses. The progressive Fish-Culturist 54:73–80.

Rottman, R. W., J. V. Shireman and F. A. Chapman. 1991. Techniques for taking and fertilizing the spawn of fish. Southern Regional Aquaculture Center Publication No. 426, 1–6.

Sakai, K., M. Nomura, F. Takashima and H. Oto. 1975. The over-ripening phenomenon of rainbow trout. II. Changes in the percentage of eyed eggs, hatching rate and incidence of abnormal alevins during the process of over-ripening. Bulletin of the Japanese Society of Scientific Fisheries 41: 855–860.

Steeby, J. A. 1987. Effects of container type and placement depth on channel catfish spawning success in ponds. The Progressive Fish-Culturist 49: 308–310.

Tiersch, T. R., C. A. Goudie and G. J. Carmichael. 1994. Cryopreservation of channel catfish sperm: storage in cryoprotectants, fertilization trials, and growth of channel catfish produced with cryopreserved sperm. Transactions of the American Fisheries Society 123:580–586.

Tucker, C. S. and E. H. Robinson. 1990. Channel catfish farming handbook. Van Nostrand Reinhold, New York, USA.

Wise, D. J., J. R. Tomasso and T. M. Brandt. 1988. Ascorbic-acid inhibition of nitrite-induced methemoglobinemia in channel catfish. The Progressive Fish-Culturist 50:77–80.