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Early out-of-season induced spawning of channel catfish *Ictalurus punctatus* (Rafinesque) conditioned in heated earthen ponds

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Abstract

This study documents early out-of-season induced spawning of channel catfish *Ictalurus punctatus*. During the early spring (February–April) of 1999, 2000 and 2001, ponds containing (1) male and female channel catfish (mixed-sex ponds) or (2) male channel and blue catfish *I. furcatus* only, or female channel catfish only (single-sex ponds) were heated to 24–30 °C to encourage gonadal maturation and spawning. Unheated ponds were stocked with males and females and were monitored during the duration of heating. When natural spawning occurred in the heated ponds, the fish were captured by seining and unspawned females were injected with 100 μg kg^{-1} of synthetic leutenizing hormone-releasing hormone. Injected females were either paired with males or held in communal all-female groups, and monitored for ovulation. Eggs were collected and fertilized with sperm of channel catfish or blue catfish. Females paired with males were induced to spawn 44 days (mixed-sex ponds) and 50 days (single-sex ponds) before natural spawning occurred in unheated ponds. Spawning latency (the time between injection and ovulation) and the percentage of neurulated embryos from eggs fertilized using channel catfish sperm was not different between spawning before the natural season ($P = 0.68$) and during the natural season in fish from mixed-sex ponds ($P = 0.57$). Females held in all-female groups produced eggs 34 days before the onset of spawning in unheated ponds. Spawning latency was not different between spawns before and during the natural season ($P = 0.16$), and the percentages of neurulated embryos from eggs fertilized with channel catfish sperm ($P = 0.76$) or blue catfish sperm ($P = 0.77$) before or during the natural season were not different. This study demonstrates the feasibility of conditioning of channel catfish females for early out-of-season induced spawning in the laboratory.

Keywords: catfish, spawning, induced, out-of-season

Introduction

Spawning of channel catfish *Ictalurus punctatus* in ponds is the typical method of fry production employed by the catfish industry (Huner & Dupree 1984; Busch 1985; Dupree 1995). Females and males are placed in ponds containing spawning containers that simulate natural nesting sites (Clapp 1927), and are allowed to form spawning pairs. This method precludes the collection of unfertilized eggs for research and inhibits the production of improved stocks. In induced paired spawning, catfish are injected with gonadotropic hormones such as human chorionic gonadotropin (Sneed & Clemens 1959; Goudie, Simco, Davis & Parker 1992), or with synthetic leuteinizing hormone-releasing hormone (LH-RHa; DesGly^{10} [D-Ala^{6}], LH-RH-ethylamide) (Busch & Steeby 1990; Silverstein, Bosworth & Wolters 1999). Injected fish are paired in flow-through aquaria or in cages in ponds, and monitored for spawning activity. When the female begins to actively lay eggs, she can be anaesthetized and eggs can be hand collected (stripped) and fertilized using sperm that have been collected from the crushed testes of donor males, or by using cryopreserved sperm (Tiersch, Goudie & Carmichael 1994).

Induced spawning (sometimes referred to as artificial spawning) is an important tool for channel catfish research. Specific pair-matings for genetic improvement is not guaranteed unless females can be induced to spawn for collection of unfertilized eggs. The production of interspecific or intergeneric catfish hybrids is generally only possible using induced spawning (Tave & Smitherman 1982), and such crosses can be superior in production traits and are thus valuable for culture. For example, the hybrid of channel catfish by blue catfish *Ictalurus furcatus* is sought after for production because of its superior growth and resistance to stress compared with either parental species (Yant, Smitherman & Green 1975; Brooks, Chappell, Williams & Dunham 1982; Dunham, Smitherman, Brooks, Benchakan & Chappell 1982; Dunham, Smitherman, Goodman, & Webber 1983). However, induced spawning of channel catfish is capital intensive, labour intensive and complicated in comparison with pond spawning, and is thus currently limited to research settings and to a few commercial operations. Tools that enhance induced spawning are therefore of value to catfish production and research.

Seasonal regression and recrudescence of channel catfish gonads follow an endogenous rhythm, and although the exact effect of photoperiod on reproduction in channel catfish is not fully understood, it appears that temperature is the primary environmental cue that induces springtime gonadal maturation (Davis, Goudie, Simco, MacGregor & Parker 1986; Lang, Romaire & Tiersch 2003). Channel catfish can be conditioned to spawn in broodstock ponds during winter months (1–2 months before the natural season) by maintaining pond water at temperatures conducive to spawning (24–30 °C) (Lang *et al.* 2003). In such a scenario, gonadal maturation is sufficient for natural reproduction in spawning containers in ponds, and early out-of-season induced spawning is possible. Reliable early spawning would be a valuable research tool and would enable early production of fry and hybrids for commercial culture.

Our overall goal is to develop a reliable, early spawning regime that requires little or no male input, by heating of earthen ponds before the natural spawning season. Females injected with LH-RHa and placed in aquaria will release viable eggs in the absence of males (Bates & Tiersch 1998); however, it is unknown whether the presence of males in ponds would be required to ripen females during early out-of-season conditioning. The goal of this study was to demonstrate that fish could be conditioned for out-of-season induced spawning before the natural spawning season, and to evaluate the quality of the resultant eggs. Specifically, we documented egg production, compared spawning latency (hours between injection and spawning) after hormone injection and embryo neurulation (per cent) between early out-of-season induced spawns and natural-season induced spawns collected from: (1) channel catfish females conditioned in heated ponds in the presence of males (mixed-sex ponds) and paired with males in the laboratory (paired spawns) for spawning; (2) channel catfish females conditioned in the absence of males (single-sex ponds) and pair-spawned and (3) channel catfish females conditioned in single-sex ponds, and held in the laboratory in all-female groups for egg collection following hormonal stimulation (grouped spawns). Additionally, for grouped spawns, we compared spawns before and during the natural season for: (1) the volume of eggs stripped and (2) neurulation in embryos from eggs fertilized using either blue catfish or channel catfish sperm. We are not aware of other reports that address the conditioning of channel catfish separated by sex for induced early out-of-season induced spawning, or the grouped spawning of females conditioned in heated ponds for induced early out-of-season induced spawning.

Methods

Broodstock care

During February to April of the years 1999, 2000 and 2001, 0.04 ha earthen ponds located at the Aquaculture Research Station of the Louisiana State University Agricultural Center in Baton Rouge were stocked with mature channel catfish broodstock (1.3–4.1 kg; 440–680 mm total length). Ponds stocked as 'mixed-sex' ponds received females and males at a 2:1 female-to-male ratio (20 females and 10 males); or ponds stocked as 'single-sex' ponds received only females ($n = 30$), or only male channel catfish ($n = 22$) and blue catfish ($n = 8$). To condition the fish for early spawning, pond temperatures were raised by 2 °C day^{-1} and were subsequently maintained at 27 ± 2 °C by addition of water from a 600 m geothermal well (Lang *et al.* 2003). Temperature was monitored daily using a hand-held digital thermometer (Model HH-21, Omega Engineering, Stamford, CT, USA), and hourly using data loggers (Hobo Data Logger, Onset Computer Corporation, Pocasett, MA, USA) submerged ~ 1 m below the water surface.

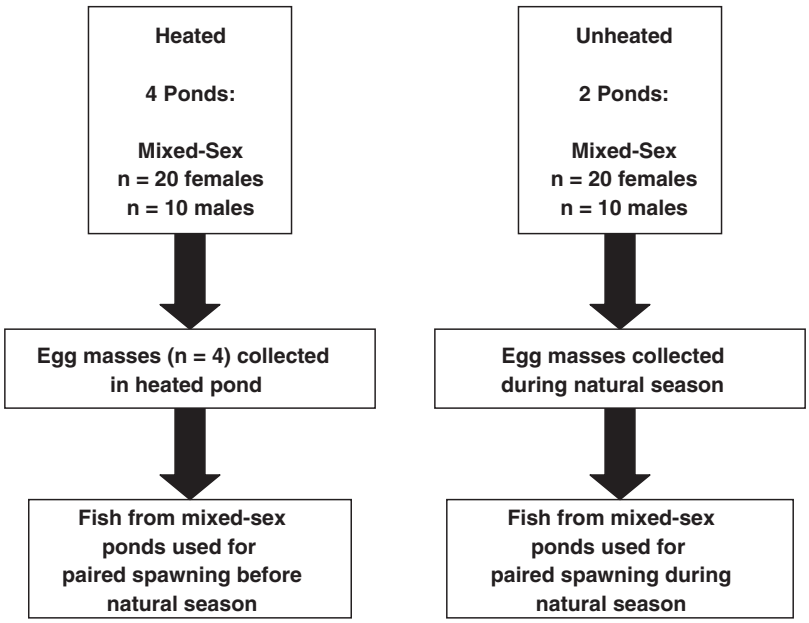

Figure 1 Schematic diagram illustrating spawning practices for 1999. Fish from six mixed-sex ponds were utilized for early out-of-season induced spawning. When spawning occurred in a heated mixed-sex pond, fish were collected from two heated mixed-sex ponds by seining and were induced to pair-spawn in the laboratory before the onset of the natural spawning season. The following week, fish from the remaining two heated mixed-sex ponds were collected and used for induced early spawning. When spawning occurred in unheated ponds, unspawned fish from those ponds were collected and used for induced natural season spawning.

The occurrence of spawning in heated ponds containing channel catfish broodstock was used to indicate the physiological readiness of fish to spawn (e.g. conditioned sufficiently to produce eggs in the laboratory). To determine this timing, spawning cans were placed in heated and unheated mixed-sex ponds and were checked for evidence of spawning at 3-day intervals (Busch 1985). After four egg masses were collected from a heated mixed-sex pond, fish in that pond or in the concurrently heated all-female and all-male ponds were collected by seining for induced spawning trials.

The nature of this research was exploratory, and thus the experimental design was modified slightly each season. During 1999, six ponds were stocked as mixed-sex ponds (Fig. 1). Four of the six ponds were heated, and induced paired spawning was attempted with all fish from all six ponds. During 2000, five ponds were stocked as mixed-sex and six ponds were stocked as single sex, of which three contained only males and three contained only females (Fig. 2). Induced paired-spawning was attempted with all fish from all five mixed-sex ponds (three from heated ponds and two from unheated ponds), and with all fish from three sets of single-sex ponds (three heated all-female ponds and three heated all-male ponds). During 2001, induced spawning of females as female–male pairs and in groups was attempted with fish from six single-sex ponds (six heated all-female ponds and three heated all-male ponds) (Fig. 3).

Induced spawning practices

Fish were transferred from a hauling tank to 1000 L tanks in a recirculating system equipped with a 0.60 m^3 upwelling bead filter (Water Garden Gems, Marion, TX, USA) within 30 min of capture, and held for 2 days to allow for purging of stomach contents and acclimation. Ambient water temperature in all culture facilities ranged from 23 to 27 °C. Warm (30 °C) water was added if possible to attempt to maintain water temperatures within the defined spawning range. All fish were anaesthetized with tricane methanesulphonate (MS-222; Argent Chemical Laboratories, Redmond, WA, USA), weighed, measured for length and marked for identification before pairing. Females that appeared ripe (as indicated by

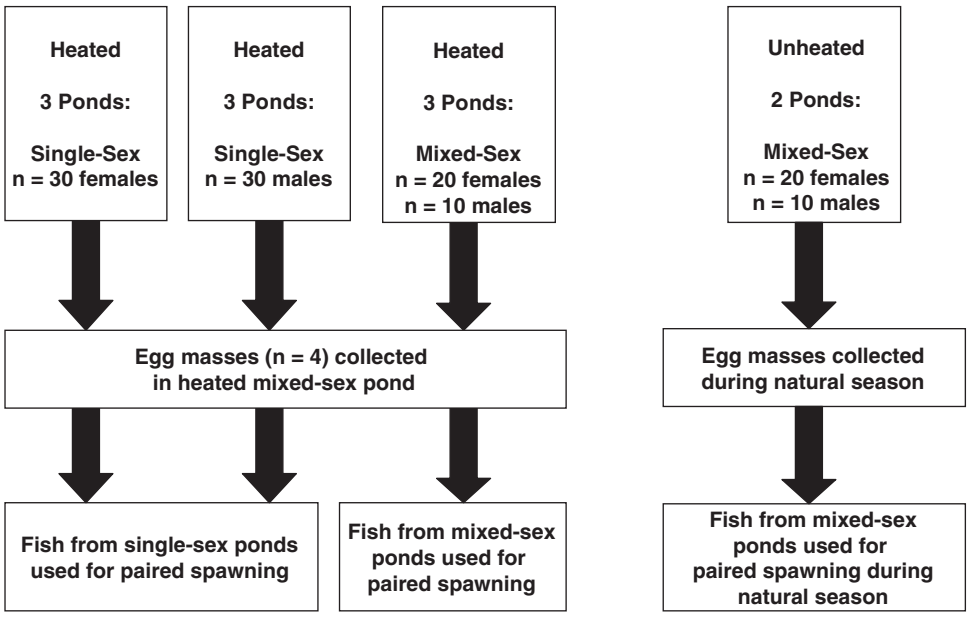

Figure 2 Schematic diagram illustrating spawning practices for 2000. Fish from five mixed-sex ponds, and fish from six single-sex ponds (three all-female and three all-male) were utilized for induced spawning. When spawning occurred in a heated mixed-sex pond, fish from both mixed-sex ponds and from single-sex ponds were induced to pair-spawn in the laboratory, before the onset of the natural spawning season.

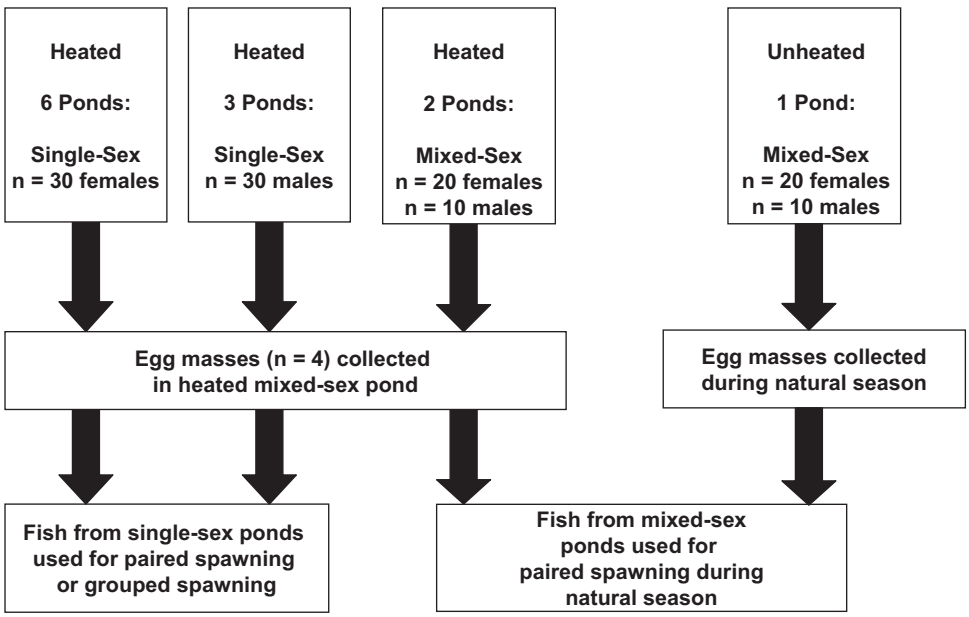

Figure 3 Schematic diagram illustrating spawning practices for 2001. Fish from two heated and one unheated mixed-sex ponds were utilized, and fish from nine single-sex ponds (six all-female, three all-male) were utilized for induced spawning. When spawning occurred in a heated mixed-sex pond, fish from two heated all-female ponds and one all-male pond were collected by seining and used for early induced group-spawning. The following week, fish from the next set of three ponds (two all-female, one all-male) were collected for spawning until all ponds had been utilized.

rounded bellies) were injected intraperitoneally with synthetic leuteinizing hormone-releasing hormone (LH-RHa) at a dosage of 100 μg for each kilogram of weight (Busch & Steeby 1990). Males were not injected. Females were either paired with males or held in all-female groups.

For paired spawns, an injected female and a male were placed in each of eight individual 120 L fibreglass tanks equipped with Plexiglas viewing windows to monitor spawning behaviour and egg release (Bates & Tiersch 1998). This system was equipped with a 0.30 m^3 upwelling biofilter (Armant Aquaculture, Vacherie, LA, USA) during 1999 and 2000, and a 0.60 m^3 upwelling bead filter (Water Garden Gems) during 2001. Females were placed in the spawning tanks first, followed by males (to minimize the risk of aggressive territorial behaviour by the male), and were paired based on similar weight and length to minimize broodstock injury. Pairs were monitored every 2 h for signs of spawning activity. When a female had released $\sim$ 100 mL eggs, she was removed and anaesthetized with MS-222 and stripped. Fish that had released over $\sim$ 500 mL eggs into the tank were not stripped.

For group spawns, females were injected and were held in groups of eight to 12 in the 1000 L acclimation tanks. One to three males were held concurrently in a 2500 L tank in the same system, but not in the same tanks as the females. At 6- or 12-h intervals after injection, females were netted individually and brought to the surface for examination of the urogential region and belly, and expulsion of eggs. If eggs were released through the application of gentle abdominal pressure, the female was anaesthetized and stripped. During the first 2 weeks of grouped spawning, all females were injected on the same day (the Monday following Friday capture). During the remaining 4 weeks of grouped spawning, approximately half of the fish were injected initially, and the remaining fish were injected the following day. Fish for grouped spawning were monitored for evidence of readiness to ovulate at 6-h intervals during Weeks 1 and 2, and at 12-h intervals during Weeks 3–6.

Sperm collection and refrigerated storage

Channel catfish and blue catfish males cannot be stripped of milt (Bart & Dunham 1990), and the male must be killed so that testis can be removed and macerated to collect the sperm as a suspension (Tiersch *et al.* 1994). Male catfish were killed by administration of a lethal dose of MS-222. Males were weighed, measured for length and the testes surgically removed. Sperm were collected by crushing the testis in 20 mL of Hanks' balanced salt solution (HBSS) prepared at 290 mOsmol kg^{-1} (Bates, Wayman & Tiersch 1996) for each gram of testis and straining of the resulting suspension with a 26 μm screen (Christensen & Tiersch 1996). Sperm suspensions were stored at 4 °C, and monitored daily for motility (according to Bates *et al.* 1996).

Collection and fertilization of eggs

Eggs were collected in greased bowls (vacuum grease, Dow Corning, Midland, MI, USA) (Goudie *et al.* 1992; Tiersch *et al.* 1994) by applying gentle pressure to the area surrounding the urogential pore, and to the abdomen. After stripping, the fish was returned to the tank and revived from sedation by orienting the flow of water along the gills. Eggs were poured from greased collection bowls into greased cylinders, and fresh HBSS (290 mOsmol kg^{-1}) was added to rinse away ovarian fluid or blood. Within 30 min of collection, a 5 mL sample of eggs (125–150 eggs) was poured into 450 mL greased beaker (and replicated as necessary) forming a single layer. Using a clean pipet, 1 mL of sperm suspension from channel catfish or blue catfish was added to each sample of eggs. Eggs were activated using water (enough to cover the eggs) from the incubation system. After 5–10 min, additional tank water was added to bring the total volume to $\sim$ 200 mL.

Evaluation of early out-of-season induced spawning

Spawning events (pond or induced) were classified as early only when they occurred before the date when natural spawning began in unheated ponds; all spawns thereafter were considered to be natural-season spawns, regardless of whether the pond was heated or not. Egg production by individual females was considered successful if eggs appeared normal (yellow and $\sim$ 5 mm in diameter) (Markmann & Dorosvhov 1983), and formed a cohesive mass following activation. Spawning latency (Bates & Tiersch 1998), defined as the time elapsed between injection and ovulation, indicated the spawning readiness of grouped and paired females. Paired or grouped females that spawned completely in the tank were not evaluated for latency. Eggs stripped from paired or grouped females that were fertilized using fresh sperm of channel catfish or blue catfish were evaluated for quality based on rates of embryo neurulation at 27 h after fertilization. Females that died before spawning were excluded from analyses.

Table 1 Summary of females from heated and unheated mixed-sex ponds that were induced to spawn in female–male pairs during 1999 and 2000. Spawning latency* ($P = 0.68$) and mean neurulation† ($P = 0.57$) were not significantly different ($P \leq 0.05$) during the early season and the natural season

Year	Number injected	Number spawned	Early spawns	Natural season spawns	Spawning latency (h)		Mean neurulation (%)	
					Early	Natural	Early	Natural
1999	35	17	9	8	32 ± 13	47 ± 12	90 ± 3	82 ± 11
2000	29	21	8	13	48 ± 15	38 ± 8	82 ± 18	87 ± 8
Total	64	38	17	21	39 ± 16	41 ± 10	87 ± 12	84 ± 9

*The number of hours elapsing between injection and ovulation.
†The percentage of eggs to develop into neurulated embryos ~ 27 h after fertilization.

Statistical analysis

The generalized linear model (GLM) procedure of the Statistical Analysis Software system version 8 for Windows® (SAS Institute , Cary, NC, USA) with unbalanced design was used to detect differences in: (1) spawning latency among fish spawned as female–male pairs, and among females spawned in groups; (2) the amount of eggs stripped from females spawned in groups and (3) the per cent neurulation in embryos produced early and during natural season for females spawned as pairs or in groups, and fertilized using channel catfish sperm or blue catfish sperm. The model used year of spawning as a blocking factor. Tukey's studentized range test was used to compare means. Differences were considered significant at $P \leq 0.05$. Owing to the non-normal distribution of the percentage data, they were arcsine-root transformed before statistical analysis.

Differences between neurulation of embryos fertilized using either blue catfish or channel catfish sperm were analysed using a two-way analysis of variance (Statistical Analysis Software system version 8 for Windows®, SAS Institute , Cary, NC, USA). Tukey's studentized range test was used to compare differences between the factors season and paternal origin. Differences were considered significant at $P \leq 0.05$.

Results

Egg production of channel catfish from mixed-sex ponds

During 1999 and 2000, 60% of injected females (38 of 64) taken from mixed-sex ponds and paired with males produced eggs (Table 1). Eggs were first collected on 19 April 1999 (7 days before spawning began in unheated ponds), and 13 March 2000 (44 days before spawning began in unheated ponds). Overall, 45% of females (17 of 38) produced eggs before the onset of spawning in unheated ponds. Spawning latency was 39 ± 16 h for females stripped early in the season, and 41 ± 10 h for females stripped during the natural season. Neurulation was 87 ± 12% in embryos produced early in the season and 84 ± 9% in embryos produced during the natural season. There was no significant difference in spawning latency ($P = 0.68$) or neurulation percentage ($P = 0.57$) between eggs collected and fertilized before or during the natural season.

Egg production of channel catfish from single-sex ponds

During 2000 and 2001, 36% of injected females (18 of 50) taken from single-sex ponds and paired with males produced eggs (Table 2). Of these females, 61% (11 of 18) produced eggs before the onset of spawning in unheated ponds. Eggs were first collected on 6 March 2000 (50 days before spawning began in unheated ponds) and on 26 March 2001 (34 days before spawning began in unheated ponds). Spawning latency was 55 ± 16 h for females stripped before, and 47 ± 5 h for females stripped during the natural season. Neurulation was 82 ± 18% in embryos produced before the natural season and 83 ± 10% in embryos produced during the natural season.

Overall, 31% (34 of 108) of grouped females yielded eggs from stripping (Table 3). In 2001, spawning occurred in unheated ponds while the third group of females from single-sex ponds was being spawned in the laboratory. Egg production was 18% (nine of 50) for fish spawned in groups before the onset of spawning in unheated ponds, and was 43% (25 of 58) for the

Table 2 Summary of females from heated and unheated single-sex ponds that were spawned as female–male pairs during 2000 and 2001

Year	Number injected	Number spawned	Early spawns	Natural season spawns	Spawning latency* (h)		Mean neurulation† (%)	
					Early	Natural	Early	Natural
2000	14	6	6	0	15 ± 11	0	77 ± 23	0
2001	36	12	5	7	69 ± 1	47 ± 5	90 ± 0	83 ± 10
Total	50	18	11	7	55 ± 16	47 ± 5	82 ± 18	83 ± 10

*The number of hours elapsing between injection and ovulation.
†The percentage of eggs to develop into neurulated embryos ∼ 27 h after fertilization.

Table 3 Summary of grouped induced spawning of fish from heated single-sex ponds. Embryo neurulation* is reported for eggs fertilized with either channel catfish (CCF) or blue catfish (BCF) sperm. In comparison with females spawned during the natural season, there was no difference ($P \leq 0.05$) for fish spawned early in the amount of eggs stripped ($P = 0.07$), spawning latency† ($P = 0.16$), and embryo neurulation of eggs fertilized with channel catfish sperm ($P = 0.76$) or blue catfish sperm ($P = 0.77$)

Groups	Season spawned	Number injected	Number stripped	Volume of eggs (mL)	Per cent spawned	Spawning latency (h)	Mean neurulation (%)	
							CCF × CCF‡	CCF × BCF
3	Early	50	9	199 ± 118	18	64 ± 14	71 ± 30	73 ± 31
3	Natural	58	25	281 ± 109	43	56 ± 13	68 ± 31	69 ± 29

*The percentage of eggs to develop into neurulated embryos ∼ 27 h after fertilization.
†The number of hours elapsing between injection and ovulation.
‡CCF × CCF indicates a channel catfish female crossed with a channel catfish male; CCF × BCF indicates a channel catfish female crossed with a blue catfish male.
There was no significant difference overall between eggs fertilized using blue catfish sperm or channel catfish sperm ($P = 0.84$).

remaining three groups that spawned during the natural season.

Spawning latency was 58 ± 14 h, and was not different for females stripped before or during the natural season ($P = 0.16$). Mean volume of eggs stripped was 250 ± 116 mL, and was not different in females that were stripped before or during the natural season ($P = 0.07$). The average number of eggs produced by grouped females (that produced eggs) was 6379 ± 3151 eggs per female (assuming 30 eggs mL^{-1}), or 3370 ± 1831 eggs kg^{-1} of female. However, stress and damage of the ovaries of females can occur during stripping, and to preserve broodstock health, fish were stripped only as long as eggs were freely released.

Neurulation was 69 ± 30% in embryos produced using channel catfish sperm and 69 ± 28% in embryos produced using blue catfish sperm. Neurulation percentage was not different in embryos between eggs collected before and during the natural season and fertilized with channel catfish sperm ($P = 0.76$) or blue catfish sperm ($P = 0.77$). Furthermore, there was no significant difference overall between the neurulation of embryos produced using blue catfish sperm or channel catfish sperm ($P = 0.84$).

Discussion

This report documents early out-of-season induced spawning of channel catfish females conditioned in heated mixed-sex ponds, and early out-of-season induced spawning of female channel catfish from heated single-sex ponds as female–male pairs and in groups. Compared with natural spawning, the available time during the year for collection of unfertilized eggs was extended by 6 weeks, and hybrid catfish were produced using eggs collected from fish from all-female ponds as early as in 28 March, a full month in advance of the natural spawning season. Spawning latency was not increased significantly by spawning fish early in the season, and eggs were of satisfactory quality (> 50% embryo neurulation). Out-of-season induced spawning of channel catfish

has been reported previously, but this is the first report in which induced spawning was advanced in time, and without manipulation of photoperiod. Channel catfish were induced to spawn in August ($n = 3$) and November ($n = 1$) by maintaining them in earthen ponds containing cool ($\sim 18\ ^{\circ}C$) water for 109 days (to retard gonadal maturation) followed by warmer (24 °C) water to induce late spawning (Brauhn 1971). Three of four of these fish received injections of carp pituitary extract to stimulate ovulation. Channel catfish were induced to spawn in the laboratory during November, January and February by manipulating both photoperiod and temperature in tanks to compress the 12-month spawning cycle into 9 months (Kelly & Kohler 1996).

In this study, the percentage of injected females that pair-spawned after conditioning in mixed-sex ponds (60%) was comparable with prior studies of paired and grouped spawning of channel catfish. In previous studies in our laboratory, egg production over three seasons (1994, 1995 and 1996) was 36%, 40% and 41% in females receiving one or two doses of 100 μg kg^{-1} body weight of LH-RHa and paired with males (Bates & Tiersch 1998). Other reports include 76% ovulation in grouped females injected with carp pituitary extract (Dunham, Liu & Argue 1998) and 58% in females injected with a single dose of LH-RHa (Bates & Tiersch 1998), although it is often difficult to directly compare studies due to differences in procedures and terminology, and in broodstock selection criteria.

Egg production in fish from single-sex ponds was variable, and it is possible that external factors affected the results. Disease outbreaks were a problem during each of the seasons. During the 1999 season, 65% (six females and two males) of fish brought inside for spawning on April 29 died within 24 h of collection. During 2000, 55% of fish (eight females and two males) from one heated mixed-sex pond died within 48 h of harvest. Of the remaining five females, two released putrescent (presumably overripe) eggs upon being handled. During the 2001 season, deaths occurred in each group of fish spawned. Fifty-six of the 182 females (31%) used for induced spawning died after injection and pairing or grouping. During the first week of April, 28 of 32 females died within 1 week of harvest. Live and dead specimens were submitted during 2000 and 2001 to the Louisiana State University School of Veterinary Medicine Aquatic Animal Diagnostic Laboratory for formal pathological examinations. Fish were diagnosed with infections of *Ichthyoptherius multifilis* and *Cleidodiscus* sp. during 2000, and were diagnosed with *Flexibacter columnaris* sp. and *Cleidodiscus* sp. during 2001 (J. Hawke, Louisiana State University School of Veterinary Medicine, pers. comm.). Such problems are not limited to heated ponds, and can be induced by stress due to seining and handling for induced spawning. Further research is needed to evaluate whether there is a relationship between disease outbreaks and induced spawning of catfish conditioned in heated ponds before the natural spawning season.

In addition to the problems with disease, the low overall success of early grouped-spawning of females from single-sex ponds before the natural spawning season in 2001 was skewed by the first week, during which time no females spawned. In that case, ambient air temperature began to drop steadily on the day these females were harvested, and during the following 2 weeks, average daily ambient pond temperatures were below 17 °C (Fig. 4). During this time, one of two heating units in the laboratory failed and for 2 days before injection fish experienced a significant decline in water temperature below the defined range of spawning (24–30 °C) despite addition of heated water to the tank system, and no spawning took place. Grouped spawning of females was more successful during the natural season (as in Bates & Tiersch 1998), but given the documentation of biological feasibility in this study, conditioning of females in heated ponds for use in grouped spawning before the natural season warrants further study.

It has been previously demonstrated that male and female channel catfish will spawn in heated ponds before the natural spawning season (Lang *et al.* 2003). The results presented here demonstrate that channel catfish females can be conditioned and induced to produce eggs earlier in the calendar year (before the start of natural spawning) than previously thought, and without direct stimulation from males. In addition, this study documents that male and female channel catfish and male blue catfish can be conditioned by heating in mixed-sex and single-sex ponds for hormone-induced spawning in the laboratory before the natural spawning season. This provides benefits to research, as it extends the available time for study each year. At present, early spawning of channel catfish, with or without males, requires refinement before it would be suitable for application on a commercial scale. For example, methods for heating of larger (> 0.04 ha) ponds would need to be tested and proven cost-effective, potential issues with diseases must be addressed and spawn-

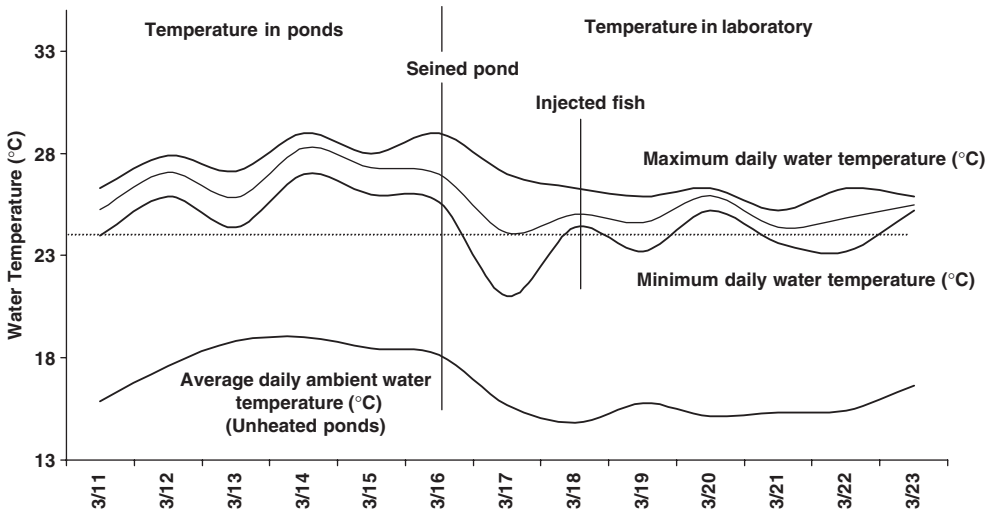

Figure 4 Water temperature (°C) of heated ponds and grouped spawning tanks during March 2001. Channel catfish females from heated ponds experienced decreased water temperatures when brought inside for induced spawning. The uppermost plot indicates the maximum, average daily and minimum water temperatures experienced by fish before and after hormone injection. The bottom-most plot represents the average daily ambient pond temperature of unheated ponds. The dashed line represents the minimum temperature for spawning activity of channel catfish (24 °C).

ing techniques would require optimization of egg yield and broodstock survival.

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