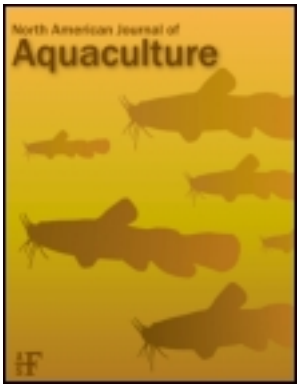

North American Journal of Aquaculture
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/unaj20

Effect of Carp Pituitary Extract and Luteinizing Hormone Releasing Analog Hormone on Reproductive Indices and Spawning of 3-Year-Old Channel Catfish

Marinela Barrero [a], Brian C. Small [b], Louis R. D'Abramo [a], Geoffrey C. Waldbieser [b], Larry A. Hanson [c] & Anita M. Kelly [d]

[a] Department of Wildlife and Fisheries, Mississippi State University, Box 9690, Mississippi State, Mississippi, 39762, USA

[b] U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Post Office Box 38, Stoneville, Mississippi, 38776, USA

[c] College of Veterinary Medicine, Mississippi State University, Box 6100, Mississippi, Mississippi State, 39762, USA

[d] Fisheries and Illinois Aquaculture Center, Southern Illinois University, Mailcode 6511, Carbondale, Illinois, 62901, USA

Version of record first published: 09 Jan 2011.

North American Journal of Aquaculture 70:138–146, 2008

DOI: 10.1577/A06-072.1

[Article]

Effect of Carp Pituitary Extract and Luteinizing Hormone Releasing Analog Hormone on Reproductive Indices and Spawning of 3-Year-Old Channel Catfish

MARINELA BARRERO[1]

Department of Wildlife and Fisheries, Mississippi State University, Box 9690, Mississippi State, Mississippi 39762, USA

BRIAN C. SMALL*

U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Post Office Box 38, Stoneville, Mississippi 38776, USA

LOUIS R. D'ABRAMO

Department of Wildlife and Fisheries, Mississippi State University, Box 9690, Mississippi State, Mississippi 39762, USA

GEOFFREY C. WALDBIESER

U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Post Office Box 38, Stoneville, Mississippi 38776, USA

LARRY A. HANSON

College of Veterinary Medicine, Mississippi State University, Box 6100, Mississippi State, Mississippi 39762, USA

ANITA M. KELLY

Fisheries and Illinois Aquaculture Center, Mailcode 6511, Southern Illinois University, Carbondale, Illinois 62901, USA

Abstract.—The efficacy of pituitary extract from common carp *Cyprinus carpio* (CPE) and luteinizing hormone releasing hormone analog (LHRHa) treatments in inducing spawning in channel catfish *Ictalurus punctatus* undergoing their first oogenesis just before the spawning season was evaluated in four commercial strains of channel catfish. Before the hormones were injected, the average oocyte size (1,624 μm) in May, the typical start of the spawning season, was smaller than that considered normal for mature oocytes (3,000 μm) in this species. Priming and resolving doses of CPE, LHRHa, or saline were administered in early May. Plasma estradiol (E_2) and testosterone (T) levels were measured before the priming (0 h) and resolving (20 h) doses and 4 h later (24 h). Oocyte germinal vesicle migration and cathepsin B, D, and L activities were assessed at 0 and 24 h. At 20 h, estradiol levels were significantly higher in CPE- and LHRHa-treated fish. For LHRHa-treated fish, 20-h levels of T were also significantly higher in LHRHa-treated fish at 24 h. Cathepsin B and L activities were also significantly higher at this time. No differences were observed among the strains evaluated for any of the measured parameters. As oocytes did not yet appear to have entered the final stage of maturation, the fish were returned to ponds with noninjected cohorts to evaluate spawning success. None of the fish injected with saline spawned. On average, 12.5% and 18.8% of those injected with CPE and LHRHa, respectively, spawned. The LHRHa-injected fish had a 14% greater incidence of spawning than noninjected fish. Fertilization rates were not significantly different among treatments. Of the treated fish, those that spawned had significantly higher plasma E_2 concentrations at 20 h. Thus, LHRHa injection of early-egg-stage channel catfish could serve to increase the spawning rates of young adults without resorting to intensive spawning techniques.

One facet of the efficient use of broodstock in breeding programs for channel catfish *Ictalurus punctatus* is the optimization of spawning success in young breeding populations. Lack of spawning by some females may be the result of variations in the age of puberty. Most producers use fish that are age 3 or older because older fish tend to be more reliable spawners; however, some channel catfish females spawn at 2 years of age. During the past few decades, several strategies have been developed and evaluated in

* Corresponding author: bsmall@ars.usda.gov
[1] Present address: Food Science and Technology Institute, Central University of Venezuela, Post Office Box 47.212, Los Chaguaramos, Caracas 1041-A, Venezuela.

Received September 25, 2006; accepted April 16, 2007
Published online February 7, 2008

an attempt to control the timing of sexual maturation and improve spawning success in cultured catfish. Among them, hormonal and environmental manipulations and genetic selection have been used successfully to accelerate maturation (Bates and Tiersch 1998; Ponthier et al. 1998).

Hormonal manipulation using exogenous pituitary substances to induce spawning in fish has been very successful (Clemens and Sneed 1962). In most cases, ripe female broodfish of different species spawn 12–24 h after an injection of pituitary extract from common carp *Cyprinus carpio* (CPE). Successful induced ovulation improves the ability to predict when spawning will occur, thereby aiding fish culturists in achieving greater efficiency in broodstock management and egg production (Degani and Gallagher 1996; Brzuska and Grzywaczewski 1999).

An alternative hormonal induction method is the use of luteinizing hormone releasing hormone analog (LHRHa), an oligopeptide that can be produced in large quantities at low cost. Several researchers have demonstrated that LHRHa administered at different stages of gonadal development stimulates chronic gonadotropin secretion by the pituitary. Moreover, in prespawning fish, ovulation and spermiation are often accelerated by LHRHa treatment. Overall, LHRHa treatment appears to result in a short-term elevation of plasma estradiol and testosterone levels and to stimulate final oocyte maturation and ovulation (Crim and Evans 1983; Hobby and Pankhurst 1997; Tan-Fermin et al. 1997).

In channel catfish, as in other teleosts, oocytes reach their final size during vitellogenesis and then undergo maturation and ovulation (Silverstein and Small 2004). Oocyte maturation and ovulation are controlled by appropriate hormonal stimuli. Toward the final vitellogenic phase, the germinal vesicle, centrally located up to that point, moves to the periphery, indicating the initiation of final maturation. Typically, the last phase of oocyte maturation is associated with additional proteolysis of yolk proteins by lysosomal enzymes (Carnevali et al. 2006). Depending on the species of fish, cathepsins B, D, and L have been implicated as the proteases responsible for maturation-associated proteolysis of vitellogenin-derived proteins.

The objective of this study was to evaluate the efficacy of CPE and LHRHa injections in increasing the incidence of spawning in young-adult female channel catfish. In association with an assessment of the possible effect on spawning, both plasma estradiol and testosterone concentrations and cathepsin B, D, and L activities in follicular oocytes were monitored.

Methods

Animals and husbandy.—Two-year-old female channel catfish (weight, 1.2 ± 0.2 kg [mean ± SE]) were acquired from each of four different selectively bred strains (36 fish per strain): the NWAC103 commercial strain (A) and three proprietary commercial strains (B, C, and D). In May 2003, nine fish per strain were stocked into each of four 0.04-ha earthen ponds at the Thad Cochran National Warmwater Aquaculture Center, Stoneville, Mississippi. Pond temperatures averaged 22 ± 1°C at the time of stocking. Average pond temperatures for May and June were 25 ± 1°C and 28 ± 1°C, respectively. Before stocking, all fish were tagged with a passive integrated transponder (PIT) for individual identification. Throughout the study, fish were fed a 36% crude protein diet at a rate of up to 17 kg/ha daily. Oocyte size of fish from each strain was monitored monthly, from October 2003 to May 2004, by seining 20 fish from each pond and transferring them to four respective concrete raceways (5.44 m^3) containing well water. Before oocyte sampling, the fish were rapidly anesthetized in a bath of 250 mg of MS-222 (tricaine methanesulfonate)/L of water and identified with a PIT tag reader (Power Tracker II; AVID, Norco, California). Ovarian tissues were obtained by cannulation from three fish per strain from each pond as described by Markmann and Doroshov (1983). Thereafter, the fish were allowed to recover in freshwater and returned to their respective ponds. In May 2004, equal numbers of each strain were injected with induction hormones or a saline placebo. To facilitate spawning, seven 3-year-old male channel catfish (Norris strain) and spawning containers were added to each pond after the induction treatments. The Norris strain was selected to avoid strain preference potentially associated with pure versus interstrain pairings. Every 2–3 d during the spawning season, the spawning containers were checked for the presence of eggs. Egg masses were removed from the ponds and reared in a hatchery under standard commercial conditions.

Sampling and hormone injection.—On May 10, 2004, before the start of the spawning season, ponds were seined and 20 fish (5/strain) from each pond were transferred to separate raceways assigned according to pond. Average weight of all the fish sampled was 1.5 ± 0.2 kg. Preinjection samples of blood and follicle-enclosed oocytes were collected. Oocytes were collected by cannulation of the oviduct. The procedure for hormone injection followed the general recommendations of Sneed and Clemens (1960) and Lambert et al. (1999). Females of each strain from each pond were injected as follows: two fish received intraperitoneal

(i.p.) injections of CPE (Sigma P3034; Sigma Chemical Company, St. Louis, Missouri) at 2 mg/kg body weight (BW) followed by a resolving dose of 8 mg/kg BW 20 h later; two fish received i.p. injections of LHRHa (Sigma L7134) at 30 μg/kg BW followed by a resolving dose of 150 μg/kg BW 20 h later; and one fish received two i.p. injections (0 and 20 h) of saline solution (0.9% sodium chloride) and served as the control. At 20 and 24 h postinjection, blood samples were collected to evaluate whether the hormone treatment affected the levels of plasma estradiol and testosterone. Also at 24 h, ovarian samples were collected to monitor the effects of the injections on oocyte maturation and cathepsin activities. After the 24-h blood and ovarian samples were collected, all fish were returned to their respective ponds and spawning activity was monitored.

Estradiol and testosterone.—A syringe fitted with an 18-gauge needle was used to obtain a 1-mL blood sample from the caudal vasculature of each fish. Each sample was placed into a 1.5-mL heparinized tube and stored on ice until centrifuged. The separated plasma was collected and then stored at −80°C. Commercially available enzyme-linked immunosorbent assay (ELISA) kits (Oxford Biomedical Research, Oxford, Michigan) were used to conduct steroid analyses. Steroids were extracted from plasma samples with 1 mL of ethyl ether and then incubated with 50 μL of estradiol– or testosterone–horseradish peroxidase conjugate in antibody-coated plates for 1 h at room temperature. Any bound enzyme conjugate was detected by addition of 150 μL of the chromogenic substrate (3,3′,5,5′ tetramethylbenzidine) and allowing 30 min for color development. The intensity of color development was inversely proportional to the amount of E_2 or T in the sample. Sample absorbencies were read against standards at both 650 nm and 490 nm with a microplate reader (Model 550; Bio-Rad, Hercules, California).

Oocyte maturation.—To follow oocyte maturation visually, samples of follicle-enclosed oocytes were collected by cannulation and stored in a 10% solution of formalin until evaluation. When removed from the formalin solution, oocytes were placed into Serra's fixative to clear the eggs for visualization of the germinal vesicle according to the procedure described by Stoeckel (2000). A compound microscope with transmitted light was used to observe the germinal vesicles of the oocytes. The diameters of oocytes within the samples were measured with an ocular micrometer. An oocyte was classified as mature when the germinal vesicle was clearly differentiated and had moved from the middle to the periphery of the oocyte (Nagahama 1983).

Enzymatic activity of cathepsins.—The enzymatic activities of cathepsins B, D, and L were determined in samples of follicular oocytes that were flash-frozen in liquid nitrogen and then transported to the laboratory on dry ice. All samples were stored at −80°C until analysis.

Cathepsin B.—The enzymatic activity of cathepsin B was assayed using Z-Arg-Arg-NNap (Sigma C5520) as a substrate according to the method described by Barrett and Kirschke (1981). Hydrolysis of the substrate (Z-Arg-Arg-NNap liberated 2-Naphthylamine) was assayed colorimetrically after it was coupled with Fast Garnet (Sigma F8761), a diazonium salt. This colored product was maintained in a 4% solution of Brij 35 (Sigma B250010), a nonionic detergent, and read at 520 nm on the spectrophotometer. The amount of naphthylamine produced was determined by use of a standard curve based on serial dilutions of 20 mM of pure 2-naphthylamine (Sigma A6640–5). The generation of 10 nmol of 2-naphthylamine corresponds to 1 milliunit of cathepsin B activity.

Cathepsin D.—The proteolytic activity of cathepsin D was measured by spectrophotometric methods using bovine hemoglobin (5% weight per volume [w/v]) as a substrate according to the method described by Takahashi and Tang (1981). The hemoglobin is hydrolyzed by cathepsin D to liberate peptides that are soluble in a solution of trichloroacetic acid (TCA). The concentration of peptides was determined by recording the absorbance of the solution at 280 nm with a spectrophotometer (Spectronic 2000; Bausch & Lomb, Hilliard, Ohio). All determinations were performed within the linear range of the assay, up to a net absorbance of 0.3.

Cathepsin L.—To assay the enzymatic activity of cathepsin L, azocasein (2% w/v) containing 6 M urea was added to the sample that was incubated for 30 min at 37°C as described by Barrett and Kirschke (1981). The azo-coupling groups confer an intense yellow color to the azocasein, which is hydrolyzed by cathepsin L to liberate peptides that are soluble in a solution of TCA. The concentrations of peptides were then quantified by recording the absorbance of the solution at 366 nm on the spectrophotometer. Enzymatic activity of cathepsin L was obtained according to the relationship of μmol of products formed per mg of protein.

Fertilization rate.—Egg samples collected from individual spawns were held in the hatchery for 2 d and then removed and placed in Stockard's solution. Using light microscopy, fertilized eggs were identified by observing the stages of embryonic development as described by Silverstein and Small (2004). Eggs that

showed no sign of embryonic development were recorded as unfertilized.

Genotyping.—To determine the potential strain and treatment effects on spawning success, whole-blood samples from each female broodfish and swim-up fry from each spawn were collected. Fifteen microliters of whole blood, or less than 100 mg of whole fry (euthanized by anesthetic overdose), were placed into 500 μL of digestion buffer (10 mM tris-Cl [pH 8.0], 25 mM EDTA, 100 mM NaCl, 0.5% sodium dodecyl sulfate, and 100 μg/mL proteinase K) and incubated overnight at 50°C. After addition of 250 μL of 7.5 M ammonium acetate, the solutions were mixed and proteins were pelleted by microcentrifugation. The supernatant was transferred to a new tube and genomic DNA was precipitated with an equal volume of isopropanol. The DNA pellet was washed with a 70% solution of ethanol and resuspended in water. Forty nanograms of genomic DNA were used to amplify five microsatellite loci (*IpCG0001*, *IpCG0018*, *IpCG0035*, *IpCG0070*, and *IpCG0189*; Waldbieser et al. 2001), and the reaction products were resolved by capillary electrophoresis on an ABI PRISM 3100 Genetic Analyzer (Waldbieser et al. 2003). Parental genotypes were deduced assuming Mendelian inheritance of microsatellite alleles from full-sib fry.

Statistical analysis.—Statistical analyses were conducted using the mixed procedure of Statistical Analysis System version 9.1 software (SAS Institute, Inc., Cary, North Carolina) and the P-diff option in Proc Mixed to separate the means post hoc. A priori separations of the means were conducted using a Student's *t*-test as appropriate (Sokal and Rohlf 1969). The main effects of strain, time, and treatment on steroid concentrations and cathepsin activities were analyzed by analysis of variance (ANOVA), and individual fish were used as the experimental unit. Percent incidence of spawning and fertilization were arcsine transformed before ANOVA; strain and treatment were the fixed effects. Ponds and individual spawns served as the experimental units for analysis of spawning incidence and fertilization rate, respectively. Differences were assumed to be significant at $P < 0.05$ when individuals were the experimental units and at $P < 0.10$ when ponds were the experimental units to allow for greater variability between ponds.

Results

Each spawn was positively identified to individual females based on unique genotypic matches. Strain had no effect ($P > 0.05$) on plasma estradiol and testosterone concentrations within treatment or time. Injection with saline had no effect on plasma estradiol levels at 20 and 24 h compared with 0-h concentrations (Figure 1). Injection with either CPE or LHRHa resulted in a significant increase ($P < 0.05$) in plasma estradiol levels 20 h after the priming dose. Fish that spawned had significantly higher ($P < 0.05$) circulating concentrations of estradiol (0.63 ± 0.10 ng/mL) at 20 h after the priming dose than fish that did not spawn (0.19 ± 0.05 ng/mL). Treatment with either saline or CPE had no effect ($P > 0.05$) on circulating testosterone concentrations (Figure 1). However, plasma testosterone levels significantly increased ($P < 0.05$) 20 h after injection with the priming dose of LHRHa compared with 0-h plasma levels. Circulating levels of testosterone at any given time point were not significantly different ($P > 0.05$) between spawning fish and nonspawning fish.

No difference ($P > 0.05$) in the activity of cathepsin B, D, or L was observed among the strains. Injection with saline had no effect ($P < 0.05$) on cathepsin B, D, or L activity (Figure 2). At 24 h, activities of cathepsins B and L in ovarian tissue of fish injected with either CPE or LHRHa were observed to have significantly increased ($P < 0.05$) compared with 0-h activities. Treatment of broodfish with either CPE or LHRHa had no effect ($P < 0.05$) on the activity of cathepsin D in 24-h ovarian samples. Activities of cathepsins B, D, and L at any given time point were not significantly different ($P > 0.05$) between spawning fish and nonspawning fish.

Average oocyte diameter increased steadily from 755 ± 53 μm (when fish were obtained in October) to 1,624 ± 80 μm at the time of injection the following spring. Twenty-four hours after the priming dose, the germinal vesicle remained in the center of the oocytes sampled from each injected fish, and no sign of maturation or ovulation was apparent. No mortalities were observed among injected broodfish, regardless of treatment. No significant differences ($P > 0.10$) in the incidence of spawning were observed among the strains. The effect of treatment on average incidence of spawning, pooled across strains, is presented in Figure 3. Catfish injected with LHRHa spawned from May 25 to June 7 and had a higher incidence of spawning than saline-injected fish ($P = 0.01$) and noninjected fish ($P = 0.08$). Fish injected with CPE spawned from June 5 to June 25 and had a higher incidence of spawning than the saline-injected fish ($P = 0.07$) but spawned at a rate similar to that of noninjected fish ($P = 0.55$). Noninjected fish began spawning on May 26 and continued until June 10. None of the saline-injected fish spawned. Fertilization rates were not significantly different ($P > 0.05$) among the treatments (Figure 3).

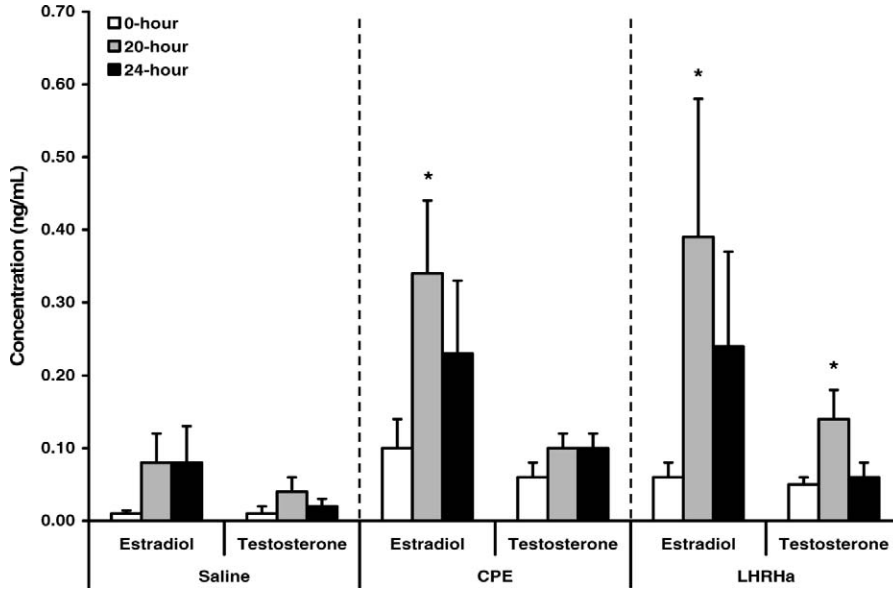

FIGURE 1.—Concentrations (mean + SE) of plasma estradiol and testosterone for young-adult female channel catfish injected with a priming dose and a resolving dose 20 h later of saline, carp pituitary extract (CPE), or luteinizing hormone releasing hormone analog (LHRHa). Mean steroid concentrations that are significantly different ($P < 0.05$) from the preinjection (0-h) concentrations in the same treatments are denoted by asterisks.

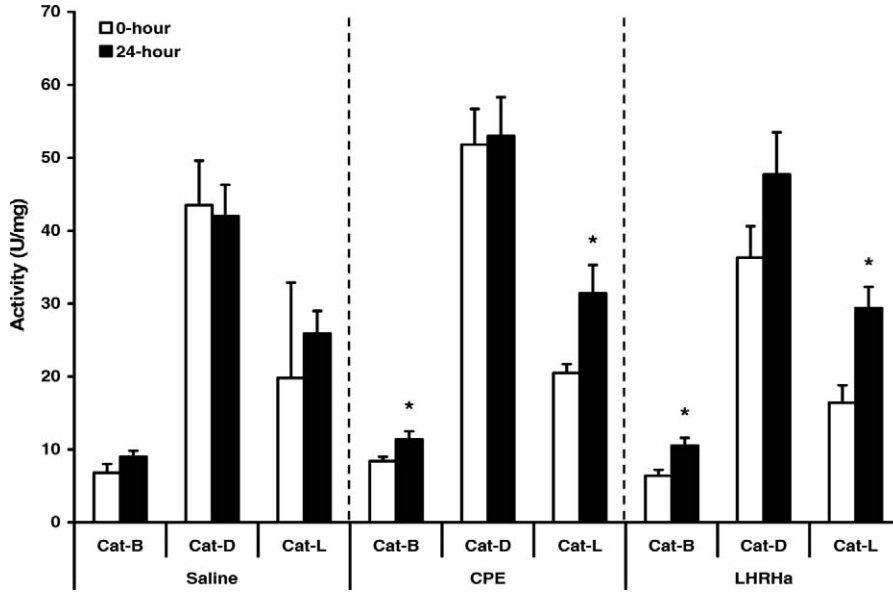

FIGURE 2.—Preinjection (0-h) and 24-h postinjection activities (mean + SE) of cathepsin B (Cat-B), D (Cat-D), and L (Cat-L) in the ovaries of young-adult female channel catfish injected with a priming dose and a resolving dose 20 h later of saline, carp pituitary extract (CPE), or luteinizing hormone releasing hormone (LHRHa). Mean 24-h cathepsin activities that are significantly different ($P < 0.05$) from the preinjection (0-h) activities in the same treatments are denoted by asterisks.

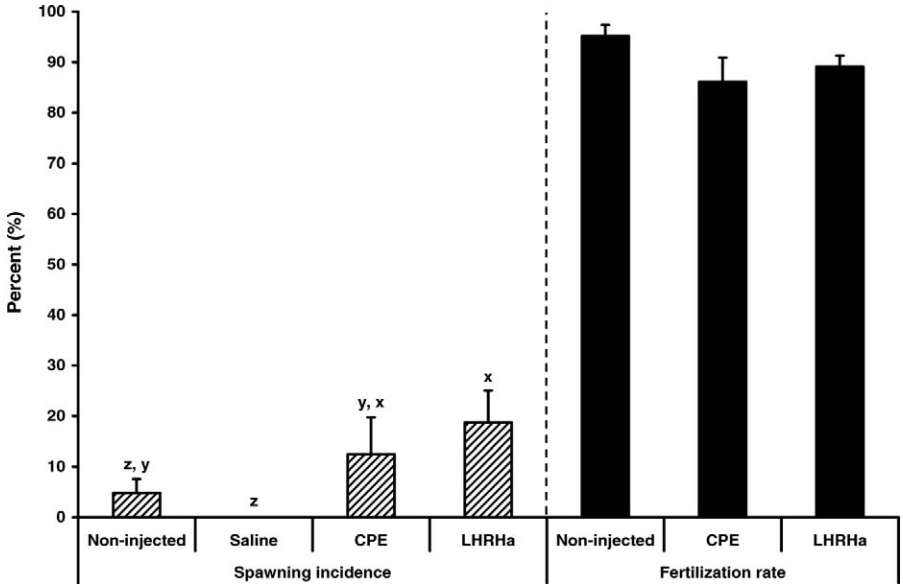

FIGURE 3.—Percent spawning incidence (mean + SE) and corresponding fertilization rates of eggs resulting from the injection of young-adult female channel catfish with a priming dose and a resolving dose 20 h later of saline, carp pituitary extract (CPE), or luteinizing hormone releasing hormone analog (LHRHa) compared with those of noninjected fish. Means with different letters are significantly ($P < 0.10$) different.

Discussion

Modern strains of farm-raised channel catfish have ancestry from many river systems. In the United States, 92 channel catfish stocks have been documented (Dunham and Smitherman 1984). A variety of breeding programs to improve channel catfish traits for commercial aquaculture have been pursued over the years, but few comparisons of reproductive traits have been made among selectively bred commercial strains. This study is the first to conduct such comparisons among young-adult channel catfish from commercially reared strains.

Spawning traits of pure- and interstrain channel catfish were previously compared by Dunham et al. (1983), who observed that the interstrain catfish typically spawned earlier than the pure strain. Spawning rate and fecundity of 3-year-old interstrain catfish were also higher than those of pure-strain fish. Four-year-old pure-strain catfish, however, demonstrated improved spawning performance, which may be due to delayed maturation relative to the interstrain fish. Associated advantages or disadvantages potentially resulting from pure-strain matings were avoided in the present study by using Norris strain male channel catfish. The average spawning rate reported by Dunham et al. (1983) for pure-strain matings was 28%. Silverstein and Small (2004) examined data obtained from the U.S. Department of Agriculture, Agricultural Research Service, in Stoneville, Mississippi, and estimated that typical spawning rates in broodfish ponds ranged from 8% to 80%, averaging 29%. The incidence of spawning in the present study was only 4.8% for young-adult catfish that were not injected with CPE or LHRH but increased to 18.8% among the LHRHa-injected fish. These rates are below the average suggested by Silverstein and Small (2004) and reflect the uncertainty of spawning success among young broodfish. For this reason, LHRHa injection may prove to be useful in stimulating spawning in young broodfish.

The present study is unlike other studies using 3- and 4-year-old channel catfish to evaluate hormone induction techniques because the females used in other studies were individually selected based on the roundness of their abdominal area and coloration and swelling of the genital area and the males were selected based on head size and prominence of genital papillae (Sneed and Clemens 1960; Busch and Steeby 1990; Bates and Tiersch 1998; Silverstein et al. 1999). In each of these studies, fish with the best morphological characteristics indicative of spawning were selected, with the goal of inducing spawning in artificial environments (aquaria and cages). In the present study, no effort was made to select fish most likely to spawn. Instead, the female broodfish were randomly assigned to hormone treatment groups in an effort to assess

whether young broodfish that might not spawn naturally could be hormonally induced to spawn in ponds.

The differences in fish response to hormonal induction in this study are probably functions of reproductive maturity and seasonal responsiveness because body weight and age were similar and no effect of strain was detected. When coupled with small oocyte size and a lack of germinal vesicle migration, the failure of a significant portion of the hormonally induced fish to ovulate 24 h after treatment is further evidence that these fish were not preovulatory at the time of injection. Although others have demonstrated the efficacy of CPE and LHRHa for inducing ovulation in channel catfish (Sneed and Clemens 1960; Busch and Steeby 1990; Bates and Tiersch 1998; Silverstein et al. 1999), those studies used fish selected for the best morphological characteristics indicative of spawning, and the majority were probably preovulatory at the time of injection. The present study demonstrates an ability to induce pond-spawning in young-adult catfish with early-stage eggs after a single priming and resolving dose regimen of LHRHa. The reproductive axis of these young-adult catfish responded to hormonal stimuli with short-term elevations of plasma E_2 and T, which may have aided the induction of final oocyte maturation and ovulation. Similar increases in circulating E_2 and T associated with an induction of final oocyte maturation and ovulation have been previously observed in fish (Crim and Evans 1983; Hobby and Pankhurst 1997; Tan-Fermin et al. 1997). Increases in the concentrations of plasma steroids during the postinjection period probably resulted from increased pituitary gonadotropin production (Singh and Singh 1987).

A limited number of studies have investigated the relationships among hormone treatments, lysosomal enzymatic activity, and oocyte maturation. This is the first report of the effects of exogenous CPE and LHRHa administration on cathepsin activities in the ovaries of channel catfish. Similar to the observed reproductive steroid response, the activities of cathepsins B and L increased in the ovaries of young-adult catfish treated with CPE and LHRHa. Cathepsin D, however, did not respond to hormonal stimuli at this stage. The lack of a cathepsin D response to hormone injection is supported by the investigations of Carnevali et al. (1999) and Kestemont et al. (1999), who concluded that cathepsin D was highest in early vitellogenic oocytes when yolk protein was being deposited at a high rate. Carnevali et al. (1999) stated that, typically, second proteolysis, the last phase of oocyte maturation, occurs just before ovulation and is associated with the cleavages of higher molecular weight yolk proteins by the proteolytic action of lysosomal enzymes. In barfin flounder *Verasper moseri*, Matsubara et al. (2003) observed that a dramatic increase in protease activity occurred during the late maturational stage when homogenates of oocytes at different maturational stages were assayed. Furthermore, among lysosomal enzymes, cathepsin B was observed to be the protease responsible for maturation-associated proteolysis of yolk proteins in this species. In gilthead seabream (also known as the gilthead bream) *Sparus auratus*, cathepsin L was reported to have the highest level of protease activity before final oocyte maturation (Carnevali et al. 2006). Similarly, Kestemont et al. (1999) reported a significant increase in cathepsin L activity during the spawning season of the Eurasian perch *Perca fluviatilis*, and Hiramatsu et al. (2002) concluded that cathepsin L was involved in secondary proteolysis of the yolk proteins during final maturation of oocytes in the white perch *Morone americana*. The observed changes in cathepsin activities following hormone injection in the present study suggest that, although final maturation may have occurred several days later, LHRHa and CPE acted to stimulate cathepsin B and L activity before final oocyte maturation and ovulation.

Conclusions

Genetic improvement of channel catfish is slow owing to the long generation time for this species, typically 3–4 years. Results of the present study, demonstrating an increase in the incidence of spawning of young adult catfish injected with LHRHa, suggest that young fish that might not spawn naturally in ponds could be hormonally induced to spawn. This methodology could be used to increase spawning rates in young broodfish and decrease the time between generations for selective breeding. Further, the lack of differences in any of the reproductive parameters measured among the four strains evaluated suggests that there have been few changes in reproductive traits as a result of past selective breeding practices or that the changes have been similar across strains. Furthermore, this is the first report of the effects of exogenous CPE and LHRHa administration on cathepsin activities in the ovaries of channel catfish. Thus, these data represent the foundation for future investigations into the roles of cathepsins in channel catfish oocyte maturation.

Acknowledgments

This article is based, in part, on research conducted by the senior author for the doctor of philosophy degree in the Department of Wildlife and Fisheries at Mississippi State University, Starkville, Mississippi.

We thank the Scientific and Humanistic Council, Central University of Venezuela, Mississippi State University, and the Southern Regional Aquaculture Center for the funds to support this investigation. We sincerely appreciate the cooperation of Harvest Select Farms and Thompson Fisheries in providing the fish to conduct this investigation. Mention of trade names, proprietary products, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply approval to the exclusion of other products that may be suitable.

References

Barrett, A. L., and H. Kirschke. 1981. Cathepsin B, cathepsin H, and cathepsin L. Pages 535–559 *in* L. Lorand, editor. Methods in enzymology, volume 80. Academic Press, New York.

Bates, M. C., and T. R. Tiersch. 1998. Preliminary studies of artificial spawning of channel catfish as male–female pairs or all-female groups in recirculating systems. Journal of the World Aquaculture Society 29:325–334.

Brzuska, E., and R. Grzywaczewski. 1999. Artificial spawning of carp *Cyprinus carpio* L.: differences between the effects on reproduction in females of Israeli strain Dor-70 and its cross-breed treated with carp pituitary and Ovopel. Aquaculture Research 30:559–570.

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

Carnevali, O., R. Carletta, A. Cambi, A. Vita, and N. Bromage. 1999. Yolk formation and degradation during oocyte maturation in seabream *Sparus aurata*: involvement of two lysosomal proteinases. Biology of Reproduction 60:140–146.

Carnevali, O., C. Cionna, L. Tosti, E. Lubzens, and F. Maradonna. 2006. Role of cathepsins on ovarian follicle growth and maturation. General and Comparative Endocrinology 146:195–203.

Clemens, H. P., and K. E. Sneed. 1962. Bioassay and use of pituitary materials to spawn warmwater fishes. U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Research Report 61, Washington, D.C.

Crim, L. W., and D. M. Evans. 1983. Manipulation of the seasonal reproductive cycle of the landlocked Atlantic salmon (*Salmo salar*) by LHRH analogues administered at various stages of gonadal development. Canadian Journal of Fisheries and Aquatic Sciences 40:61–67.

Degani, G., and M. L. Gallagher. 1996. Steroid and carp pituitary extract influence on follicle frequency in vitellogenesis and maturation of *Trichogaster trichopterus*. Journal of Aquaculture in the Tropics 11:39–48.

Dunham, R. A., and R. O. Smitherman. 1984. Ancestry and breeding of catfish in the United States. Alabama Agricultural Experiment Station, Auburn University, Auburn.

Dunham, R. A., R. O. Smitherman, J. L. Horn, and T. O. Brice. 1983. Reproductive performances of crossbred and pure-strain channel catfish brood stocks. Transactions of the American Fisheries Society 112:436–440.

Hiramatsu, H., N. Ichikawa, H. Fukada, T. Fujita, C. V. Sullivan, and A. Hara. 2002. Identification and characterization of proteases involved in specific proteolysis of vitellogenin and yolk proteins in salmonids. Journal of Experimental Biology 292:11–25.

Hobby, A. C., and N. W. Pankhurst. 1997. Postovulatory egg viability in the snapper *Pagrus auratus* (Sparidae). Marine Freshwater Research 48:385–389.

Kestemont, P., J. Cooremas, A. Abi-Ayad, and C. Melard. 1999. Cathepsin L in eggs and larvae of perch *Perca fluviatilis*: variations with developmental stage and spawning period. Fish Physiology and Biochemistry 21:59–64.

Lambert, D. M., B. J. Argue, Z. Liu, and R. A. Dunham. 1999. Effects of seasonal variation, thyroid and steroid hormones, and carp pituitary extract on the artificial production of channel catfish *Ictalurus punctatus* × blue catfish *I. furcatus* hybrids. Journal of the World Aquaculture Society 30:80–86.

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

Matsubara, T., M. Nagae, N. Ohkubo, N. Andoh, S. Sawaguchi, N. Hiramatsu, C. V. Sullivan, and A. Hara. 2003. Multiple vitellogenins and their unique roles in marine teleosts. Fish Physiology and Biochemistry 28:295–299.

Nagahama, Y. 1983. The functional morphology of teleost gonads. Pages 223–275 *in* W. S. Hoar, D. J. Randall, and E. M. Donaldson, editors. Fish physiology, volume 9A. Academic Press, New York.

Ponthier, J. L., C. H. L. Shackleton, and J. M. Trant. 1998. Seasonal changes in the production of two novel and abundant ovarian steroids in the channel catfish (*Ictalurus punctatus*). General and Comparative Endocrinology 111:141–155.

Silverstein, J. T., B. G. Bosworth, and W. R. Wolters. 1999. Evaluation of dual injection of LHRHa and the dopamine receptor antagonist pimozide in cage spawning of channel catfish *Ictalurus punctatus*. Journal of the World Aquaculture Society 30:263–268.

Silverstein, J. T., and B. C. Small. 2004. Reproductive physiology. Pages 69–94 *in* C. S. Tucker and J. A. Hargreaves, editors. Biology and culture of channel catfish. Elsevier, Amsterdam.

Singh, S., and T. P. Singh. 1987. Seasonal profiles of sex steroids in blood plasma and ovarian tissue of *Clarias batrachus*. General and Comparative Endocrinology 65:216–224.

Sneed, K. E., and H. P. Clemens. 1960. Use of fish pituitaries to induce spawning in channel catfish. U.S. Fish and Wildlife Service Special Scientific Report Fisheries 329.

Sokal, R. R., and F. J. Rohlf. 1969. Introduction to biostatistics. Freeman, New York.

Stoeckel, J. N. 2000. A method for viewing the germinal vesicle in oocytes of commercial catfishes. North American Journal of Aquaculture 62:240–247.

Takahashi, T., and J. Tang. 1981. Cathepsin D from porcine and bovine spleen. Pages 565–581 *in* L. Lorand, editor. Methods in enzymology, volume 80. Academic Press, New York.

Tan-Fermin, J. D., R. R. Pagador, and R. C. Chavez. 1997.

LHRHa and pimozide-induced spawning of Asian catfish *Clarias macrocephalus* (Gunther) at different times during an annual reproductive cycle. Aquaculture 148:323–331.

Waldbieser, G. C., B. G. Bosworth, D. J. Nonneman, and W. R. Wolters. 2001. A microsatellite-based genetic linkage map of the channel catfish, *Ictalurus punctatus*, genome. Genetics 158:727–734.

Waldbieser, G. C., S. M. A. Quiniou, and A. Karsi. 2003. Rapid development of gene-tagged microsatellite markers from BAC clones using anchored TAA-repeat primers. BioTechniques 35:976–979.